Study Title:
Inhibition of Rho Kinase (ROCK) with Fasudil as disease-modifying treatment for ALS

Protocol Code:
ROCK-ALS

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Protocol

Version: 3.0_final Date: 27.06.2019

Sponsor of the Clinical Study
[according to § 4 Abs. 23 AMG]

Georg-August-Universität Göttingen
Stiftung Öffentlichen Rechts
Universitätsmedizin Göttingen

Coordinating Investigator (LKP)
[according to § 4 Abs. 25 AMG]

Prof. Dr. Jens Schmidt
Klinik für Neurologie
Universitätsmedizin Göttingen

- Confidential -

Protocol Approval Signature Page

The following persons agree with the conduct of the study according to this protocol.

Approved by:

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06-Aug-2019
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15 July 2019
Date

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Head of Studienzentrum UMG
Universitätsmedizin Göttingen

24/7/19
Date

The present study protocol was prepared in accordance with the relevant ICH-GCP criteria.

Investigator Signature Page

I have read the clinical study protocol and I confirm that it contains all information to accordingly conduct the clinical study. I know that the study will be done in agreement with GCP and I will cooperate with the respective Monitors and pledge the clinical study will be conducted at my study center according to the protocol.

The first patient will be enrolled only after all ethical and regulatory requirements are fulfilled. I pledge that written informed consent for trial participation will be obtained from all patients.

I know the requirements for accurate notification of serious adverse events and I pledge to document and notify such events as described in the protocol.

I pledge to retain all trial-related documents and source data as described. All necessary documents will be provided before trial start. I agree that these documents will be submitted to the responsible Regulatory Authorities and Ethics Committees.

_____

Investigator of the site Date

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Trial Sites
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Table of Contents

1 Abbreviations and Definitions

AD	Alzheimer's disease
ALS	Amyotrophic lateral sclerosis
ALSAQ-5	Amyotrophic Lateral Sclerosis Assessment Questionnaire (5 items)
ALAT	Alanine-Aminotransferase
ALSFRS-R	ALS Functional Rating Scale – Revised
AMG	German drug law (Arzneimittelgesetz)
ASAT	Aspartate-Aminotransferase
BP	(arterial) Blood pressure
CI	Coordinating Investigator
CK	Creatinine Kinase
CRF	Case report form
CSF	Cerebrospinal Fluid
CV	Curriculum vitae
DMP	Data Management Plan
EC/IEC	Ethics committee/Independent ethics committee
ECAS	Edinburgh Cognitive and Behavioral ALS Screen
eCRF	Electronic case report form
EEA	European Economic Area (Europäischer Wirtschaftsraum)
fALS	Familial amyotrophic lateral sclerosis
FPI	First patient in
FPLC	Fast Protein Liquid Chromatography
GCP	Good clinical practice
GCP-V	GCP regulation
GFR	Glomerular filtration rate
Hb	Hemoglobin
HCG	Human chorionic gonadotropin
Hct	Hematocrit
ICD	Informed consent document
ICH	International conference on harmonization of technical requirements for registration of pharmaceuticals for human use
IMP	Investigational medicinal product
INN	International nonproprietary name
ISF	Investigator site file
ITT	Intention-To-Treat
IUD	Intrauterine device
IV	intravenous
LKP	Leiter der Klinischen Prüfung (Coordinating Investigator)
LPI	Last patient in
LPO	Last patient out
MCH	Mean corpuscular/cellular hemoglobin
MCHC	Mean corpuscular/cellular hemoglobin concentration
MCV	Mean cell volume
MDRD	Modification of Diet in Renal Disease
MedDRA	Medical dictionary for regulatory activities terminology
MR	Magnetic resonance
MSZ	Münchner Studienzentrum

MUNIX	Motor Unit Number Index
PD	Parkinson’s disease
PLT	Thrombocytes, platelets
PPS	Per-protocol analysis set
RBC	Red blood cells
ROCK	Rho Kinase
SAE	Serious adverse event
SAP	Statistical analysis plan
SDV	Source data verification
SMB	Safety Monitoring Board
SmPC	Summary of product characteristics
SOP	Standard operating procedure
SUSAR	Suspected unexpected serious adverse reaction
sVC	Slow vital capacity
TMF	Trial master file
UMG	Universitätsmedizin Göttingen
WBC	White blood cells

2 Synopsis

Study Title	**Inhibition of Rho Kinase (ROCK) with Fasudil as disease-modifying treatment for ALS**
Type of Project	Phase IIa, multi-center, international, randomized, double-blind, controlled, prospective, dose-finding, exploratory, interventional
Sponsor	Universitätsmedizin Göttingen (UMG) Georg-August-Universität Rainer Bredenkamp Head of Studienzentrum UMG Von-Bar-Str. 2/4, 37075 Göttingen
Coordinating Investigator	Prof. Dr. Jens Schmidt Klinik für Neurologie Universitätsmedizin Göttingen (UMG) Robert-Koch-Str. 40 37075 Göttingen Tel.: +49 (0) 551 39 22355 Fax: +49 (0) 551 39 8405 j.schmidt@med.uni-goettingen.de
(Sub-) Investigators	A list of sub-investigators participating in this study is kept at the Trial Office (UMG, Göttingen)
Participating Trial Sites	This is a multicenter, international study, which will be conducted in approximately 15 trial sites in Germany, France and Switzerland. A list of investigators and participating trial sites is kept on file at the Trial Office (UMG, Göttingen).

Hypothesis	Fasudil is safe and well tolerated, and its application will significantly improve the clinical outcome in patients with Amyotrophic lateral sclerosis (ALS).
Rationale	Rho Kinase (ROCK) inhibition is a completely novel and yet untargeted therapeutic strategy for the treatment of neurodegenerative disorders, including ALS. In previous studies, the ROCK inhibitor Fasudil was neuroprotective, induced axonal regeneration and improved survival and behavioral outcome in models of ALS and other neurodegenerative diseases. The participating centers have thorough experience in the conduct of clinical trials and the trial drug is licensed in Japan. Therefore, the trial can be rapidly initiated. Since Fasudil is a kinase inhibitor, the activity of ROCK can be measured to ensure target engagement. In addition, Fasudil and its metabolites can be detected and quantified using Fast protein liquid chromatography (FPLC) in blood and Cerebrospinal Fluid (CSF). We propose to collect biological samples for biomarker studies, including blood, CSF, urine, and saliva. There is ample published preclinical and clinical data on pharmacology and toxicology.
Study Medication	Fasudil hydrochloride hydrate IV solution 30 mg (2 x 1 ml, 15 mg/ml Fasudil hydrochloride hydrate), Fasudil hydrochloride hydrate IV solution 15 mg (1 ml, 15 mg/ml Fasudil hydrochloride hydrate and 1 ml NaCl 0.9%), Placebo to Fasudil hydrochloride hydrate (2 x 1 ml, NaCl 0.9%) Manufacturer: Universitätsklinikum Leipzig AöR, Apotheke
Study Design	Intervention: Fasudil IV in two doses administered two times daily for 20 treatment days. The treatment days may be distributed within a period of 26 (minimum) to 30 (maximum) subsequent days with a maximum of three consecutive days without treatment. Arm A: dose = 30 mg (2 x 15mg); number of subjects to be enrolled: 40 Arm B: dose = 60 mg (2 x 30mg); number of subjects to be enrolled: 40 Arm C: Placebo; number of subjects to be enrolled: 40 IV application of Fasudil and placebo will be performed over 45 min. Monitoring of blood pressure and pulse before and at 0, 10, 20, 30, 45, and 60 minutes after

	each intravenous infusion of the drug for each of the IV administrations. Duration of intervention per patient: 20 days treatment + follow up until day 180 ± 5.
Timetable	Start of recruitment: Q1 2019 End of recruitment: anticipated: Q2 2020 Last patient last visit: anticipated: Q4 2020
Total Number of Patients	Total: 120
Study population	ALS patients (early stage)
Inclusion Criteria	(1) Probable (clinically or laboratory) or definite ALS according to the revised version of the El Escorial World Federation of Neurology criteria (2) Disease duration more than 6 months and less than 24 months (inclusive). Disease onset defined as date of first muscle weakness, excluding fasciculations and cramps (3) Vital capacity more than 65% of normal (slow vital capacity; best of three measurements) (4) Age: ≥ 18 years (5) Patients have to be treated with Riluzole (2 x 50mg/d), must be stable for at least four weeks before randomization (6) Patients who have started on Edaravone therapy shall continue Edaravone treatment. Edaravone treatment must not be discontinued for reasons of trial participation. (7) Women of childbearing age must be non-lactating and surgically sterile or using a highly effective method of birth control and have a negative pregnancy test. Acceptable methods of birth control with a low failure rate i.e. less than 1% per year) when used consistently and correct are such as implants, injectables, combined oral contraceptives, hormonal intrauterine devices (IUDs), sexual abstinence or vasectomized partner. For Switzerland sexual abstinence is not allowed as a contraceptive method. (8) Capable of thoroughly understanding all information given and giving full informed consent according to GCP (9) Patients have to have a valid health insurance, when recruited in a center in France

Exclusion Criteria	(1) Previous participation in another clinical study involving trial medication within the preceding 12 weeks or five terminal half times of the longest to be eliminated trial medications (whichever is longer) or previous participation in this trial

	(2) Tracheostomy or continuous assisted ventilation of any type during the preceding three months before randomization or a significant pulmonary disorder not attributed to ALS, which may complicate the evaluation of respiratory function, intermittent non-invasive ventilation is permitted, (3) Patients with a history of intracranial bleeding, known intracerebral aneurysms or Moyamoya disease, or positive family history for the above. If only family history positive, MR- or x-ray-based cranial imaging not older than 24 months must confirm absence of bleeding, aneurysms or Moyamoya. (4) Gastrostomy (5) Any medical condition known to have an association with motor neuron dysfunction or involving neuromuscular weakness or another neurodegenerative disease, e.g. PD or AD, which might confound or obscure the diagnosis of ALS (6) Presence of any concomitant life-threatening disease or impairment likely to interfere with functional assessment (7) Patients with known arterial hypotension (resting blood pressure <90/60 mmHg) or previous hypotensive episodes or requiring treatment for increasing of blood pressure, such as fludrocortisone, midodrine, etilefrine, cafedrine or theodrenaline (8) Patients with an uncontrollable or unstable arterial hypertensive disease (resting blood pressure >180 mmHg systolic and/or >120 mmHg diastolic under current antihypertensive medication) (9) Known pulmonary hypertension and any medication prescribed for treatment of pulmonary hypertension (10) Confirmed hepatic insufficiency or abnormal liver function (stable ASAT and/or ALAT greater than 3 times the upper limit of the normal range) and determined to be non-transient through repeat testing (11) Renal insufficiency with a glomerular filtration rate (GFR) <60 ml/min/1,73m² (calculated by MDRD equation) and determined to be non-transient through repeat testing (12) Major psychiatric disorder, significant cognitive impairment or clinically evident dementia precluding evaluation of symptoms (13) Hypersensitivity to any component of the study drug (14) Liable to be not cooperative or comply with the trial requirements (as assessed by the investigator), or unable to be reached in the case of emergency (15) Pregnant or breast-feeding females or females with childbearing potential, if no adequate contraceptive measures are used (16) Prisoners or subjects who are involuntary incarcerated

	(17) Patients subject to legal protection measures
Visit and Documentation Schedule	Screening: V0 (up to 42 days before V1) Baseline: V1 (day 1) Treatment: V1 (day 1) to V20 (day 26 to 30) Follow-up: V21 (day 45 ± 3), V22 (day 90 ± 4), V23 (day 180 ± 5) Total study visits: 23 (including 20 treatment days)
Study Objectives and Endpoints	**Study Objectives:** This study will primarily yield information on safety, tolerability and efficacy of the licensed IV formulation of Fasudil in two different doses compared to placebo. This will permit to select a safe and well-tolerated dose with the most promising efficacy potential for subsequent studies. The primary objective of this study is the assessment of tolerability and safety. **Study Endpoints:** **Primary Safety and Tolerability Endpoint:** Primary endpoints are the proportion of patients without significant drug intolerances during the treatment period (tolerability) and the proportion of patients without treatment-related SAEs through to visit V23 (safety). **Secondary Endpoints:** The survival time and the change of ALS Functional Rating Scale (ALSFRS-R), ALS Assessment Questionnaire (ALSAQ-5), Edinburgh Cognitive and Behavioral ALS Screen (ECAS), Motor Unit Number Index (MUNIX) and vital capacity (VC) from baseline to visits V20, V22, V23 after start of the intravenous application of the ROCK-inhibitor Fasudil (for 20 treatment days). Secondary safety endpoint is safety and tolerability until V20.
Safety	Safety is assessed by the recording of adverse events, laboratory parameters, vital signs, physical examination, and concomitant medication.
Discontinuation Criteria	**Premature termination of the treatment must occur in the event of:** • Personal patient wish, • Severe adverse events / toxicity (grading according to Common Terminology Criteria)

	• Significant intolerance of the study medication: assumed if the patient wishes to terminate participation in the trial due to any AE considered to be drug-related • Significant alterations of clinical or laboratory findings, e.g. persistent increase in ASAT, ALAT or GGT >3 times the upper limit of normal or persistent decrease of GFR < 45 ml/min/1,73m² (calculated by MDRD equation) • Pregnancy • Significant intercurrent illness or emergency situation requiring cessation of the study • Circumstances which, according to the study protocol, do not allow the foreseen therapeutic interventions • Cumulative SAEs that are unexpected and/or life threatening • Violation of inclusion/exclusion criteria • Loss of contact • Significant violation of the study protocol • Failure to comply with the investigational procedures • Reasonable wish of the Sponsor • Any other circumstance that, in the opinion of the investigator, warrants termination of the patient's participation in the study
Statistical Analysis	Primary endpoints (safety and tolerability): For both active treatment groups and for both, safety and tolerability, separately the difference in proportions to the placebo group will be calculated with its 95% confidence interval. Subsequent analyses will model tolerability and safety in logistic regressions adjusting for randomization stratification factors and important prognostic factors assessed at baseline. Primary analysis will be carried out on the ITT population. Secondary Endpoints: Efficacy outcomes including ALSFRS-R, ALSAQ-5, vital capacity, ECAS and MUNIX through to visit V23 will be analyzed by means of Gaussian linear model for repeated measures (so-called MMRM). The analysis will be primarily performed on the ITT population. The Kaplan-Meier method will be applied to estimate the survival time in each group. Pairwise group comparisons against placebo will be analyzed using exact log rank tests. Cox proportional hazards regression will be carried out if there is a sufficient number of events.

Risks, Adverse Drug Reactions, Drug Interactions, Restrictions, Contraindications, Procedures in Case of Emergency	Risks include potential side effects of Fasudil, e.g.: Frequent (1 – <10 %): - Abnormal elevation of liver enzymes (ALT, AST, GGT, LDH): > 5 % - Intracranial bleedings: 1.72 % - Renal dysfunction (increase of creatinine, BUN, polyuria): 0.1 – 5 % - Anemia, Leukopenia, Thrombopenia: 0.1–5 % - Hypotension: 0.1 – 5 % - Rash: 0.1 – 5 % Occasional (0.1 – <1 %): - Gastrointestinal bleeding, lung bleeding, epistaxis, hematoma: 0.27 % Rare (0.01 – <0.1 %): - Icterus: < 0.1% - Difficulty to urinate: < 0.1 % - Flatulence, nausea, vomiting: < 0.1 % - Fever: < 0.1 % - Headache, somnolence, and respiratory depression: < 0.1 % - Hot flashes: <0.1 % - Shock: 0.02 % - Paralytic ileus: 0.04 % For randomized patients treated in a double-blind fashion, emergency codes will be available to the investigator. A code, which reveals the treatment group for a specific study patient, may be opened during the study only if the choice of treatment and safety depends on the study subject's therapy assignment and in case of notification of SUSAR.
Risk-benefit Analysis	A risk-benefit assessment, including the detailed analysis of Suspected Unexpected Serious Adverse Reactions (SUSARs) will be performed by the independent Safety Monitoring Board (SMB). In case of significant safety risks, study participation may be terminated for the individual patient or for the entire study.

3 Introduction

3.1 Introduction and Background

Amyotrophic lateral sclerosis is a devastating, progressive neurodegenerative disorder resulting in muscle weakness and finally death due to respiratory failure within three to five years following the diagnosis. Until now, only the NMDA-receptor antagonist Riluzole is licensed in the EU for the treatment of ALS based on two clinical studies (1,2). Riluzole is the only approved drug in Europe for treating ALS, which has been shown to prolong patient survival by several months. This should therefore be used by all patients with the diagnosis of ALS permanently. Also during and after the ROCK-ALS study, this medication should be continued unchanged. In addition to Riluzole, Edaravone has been approved in the US for the treatment of ALS in 2017. Edaravone has slowed the decline of clinical functional parameters in a subgroup of ALS patients, but data on better survival with Edaravone are not available. Edaravone must be infused intravenously at monthly intervals for more than 10 days. Due to the form of application and the efficacy shown only in subgroups, Edaravone is by far not used as frequently as Riluzole. For ethical reasons, patients on Edaravone shall continue their therapy during this study. Riluzole increases the mean survival only by three months and thus more effective means of disease-modification are urgently needed.

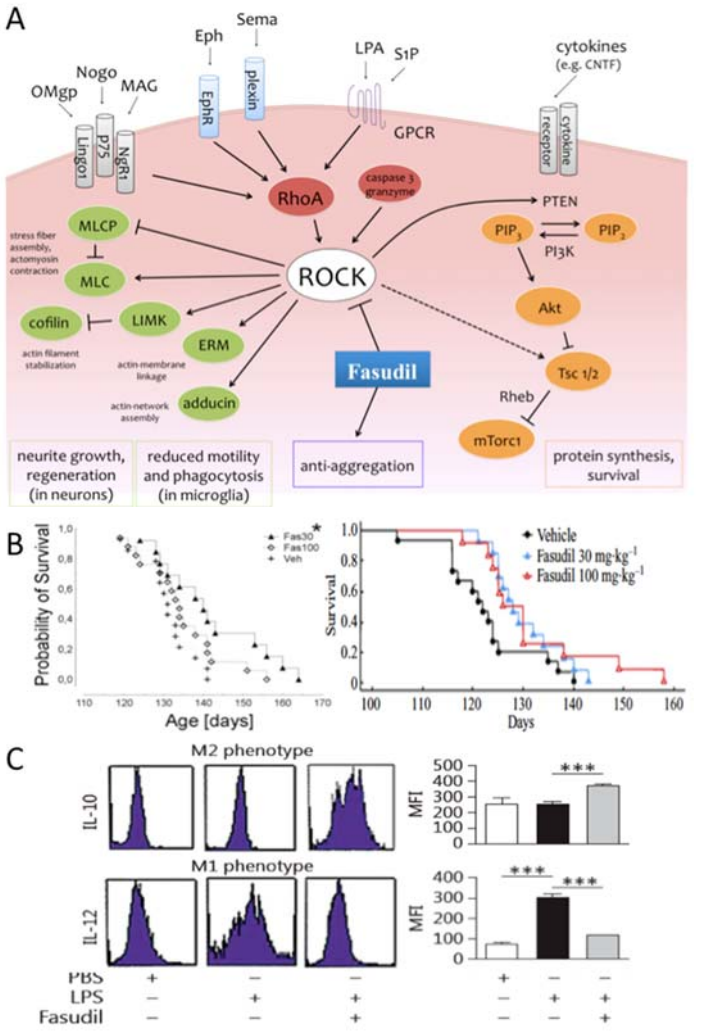

Fig. 1 A: Intracellular pathways involved in ROCK signaling influence neurite growth, cell survival, microglial function and protein aggregation (adapted from[7]) **B:** Two independent studies confirm that Fasudil increases survival in the SOD1G93A mouse model of ALS[5,6]. **C:** Fasudil shifts the microglial phenotype from the rather cytotoxic M1 to more cytoprotective M2[13].

Rho kinase (ROCK) is a serine/threonine kinase with two isoforms, ROCK1 and ROCK2. While ROCK1 is expressed in peripheral tissue, ROCK2 is highly expressed in the CNS and its expression increases with age (3). Axonal growth inhibitory molecules (e.g. Nogo, MAG, OMgp, ephrins, semaphorins), which bind to their extracellular receptors (NogoR/p75/ Lingo-1, EphR, Plexins) signal via ROCK and trigger growth cone collapse and impaired axonal regeneration. In non-neuronal structures, regulation of the actin cytoskeleton plasticity by ROCK also mediates vasoconstriction and vascular remodeling (4). Interestingly, ROCK also regulates cell survival via Akt and mTOR (5-7) (**Fig. 1A**).

Fasudil is a small-molecule inhibitor of Rho Kinase (ROCK) and was originally developed as a vasodilatatory drug and licensed in Japan in 1995 for the treatment of vasospasms following subarachnoid hemorrhage (SAH). Several thousands of patients have been treated with Fasudil since. However, it has also been tested in numerous clinical trials for other applications, most frequently in cardiovascular disease, such as angina pectoris, Raynaud's syndrome, pulmonary hypertension and arterial hypertension (4). Fasudil has not been approved in any country besides Japan and China. In the CNS, the effects of Fasudil were assessed in a phase III trial in patients with acute ischemic stroke, where Fasudil treatment significantly improved clinical outcome (8). Significant amounts of Fasudil are taken up in the brain (9). An intravenous formulation of Fasudil is licensed in Japan, but oral (tablet) formulations, including extended release (ER) formulations, were used in clinical trials in humans. The longest published exposure to Fasudil in humans (clinicaltrials.gov and PubMed, all entries for "Fasudil, clinical, trial") was 8 and 12 weeks (angina pectoris and pulmonary arterial hypertension). Side effects included allergic skin reactions, a slight drop in systolic blood pressure and reversible renal impairment without major safety concerns arguing against longer dosing as required for the treatment of ALS (10,11). Because of ample clinical experience with Fasudil and its well-known safety profile it represents an excellent candidate for repositioning as a disease-modifying therapy in ALS. Our group recently showed that oral Fasudil exerts neuroprotective and pro-regenerative effects in Parkinson's disease (PD) models in vitro and in vivo: delivery of Fasudil in drinking water attenuated MPTP-induced dopamine (DA) neuron death in a mouse model of PD, preserved nigrostriatal projections, striatal DA and metabolite levels and improved motor function, independently from its vasodilatatory effects (5). Most importantly, in the SOD1(G93A) mouse model of amyotrophic lateral sclerosis (ALS), Fasudil prolonged survival, improved motor function and a regenerative response in the neuromuscular junction and this was accompanied by a modulation of microglial activity (6,12,13) (Fig. 1B and C). ROCK inhibition not only influenced morphology of microglia, but also attenuated the LPS-induced release of chemokines and cytokines (12,14). Other groups showed beneficial effects in the SMN1 knockdown model of spinal muscular atrophy (SMA): here, Fasudil-treated animals showed a longer survival time, which was accompanied by increased postsynaptic endplate and muscle fiber size (15). We have also recent evidence for a beneficial effect of ROCK inhibition on protein aggregation, a major hallmark in neurodegenerative disorders. Although the underlying mechanisms are not fully understood, there is evidence to suggest that ROCK plays an important role in the pathogenesis of numerous neurodegenerative disorders and that inhibition of ROCK could represent a promising novel therapeutic target. We therefore propose to evaluate the safety, tolerability and efficacy of Fasudil IV in a human clinical trial on ALS patients.

3.2 Rationale and Justification for the Study

This is the first controlled trial to assess the effects of the ROCK inhibitor Fasudil in ALS. We employ a three-armed trial design to establish the best-tolerated dosage of Fasudil IV. The concept of utilizing a drug, which simultaneously beneficially affects survival of motoneurons, axonal regeneration and modulates glial function, is highly innovative. ROCK inhibition is a completely novel and yet untargeted therapeutic strategy for the treatment of neurodegenerative disorders, including ALS. The participating centers have thorough experience in the conduct of clinical trials. Therefore, the trial can be rapidly initiated. Since Fasudil is a kinase inhibitor, the activity of ROCK can be measured to ensure that the pathway of interest has been affected. In addition, Fasudil can be detected and quantified using FPLC. There is ample published preclinical and clinical data on pharmacology and toxicology

(6,11,12,16,17). We propose to collect biological samples for biomarker studies, including blood, CSF, urine and saliva.

Since Fasudil IV has never been used in ALS patients in a controlled trial, we propose an exploratory Phase IIa study, focusing on safety and tolerability, as well as on efficacy as secondary endpoint. Although the trial duration may not be sufficient to detect significant changes in functional scales or survival, this study is a prerequisite for any larger phase IIb/phase III study. For rationale on dose selection, please refer to 6.1.

3.3 Study Objectives

The primary objective of this phase IIa study (proof-of-concept study) is to evaluate the safety and tolerability until V23 after start of the intravenous application of the ROCK-inhibitor Fasudil (for 20 treatment days) in two different doses compared to placebo in patients with ALS.
A secondary aim is to assess the survival time and the change of ALSFRS-R, ALSAQ-5, ECAS and of vital capacity (VC) from baseline to V20, V22, and V23 days and MUNIX from baseline to V20, V22 and V23 after start of the intravenous application of the ROCK-inhibitor Fasudil. A further secondary aim is to determine safety and tolerability until V20.
This study will assess the safety and tolerability of two Fasudil doses with the most promising efficacy potential for subsequent phase IIb and phase III studies. Results of this phase IIa study will allow estimating if a longer treatment period could be favorable and if an oral formulation (e.g. with extended release tablets) can be implemented.
Insufficient tolerance or significant safety concerns after application of Fasudil IV in both doses will argue against a subsequent phase IIb or III study. On the other hand, lack of significantly improved efficacy readouts will not argue against a follow-up study, because we can only estimate the magnitude of improvement at this stage and the study may be underpowered to detect a significant improvement.
In order to achieve a long-lasting disease-modifying effect in a chronic neurodegenerative disorder, such as ALS, a future treatment most likely will have to be applied in a continuous way, which is best achieved by an extended release oral tablet medication. In the case of satisfactory tolerance and safety of IV Fasudil in ALS patients, we plan to propose a follow-up phase IIb study (extended dose-finding) exploring the safety, tolerability and clinical effects of oral extended release oral tablets of Fasudil over 52 weeks in three different dosages. This is considered to be a mandatory step before a future international, multi-site, randomized phase III study aiming at efficacy readout in a larger trial population.

During the design of this study we were taking into account the “Guideline on clinical investigation of medicinal products for the treatment of amyotrophic lateral sclerosis” of the European Medicines Agency (EMA), where appropriate.

3.4 Primary Endpoints

Primary endpoints are safety and tolerability.

The treatment with Fasudil is considered safe for an individual patient if no drug-related Serious Adverse Event (SAE) is recorded through to visit V23. The treatment with Fasudil is considered tolerable if no significant drug intolerance during the treatment period is recorded. The proportions of patients for whom the treatment is tolerable / safe are derived for each treatment group.

3.5 Secondary Endpoints

Secondary endpoints are the survival time and the change of ALS Functional Rating Scale

(ALSFRS-R), ALS Assessment Questionnaire (ALSAQ-5), Edinburgh Cognitive and Behavioral ALS Screen (ECAS), Motor Unit Number Index (MUNIX) and vital capacity (VC) from baseline to visits V20, V22, V23 after start of the intravenous application of the ROCK-inhibitor Fasudil (for 20 treatment days). Secondary safety endpoint is safety until V20.

4 Investigational Plan

4.1 Summary of Study Design, Discussion of Design and Control

Since there is a long-standing clinical experience with Fasudil in human patients, no phase I study is needed. However, it is not known, how well Fasudil is tolerated by ALS patients and there are no published reports of ALS patients treated with this ROCK inhibitor. Therefore, a phase IIa trial is performed. Since the drug is only licensed as IV formulation, a compromise in the treatment duration had to be made: the treatment duration of 20 days (until V20) is long enough to estimate safety and tolerability of Fasudil IV in ALS patients and to expect a long-lasting regulation of motoneuron survival pathways and alteration of microglial activity. At the same time, 20 treatment days are short enough to expect reasonable patient adherence for an IV therapy and to keep trial costs in a reasonable range. Concomitant treatment with Riluzole is considered standard therapy; in order to reduce bias, patients need to be treated with Riluzole for at least four weeks. Most ALS patients in the participating countries receive Riluzole and in most participating countries it is the only licensed drug, which therefore cannot be withheld.

4.2 Trial Duration/Milestones

Overall trial duration will be 36 months (Q3 2017 to Q2 2020): Set-Up period of 6 months; Recruitment period of 18 months; 20 treatment days per patient and follow-up per patient until day 180 ± 5 (V23) after first administration of the trial medication. The trial duration for the individual patient will thus be 180 ± 5 days. Database clearance, analysis and publication will be finalized latest.

5 Study Population

5.1 Inclusion Criteria

Patients are eligible to be enrolled in the study only if they meet **all of the following** criteria:

Patients meeting **all of the following** criteria will be considered for admission to the trial:

(1) Probable (clinically or laboratory) or definite ALS according to the revised version of the El Escorial World Federation of Neurology criteria,
(2) Disease duration more than 6 months and less than 24 months (inclusive). Disease onset defined as date of first muscle weakness, excluding fasciculations and cramps,
(3) Vital capacity more than 65% of normal (slow vital capacity; best of three measurements),
(4) Age: ≥ 18 years,
(5) Patients have to be treated with Riluzole (2 x 50mg/d), must be stable for at least four weeks before randomization,
(6) Patients who have started on Edaravone therapy shall continue Edaravone treatment. Edaravone treatment must not be discontinued for reasons of trial participation.
(7) Women of childbearing age must be non-lactating and surgically sterile or using a highly effective method of birth control and have a negative pregnancy test. Acceptable methods of birth control with a low failure rate i.e. less than 1% per year)

when used consistently and correct are such as implants, injectables, combined oral contraceptives, hormonal intrauterine devices (IUDs), sexual abstinence or vasectomized partner. For Switzerland sexual abstinence is not allowed as a contraceptive method,

(8) Capable of thoroughly understanding all information given and giving full informed consent according to GCP,
(9) Patients have to have a valid health insurance, when recruited in a center in France

5.2 Exclusion Criteria

Patients who meet **any of the following** exclusion criteria will not be included in the trial.

(1) Previous participation in another clinical study involving trial medication within the preceding 12 weeks or five terminal half times of the longest to be eliminated trial medications (whichever is longer) or previous participation in this trial,
(2) Tracheostomy or continuous assisted ventilation of any type during the preceding three months before randomization or a significant pulmonary disorder not attributed to ALS, which may complicate the evaluation of respiratory function, intermittent non-invasive ventilation is permitted,
(3) Patients with a history of intracranial bleeding, known intracerebral aneurysms or Moyamoya disease, or positive family history for the above. If only family history positive, MR- or x-ray-based cranial imaging not older than 24 months must confirm absence of bleeding, aneurysms or Moyamoya,
(4) Gastrostomy,
(5) Any medical condition known to have an association with motor neuron dysfunction or involving neuromuscular weakness or another neurodegenerative disease, e.g. PD or AD, which might confound or obscure the diagnosis of ALS,
(6) Presence of any concomitant life-threatening disease or impairment likely to interfere with functional assessment,
(7) Patients with known arterial hypotension (resting blood pressure <90/60 mmHg) or previous hypotensive episodes or requiring treatment for increasing of blood pressure, such as fludrocortisone, midodrine, etilefrine, cafedrine or theodrenaline,
(8) Patients with an uncontrollable or unstable arterial hypertensive disease (resting blood pressure >180 mmHg systolic and/or >120 mmHg diastolic under current antihypertensive medication),
(9) Known pulmonary hypertension and any medication prescribed for treatment of pulmonary hypertension,
(10) Confirmed hepatic insufficiency or abnormal liver function (stable ASAT and/or ALAT greater than 3 times the upper limit of the normal range) and determined to be non-transient through repeat testing,
(11) Renal insufficiency with a glomerular filtration rate (GFR) <60 ml/min/1,73m² (calculated by MDRD equation) and determined to be non-transient through repeat testing,
(12) Major psychiatric disorder, significant cognitive impairment or clinically evident dementia precluding evaluation of symptoms,
(13) Hypersensitivity to any component of the study drug,
(14) Liable to be not cooperative or comply with the trial requirements (as assessed by the investigator), or unable to be reached in the case of emergency,
(15) Pregnant or breast-feeding females or females with childbearing potential, if no adequate contraceptive measures are used,
(16) Prisoners or subjects who are involuntary incarcerated,
(17) Patients subject to legal protection measures.

5.3 Patient Recruitment Measures

It is planned to issue a patient information leaflet and an information leaflet for medical professionals (primary care physicians and neurologists). These will be distributed by each

center to all referring professionals. Further it is planned to distribute this information to national patient organizations of the participating countries and make it available on their websites, e.g. http://www.als-selbsthilfe.de/, https://www.dgm.org/, as well as on the websites of all study centers, respectively.

6 Plan for Medical Treatment

6.1 Rationale for Selection of Dose

Fasudil is already licensed in Japan for the treatment of vasospasms following subarachnoidal hemorrhage. In this indication, 30 mg of Fasudil are applied IV three times per day for a total of 14 days following aneurysm surgery, i.e. total daily dose of 90 mg (18). Test persons who were treated with 45 mg Fasudil IV reached a mean peak level of 22.8 ng/ml Hydroxyfasudil, the active metabolite of Fasudil (19). Considering linear pharmacodynamics, peak levels after application of 15 to 30 mg Fasudil IV, as proposed in this trial, should be expected to be ~10 to 15 ng/ml in the CSF. This corresponds well with the levels of Hydroxyfasudil, which were measured in mouse CSF (8.8 ng/ml) after oral treatment with Fasudil in a dosage that resulted in prolonged survival and improved behavior in two independent in vivo-studies in the SOD1.G93A mouse model of ALS (6,12). Therefore, two IV dosages, which are expected to result in Hydroxyfasudil CSF levels that were reached in functional improvement models, appear promising.

Placebo was chosen as no gold-standard disease-modifying treatment, except for Riluzole, exists to date. Riluzole co-treatment is required representing the real-life situation, since most ALS patients in the participating countries are treated with Riluzole. Since Riluzole is the only licensed medication with data on better survival for patients with ALS, it cannot be withheld on ethical grounds. Two doses of Fasudil (30 mg/d and 60 mg/d) are chosen, because some studies showed a U-shaped dose-response relationship (12,20), where the Fasudil-effect obtained with lower doses was lost at higher doses, most likely due to off-target inhibition of other kinases. The highest dose chosen here (2 x 30 mg/d) is below the dose used in clinics for the treatment of vasospasms (3 x 30 mg/d) to be on the safe side, since Fasudil has not yet been tested in ALS patients.

6.2 Description of Study Medication

Fasudil is a protein kinase inhibitor that inhibits the effects of Rho kinase. Rho kinase phosphorylates different targets, thus the effects of Fasudil are thought to be mediated by these targets, such as myosin light chain, ezrin, radixin, moesin, adducin, LIM kinase, profilin, PTEN, MAP2, CRMP2, GFAP, vimentin and many more. Through its actions on smooth muscle cells and vascular endothelium it is known to improve symptoms of cerebral ischemia associated to the development of vasospasms due to intracranial aneurysm rupture and surgical treatment. Fasudil also inhibited the migration of monocytes and suppressed the oxygen production of human neutrophils.

6.2.1 Study Medication

Name: Fasudil hydrochloride solution
Formulation:
Fasudil hydrochloride hydrate solution 30 mg for IV application
(2 x 1 ml, 15 mg/ml Fasudil hydrochloride hydrate),
Fasudil hydrochloride hydrate solution 15 mg for IV application
(1 ml, 15 mg/ml Fasudil hydrochloride hydrate and 1 ml NaCl 0.9%),

Manufacturer: Universitätsklinikum Leipzig AöR, Apotheke

6.2.2 Placebo

Placebo to Fasudil hydrochloride solution
Formulation:
2 x 1 ml, NaCl 0.9%
Placebo solution is identical in appearance to the study medication (clear, colorless fluid), but does not contain the active ingredient.
Manufacturer: Universitätsklinikum Leipzig AöR, Apotheke

6.2.3 Dosing Schedule

The trial drug or placebo is administered two times daily IV as infusion over 45 minutes. The second application starts 7 hrs +/- 1 hr after the start of the first application.

6.2.4 Instructions for Application of Study Drug

The study drug (supplied as two 1ml vials), will be diluted in 100 ml physiological solution or NaCl 0.9% or 5%-Glucose solution. It will be applied via intravenous catheter at a constant rate of infusion using a CE-certified volumetric infusion pump over a period of 45 min. Prior to the first measurement, patients have to be in a supine position for at least 5 minutes. Drug administration should be performed in a supine or semi-upright (<45°) position. Patients should get in an upright position after the end of drug administration and wait for another 15 minutes before discharge. Administration of the drug must be undertaken at a medical facility with sufficient personnel and technical emergency capabilities.
At each trial drug/placebo administration, patients will be monitored for blood pressure and pulse before and at 0, 10, 20, 30, 45 and 60 minutes after start of each drug infusion. Significant intolerance of the study medication is specified in chapter 9.1.

6.2.5 Adverse Drug Reactions, Contra-indications, Drug Interactions

Potential side effects of Fasudil in adult patients are listed in detail in the product specification file for Eril®. Briefly, significant side effects in adults include the following, stratified by frequency of occurrence:

Frequent (1 – <10 %):

- Abnormal elevation of liver enzymes (ALT, AST, AP, LDH): > 5 %
- Intracranial bleedings: 1.72 %
- Renal dysfunction (increase of creatinine, BUN, polyuria): 0.1 – 5 %
- Anemia, Leukopenia, Thrombopenia: 0.1 – 5 %
- Hypotension: 0.1 – 5 %
- Rash: 0.1 – 5 %

Occasional (0.1 – <1 %)

- Gastrointestinal bleeding, lung bleeding, epistaxis, hematoma: 0.27 %

Rare (0.01 – <0.1 %):

- Icterus: < 0.1%
- Difficulty to urinate: < 0.1 %
- Flatulence, nausea, vomiting: < 0.1 %
- Fever: < 0.1 %
- Headache, somnolence, and respiratory depression: < 0.1 %

- Hot flashes: <0.1 %
- Shock: 0.02 %
- Paralytic ileus: 0.04 %

Restrictions regarding concomitant medication are detailed in section 6.3.

6.2.6 Drug Accountability

Fasudil hydrochloride hydrate will be delivered as medicinal product to the Universitätsklinikum Leipzig AöR, Apotheke and the trial medication will be produced, packaged, stored, blinded, labeled and shipped to the trial sites in accordance with the regulations of the participating country and good manufacturing practice (GMP) (Annex 13 of the EU Guideline for GMP).
Emergency envelopes for the unblinding of study participants will be produced by the Department of Medical Statistics (Core Facility for Medical Biometry and Statistical Bioinformatics), Göttingen and provided to Universitätsklinikum Leipzig AöR, Apotheke.
The Universitätsklinikum Leipzig AöR, Apotheke has the manufacturing authorization according to § 13 AMG, for the production of investigational medicinal products.
The trial sites will be provided by Universitätsklinikum Leipzig AöR, Apotheke with study medication. After receipt of the study medication, this is to be stored according to the concomitant information in a dry and safe place at room temperature.
The study medication must be used only for the assessments specified in this protocol and must be accessible only to authorized personnel.
The Investigator is responsible for dispensing and the collection of unused study medication, and the documentation (e.g. drug accountability).
Study medication must only be used within this clinical study and must not be used after its expiry date.
Any remaining study medication will be destroyed at the respective trial site.
Dispensing to patients will be documented in the drug accountability log in the study folder at the trial site (identification of the participating person, date and amount of medication dispensed, date and amount of medication destroyed).
All study medication is to be kept out of sight and reach of children.

6.2.7 Treatment Compliance

Every attempt will be made to select patients who have the ability to understand and comply with instructions. Prior to beginning the study, patients will complete assent and informed consent, respectively (see 7.1), and the patients will undergo study-specific screening tests.

Throughout the study, patients may be subject to medical assessment and review of compliance before continuing in the study. Patients must continue to meet the inclusion criteria (except for (2) and (3)) and none of the exclusion criteria (except for (10) and (11) specified in chapter 9.1) during the study, including restrictions related to medication use. Noncompliant patients may be discontinued from the study, but will be included in the ITT analysis. Drug accountability records will be maintained by the trial site.

6.3 Concomitant Medication / Concomitant Therapy

Concomitant medication, except for Riluzole, is to be avoided during the study unless required to treat an AE or for the treatment of a pre-existing condition or an on-going medical problem.

Patients who have started on Edaravone therapy shall continue Edaravone treatment. Edaravone treatment must not be discontinued for reasons of trial participation.

If the need for concomitant medication arises, inclusion or continuation of the patient may be

at the Investigator's discretion after consultation with the Coordinating Investigator. Any additional medication used during the course of the study must be documented.

Patients, who are treated with the following concomitant treatments, are not permitted to participate in trial:

- fludrocortisone, midodrine, etilefrine, cafedrine or theodrenaline
- any medication prescribed for the treatment of pulmonary hypertension

6.4 Blinding

This study includes double-blind (patient- and investigator blind) treatment. For patients treated in a double-blind fashion, emergency codes will be available to the investigator. A code, which reveals the treatment group for a specific study patient, may be opened during the study only if the choice of treatment depends on the study subject's therapy assignment (see Section 15.2).

During the study, emergency unblinding should occur only by accessing the study patient's emergency code.

The investigator should make every effort to contact the Coordinating Investigator prior to unblinding a study patient's therapy assignment. If a study patient's therapy assignment is unblinded, the Klinische Studien Management (KSM), Göttingen, Münchner Studienzentrum (MSZ) and the trial office (UMG, Göttingen), must be notified immediately.

7 Study Procedures

7.1 Methods of Obtaining Informed Consent

The investigator is responsible for ensuring that the patient understands the objectives, implications, scope, and potential risks and benefits of participating in the study, including answering any questions that they may have throughout the study and sharing any new information that may be relevant to their willingness to continue participation in the trial in a timely manner.

All patients will be informed to the fullest extent possible about the study in language and terms they are able to understand. Informed consent documents (ICDs) explain in simple terms the objectives, implications, scope, and potential risks and benefits of study participation.

The ICD and assent documents are prepared in duplicate. If the patient decides (after appropriate consideration time) to participate in the trial, patients will sign the respective standardized ICD document in duplicate in the presence of the investigator. One original of the signed and dated ICD is handed to the patient. The other original of the ICD is filed in the Investigator Site File (ISF) at the Trial Site, one copy will be filed with the patients' medical records.

Every patient will be informed that participation in the study is voluntary and that consent can be withdrawn at any time without the need to provide reasons, and without disadvantage or prejudice.

Information on data protection will be given before the patient is entered into the study and any study-specific procedures are performed. The appropriate signatures and dates on the ICD documents are a requisite for the performance of any study specific protocol procedures and the administration of study drug.

7.2 Study-Specific Procedures

7.2.1 Trial Visits Schedule

For the planned trial visits schedule the first day (d1) of administration of trial medication is considered to be the reference date. The dates of the following visits specified in the protocol are calculated in relation to the date of this visit. If the time of a visit is modified in relation to the visits schedule, the times of the following visits will always be determined with respect to the day of first administration of trial medication visit. The time window for each planned visit is indicated in Table 1. The visits schedule is not modified by any unscheduled visits.

In the case of premature interruption of the study treatment, a complete examination identical to visit V23 should be carried out. Premature study discontinuation must be noted as such on the case report form. Patients prematurely leaving the study must not rejoin the trial. An overview including the time schedule and evaluations for all visits is given below. Evaluations performed at the study visits comply with the routine assessments for ALS patients, except that evaluations in patients outside of the clinical trial are performed less often (approximately twice a year).

Venous blood samples taken during the visits will be destroyed after determination of the required parameters. Only the samples collected for the biosample collection data bank will be stored and used as outlined in Section 7.7 of the proposal.

Action / Visit	V0 Screening	V1 BL	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16	V17	V18	V19	V20	V21	V22	V23
Day (d)		1	2	3	4	5	8	9	10	11	12	15	16	17	18	19	22	23	24	25	26	45	90	180
permitted delta in days (+/-) to d1	-42		0	0	+3	+3	+4	+4	+4	+4	+4	+4	+4	+4	+4	+4	+4	+4	+4	+4	+4	+/-3	+/-4	+/-5
		Treatment must be completed within 30 days, with a max. of 3 consecutive non-treatment days																						
Treatment IV 2 times daily		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x			
Screening assessment																								
Patient information, informed consent	X																							
Inclusion/exclusion criteria	X																							
Demographics	X																							
Medical history	X																							
Diagnosis according to revised EEC	X																							
Physical examination	X																							
Randomization#	X#																							
Recurrent additional status data																								
Concomitant treatment history	X	X		X				X						X							X	X	X	X
Vital signs (pulse, BP)**	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height (only at screening), weight	X																				X		X	X
Endpoint assessment																								
ALSFRS-R		X																			X		X	X
Vital capacity (VC) ***	X	X																			X		X	X
ALSAQ-5		X																			X		X	X
MUNIX		X§																			X		X	X
ECAS		X																			X		X	X
Safety assessment																								
Laboratory tests*,***	X	X		X				X						X							X	X	X	X
Pregnancy test***	X																							

Adverse events	X****	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Biosample collection																								
EDTA-Blood (Genotyping)		X																						
CSF (e.g. Fasudil concentration, NfL)																		(X°)	(X°)	X°				
Plasma (e.g. ROCK-activity & Fasudil concentration)	X	X																(X°)	(X°)	X°				X
Serum (e.g. pNfH, urate)		X																		X				X
Urine (e.g. soluble p75ECR)		X																		X				X
Saliva (e.g. Chromogranin A)		X																		X				X

Table 1: Trial visits schedule. ***Laboratory tests: RBC, WBC, PLT, creatinine, GFR (MDRD), ALAT, ASAT, GGT, and CK, glucose. **** BP and pulse. On V1-V20 to be captured before and at 0, 10, 20, 30, 45, and 60 minutes after each administration of trial medication. ******* If not routine please perform only after informed consent. Pregnancy tests are to be repeated in case of suspicion and/or clinical signs of pregnancy during the course of the study participation. ******** All Adverse Events must be collected from randomization. **X°** CSF (and concomitant plasma) analysis will be performed once, regularly on V20 (exceptionally on V18 or V19). IV-Treatment must have taken place at least on the two days preceding CSF/plasma analysis. On the day preceding the CSF analysis, a blood sample must be taken to ensure normal coagulation. The sample must include the analysis of RBC, WBC and PLT (1x 2.7 ml EDTA) as well as INR and PTT (1x 3 ml Citrate 3.2%). **X§** Baseline MUNIX analysis can be performed up to 2 weeks before V1. X# Randomization should be done only after all inclusion and exclusion criteria have been verified. To ensure timely delivery of trial medication, the pharmacy must be informed at least 10 days in advance. All biomaterials must be taken directly after administration of the trial drug in the morning except CSF (see above, X°).

7.2.2 Screening Visit (V0)

A signed “Informed Consent” will be obtained from each patient prior to initiating any study specific trial procedure. The patients´ eligibility will be determined in accordance with the inclusion/exclusion criteria. Demographics and a detailed medical history will be taken, and concomitant medication will be recorded. Presence of ALS diagnostic criteria will be documented as well as site of onset and ALS status at entry according to the revised El Escorial criteria. After signing of the informed consent, venous blood (1x 2.7 ml EDTA & 1 x 4.7 ml lithium-heparinate) will be taken for determination of blood cell count, creatinine, GFR, CK, ASAT, ALAT, GGT, blood glucose, and HCG or urine pregnancy test (only in women with child bearing potential; pregnancy tests are to be repeated in case of suspicion and/or clinical signs of pregnancy during the course of the study participation) in the local laboratory. Besides, one 4.7 ml tube of lithium-heparinate will be taken and immediately frozen for later analysis of ROCK-activity. A physical examination, including body weight, height, blood pressure, and radial pulse will be performed. Additionally, lung function will be assessed by measuring the patient’s vital capacity.

7.2.2.1 Randomization (Assignment of Study Medication)

Randomization takes place in a one-step procedure during the screening visit as follows:

For eligible patients, the Investigator at the trial site

1. confirms that all inclusion criteria and no exclusion criteria are met,
2. requests delivery of trial medication at the trial pharmacy at least 10 days before planned V1 to ensure timely delivery of trial medication (deviations from the time window are not considered as protocol violations),
3. informs the trial pharmacy about stratum of patient (bulbar or spinal),
4. enters the random-number into the eCRF.

Tasks 2-4 may be delegated.

Thus, each randomized patient will have a unique random-number throughout the study. Any vial of study drug for one patient must carry the same random-number.

7.2.3 Baseline Visit (V1)

Patients eligible for study participation will enter the study site within 6 weeks (42 days) after the screening visit.

7.2.3.1 Procedures during the Baseline Visit (V1)

The baseline visit is at the same time the first day of trial drug administration. The patient will receive study medication according to the randomization list. Please note that on each day of trial medication, BP and pulse are to be captured before and at 0, 10, 20, 30, 45, and 60 minutes after each administration of trial medication/placebo. For reasons of practicability, all other procedures previewed for the baseline visit will be performed after the application of the first trial medication.

Directly after completion of the first study drug infusion, venous blood samples (1x 2.7 ml EDTA, 2x 7.5 ml EDTA, 3x 4.7 ml lithium-heparinate, 2x 7.5 ml serum) will be taken. The 2.7 ml EDTA- and one 4.7 ml lithium-heparinate tube will be used for determination of blood cell count, creatinine, GFR, CK, ASAT, ALAT, GGT and blood glucose in the local laboratory. The 2x 7.5 ml EDTA-tube will be frozen at -80°C for later genotyping. The other tubes will be processed for biomarker studies and genotyping as specified in a separate protocol. Urine (50 ml) and saliva (5 ml) samples will be obtained for assessment of biomarkers and/or pharmacokinetics. They will also be processed according to a special protocol. All biosamples will be frozen at -80°C and stored in the local study center until being shipped to the trial center in Göttingen.

Body weight, blood pressure and radial pulse will be documented, and lung function will be assessed by measuring the patient's vital capacity. In addition, MUNIX, ALSFRS-R, ALSAQ-5 and ECAS will be performed.

Any changes in the concomitant medication between inclusion of the patient (written informed consent) and the baseline visit (V1) will be documented.

7.2.4 Procedures during the Treatment Phase (V2 – V20)

Treatment visits V2 and V3 must take place on the two consecutive days following V1. Visits V4 and V5 must take place on day 4 (+3 days) and day 5 (+3 days) following V1. Treatment visits V6 to V10 must take place on days 8 (+4 days) to 12 (+4 days) following V1. Treatment visits V11 to V15 must take place on days 15 (+4 days) to 19 (+4 days) following V1. Treatment visits V16 to V20 must take place on days 22 (+4 days) to 26 (+4 days) following V1. The order of the visits given in Table 1 must be kept.

During each of the treatment visits, patients will receive treatment medication two times daily. Application will be as described in 6.2 and particularly 6.2.3.
In addition to the treatment procedures, laboratory tests will be performed at V3, V7, V13, and V20. Blood samples (1x 2.7 ml EDTA and 1x 4.7 ml lithium-heparinate) for the determination of blood cell count, creatinine, GFR, CK, ASAT, ALAT, GGT and blood glucose in the local laboratory, will be obtained.
Vital signs: On each day of trial medication blood pressure radial pulse are to be captured before and at 0, 10, 20, 30, 45, and 60 minutes after each administration of trial medication/placebo. Adverse events will be documented throughout the course of the participation of the patient starting from randomisation.
Finally, on V20, ALSFRS-R, vital capacity, body weight, ALSAQ-5, MUNIX, adverse events and ECAS will be assessed. Directly after the end of the trial drug infusion in the morning, biomaterials will be obtained (31.8 ml blood, 50 ml urine, 5 ml saliva). Blood samples comprise 1x 2.7 ml EDTA and 1x 4.7 ml lithium-heparinate for determination of blood cell count, creatinine, GFR, CK, ASAT, ALAT, GGT and blood glucose in the local laboratory, as well as 2x 4.7 ml lithium-heparinate and 2x 7.5 ml serum for biomarker studies. Urine (50 ml) and saliva (5 ml) samples will be obtained again for assessment of biomarkers and/or pharmacokinetics. All biosamples are processed according to a separate protocol.
HCG or urine pregnancy test (only in women with child bearing potential) should be repeated in case of suspicion/or clinical signs of pregnancy.
One the day preceding the CSF analysis, a blood sample must be taken to ensure normal coagulation. The sample must include the analysis of RBC, WBC and PLT (1x 2.7 ml EDTA) as well as INR and PTT (1x 3 ml Citrate 3.2%). At 60 minutes after the end of the trial drug infusion in the morning of V20 (alternatively on V18 or V19 to ensure that IV-treatment has taken place at least on the two days preceding CSF/plasma analysis), a lumbar puncture will be performed and 15 ml of CSF will be drawn. In parallel, 7.5 ml serum and 4.7 ml lithium-heparinate will be taken. 2 ml of the CSF together with the 7.5 ml serum tube will be analyzed in the local laboratory for basic CSF-parameters (e.g. cell count, protein concentration, Ig-quotients, oligoclonal bands). The other 13 ml CSF and the 4.7 ml lithium-heparinate will be processed according to the biomaterials protocol and frozen at -80°C for later biomarker and pharmacokinetics analysis.
All biomaterials will be stored at -80°C in the local study center until being shipped to Göttingen.

7.2.5 Procedures during the Follow-up Phase (V21 – V23)

7.2.5.1 Short-term safety follow-up visit (V21)

Visit 21 will assess safety at day 45 (+/- 3 days) after V1. Concomitant medication changes and adverse events will be documented. Venous blood samples (1x 2.7 ml EDTA & 1x 4.7 ml lithium-heparinate) will be taken for the determination of blood cell count, creatinine, GFR, CK, ASAT, ALAT, GGT and blood glucose in the local laboratory. Blood pressure and radial pulse will be documented.

7.2.5.2 Efficacy- and safety follow-up visits (V22 and V23)

Visits 22 and 23 will be performed on days 90 days (+/- 4 days) and 180 days (+/- 5 days) after V1 (first day of administration of trial medication), respectively. Body weight, blood pressure and radial pulse will be documented, and lung function will be assessed by measuring the patient's vital capacity (VC). In addition, ALSFRS-R, ALSAQ-5, ECAS, and MUNIX will be performed. Any changes in the concomitant medication and adverse events will be documented.

At V22, venous blood samples (1x 2.7 ml EDTA & 1x 4.7 ml lithium-heparinate) will be taken for the determination of blood cell count, creatinine, GFR, CK, ASAT, ALAT, GGT and blood glucose in the local laboratory.
At V23, blood (total volume: 31.8 ml), urine (50 ml), and saliva (5 ml) samples for assessment of biomarkers and safety will be taken. Blood samples comprise 1x 2.7 ml EDTA and 1x 4.7 ml lithium-heparinate for determination of blood cell count, creatinine, GFR, CK, ASAT, ALAT, GGT and blood glucose in the local laboratory, as well as 2x 4.7 ml lithium-heparinate, and 2x 7.5 ml serum for biomarker studies. Urine (50 ml) and saliva (5 ml) samples will be obtained for assessment of biomarkers and/or pharmacokinetics. They will be processed according to a special protocol. All biosamples will be frozen at -80°C and stored in the local study center until being shipped to the trial center in Göttingen.

7.2.6 Unscheduled visits

Unscheduled visits may be carried out at any time during the study in the case of a medical emergency, when a patient would like to report a medical problem or when the investigator considers this necessary for the patient's wellbeing. These unscheduled visits must be recorded on the case report form. In no case must they lead to a modification in the study visits schedule stipulated by the protocol.

7.3 Laboratory Assessments

7.3.1 Standard Laboratory Assessments

Samples will be collected at the times specified in section 7.2.1. Unless otherwise specified, all standard laboratory tests, including chemistry, will be performed through the local laboratories.

Investigators will document their review of each safety laboratory report. Samples collected for standard laboratory tests will be destroyed according to clinical routine after the receipt of confirmed test results. Tests are run and confirmed promptly whenever scientifically appropriate. When scientific circumstances warrant, however, it is acceptable to retain samples to batch the tests run, or to retain the samples until the end of the study to confirm that the results are valid. Certain samples may be retained for a longer period, if necessary, to comply with applicable laws, regulations, or laboratory certification standards.

7.3.2 Exploratory Laboratory Assessments

At baseline (V1), the last study visit V20 and the last follow-up visit V23 (see section 7.2.3 and 7.2.4) blood, urine and saliva samples will be collected for biomarker and pharmacokinetic studies. Additionally, at V20 15 ml of CSF are collected. Exceptionally, CSF samples may be taken on V19 or V18. Trial drug must have been administered on the two days before CSF sampling. Samples will be stored at -80°C at the Trial Site until dispatch to the UMG Biobank, Göttingen. More detailed sampling, processing, storage and dispatch procedures will be defined outside of this trial protocol.

Samples will be then shipped to external collaboration partners for determination of Fasudil and its metabolites. ROCK activity on V0 and V20 will be determined in the lab of the Department of Neurology in Göttingen.

Results obtained from exploratory analyses are not required for the final study report.

7.4 Assessment of Vital Signs

Vital signs will be assessed at the times described in section 7.2.

7.5 Assessment of Efficacy

The following outcome variables will be analyzed which are measured at baseline and further points of time (see also Trial Visits Schedule, section 7.2.1):
- Survival /date of death,
- ALS-Functional Rating Scale, Revised (ALSFRS-R) (according to (21,22)),
- Vital capacity (cm^3) (according to (23)),
- ALSAQ-5 (according to (24))
- MUNIX (according to (25,26))
- ECAS (according to (27)).

7.6 Assessment of Safety

Safety and tolerability of Fasudil will be investigated using the following parameters: vital signs (blood pressure, pulse), safety laboratory parameters (ALAT, ASAT, GGT, CK, creatinine, GFR, RBC, WBC, PLT, glucose), terms and frequencies of adverse and serious adverse events (AE and SAE).

Adverse events (AE), serious adverse events (SAE), adverse reaction (AR), serious adverse reaction (SAR), suspected unexpected serious adverse reaction (SUSAR), emergency procedures will be defined according to GCP. Definition, assessment, documentation, reporting, monitoring and follow-up, as well as the emergency procedures are described in detail in chapter 10.

7.7 Detailed biomarker and sample collection plan

As part of the therapeutic trial, we will collect biosamples, which will be used for the analysis of pharmacodynamics, disease progression and additionally can be used for future studies. At V1 (baseline), V20 and V23, EDTA-blood, serum, plasma, urine, and saliva will be collected from all individuals. At V20 (or exceptionally on V19 or on V18), a CSF sample and a blood plasma sample will be collected to determine the peak concentration of Fasudil and Hydroxyfasudil as well as ROCK activity. Individual alterations of the biomarker and sample collection and analysis plan will not be classified as violations of the general study protocol. Since samples might be shipped in bulk, analysis might be performed with a delay to sample withdrawal. Storage of all biomaterial samples will be done in the UMG Biobank, a central service facility of the UMG, except for samples, that will be analyzed immediately. More detailed sampling, processing, storage and dispatch procedures will be defined outside of this trial protocol.

Action / Visit	V0 Screening	V1 BL	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16	V17	V18	V19	V20	V21	V22	V23
Day (d)		1	2	3	4	5	8	9	10	11	12	15	16	17	18	19	22	23	24	25	26	45	90	180
permitted delta in days (+/-) to d1	-42		0	0	+3	+3	+4	+4	+4	+4	+4	+4	+4	+4	+4	+4	+4	+4	+4	+4	+4	+/-3	+/-4	+/-5
		Treatment must be completed within 30 days, with a max. of 3 consecutive non-treatment days																						
Treatment IV 2 times daily		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x			
Biosample collection																								
EDTA-Blood (Genotyping)		X																						
CSF (e.g. Fasudil concentration, NfL)																			(X°)	(X°)	X°			

Plasma (e.g. Fasudil concentration & ROCK-activity)	X	X																	(X°)	(X°)	X°			X
Serum (e.g. NfL, urate)		X																			X			X
Urine (e.g. soluble p75ECR)		X																			X			X
Saliva (e.g. Chromogranin A)		X																			X			X

Table 2: Biosample collection plan. X° CSF (and concomitant plasma) analysis will be performed once 60 minutes after the end of trial drug infusion in the morning, regularly on V20 (exceptionally on V18 or V19). IV-Treatment must have taken place at least on the two days preceding CSF/plasma analysis. ******* If not routine please perform only after informed consent. All biomaterials must be taken directly after administration of the trial drug in the morning except CSF (see above, X°).

7.7.1 Whole blood, blood serum and blood plasma

At the respective time point of examination (Table 2), whole blood tubes (e.g. standard Sarstedt S-Monovette EDTA-tubes, 2.7 ml), serum tubes (e.g. Sarsted S-Monovette serum tubes, coagulation-activating, 7.5 ml) and lithium-heparinate plasma tubes (e.g. Sarstedt LH-Monovette tube, 4.7 ml) will be collected, immediately processed according to the biosample protocol and finally frozen at -80°C. They will be shipped later on dry ice to the trial center in Göttingen for analysis of biomarkers and storage in the Göttingen biobank.

7.7.2 Urine

At the respective time point of examination (see Table 2), 50 ml of urine will be collected. The urine will be tested using standard urinalysis sticks (e.g. Siemens Multistix 10 SG or similar). Readings will be recorded. Urine will be centrifuged (5 min at 2000 g at 4°C), immediately frozen at -80°C and stored at the trial center. Samples will later be shipped to the trial center in Göttingen for analysis of biomarkers and storage in the Göttingen biobank.

7.7.3 Saliva

At the respective time point of examination (see Table 2), 5 ml of saliva will be collected with the passive drool technique, will be immediately frozen at -80°C and stored at the trial center. Samples will later be shipped to the trial center in Göttingen for analysis of biomarkers and storage in the Göttingen biobank.

7.7.4 CSF

One the day preceding the CSF analysis, a blood sample must be taken to ensure normal coagulation. The sample must include the analysis of RBC, WBC and PLT (1x 2.7 ml EDTA) as well as INR and PTT (1x 3 ml Citrate 3.2%)

A minimum of 15 ml CSF will be collected at V20, or exceptionally on V19 or V18. CSF collection must take place one hour after the first trial drug application on the day of collection (to establish peak levels). The CSF will be centrifuged (20 min at 2000 g at 4°C) and aliquoted in 500 µl cryovials, frozen at -80°C and stored at the local trial center. Samples will later be shipped to the trial center in Göttingen for analysis of biomarkers and storage in the Göttingen biobank.

7.7.5 Motor Unit Number Index (MUNIX)

As was recently demonstrated, the objective determination of the motor unit number index (MUNIX) is a non-invasive, fast, and reliable electrophysiological technique allowing to track motor neuron loss in patients with ALS (25,26). The analysis of five different muscles (abductor digiti minimi (ADM), abductor pollicis brevis (APB), biceps brachii (BB), tibialis anterior (TA), extensor dig. brevis (EDB)) will be performed on both sides and the decline of the MUNIX will be assessed. MUNIX will be performed at V1 (or exceptionally up to two weeks before V1), V20, V22, and V23. A more detailed MUNIX protocol will be dispatched to all participating sites.

7.8 Individual Trial Duration

The treatment period will be a minimum of 26 and a maximum of 30 days per patient and will consist of 20 days of trial drug administration (V1 – V20). The follow-up period will be until day 180 (+/- 5 days) after the first infusion (V23). Patients should be contacted after V23 to assess disease progression.

7.9 Deviations from the Protocol

Except in cases of emergency (to protect the rights, safety and integrity of the patients), no substantial deviations from the procedures described in this protocol are permitted. If emergencies require deviations from the protocol, these must be promptly documented and reported to the Sponsor.

Instructions for the recording, reporting, and the analysis of deviations from the study protocol must be described, and requirements for reporting timelines must be observed.

8 Risk-Benefit-Assessment

8.1 Risks

ALS is a devastating disease, which dramatically reduces the physical wellbeing of the patient and life expectancy to 2 – 4 years after diagnosis. ALS is thus comparable with many malignant disorders. The potential risks of every treatment must be seen against this background.

Fasudil is approved for the treatment of vasospasms following subarachnoid hemorrhage by Japanese authorities in 1995. Several thousands of patients have been treated with this drug and the safety profile of Fasudil in humans thus is well known. In patients with a severe neurological disorder, ischemic stroke, Fasudil could also show a beneficial effect on functional outcome. Therefore, it is considered justified and safe to assess the effect of Fasudil in patients with the neurodegenerative disease ALS.

Placebo is acceptable in this case since both placebo and Fasudil are given as add-on to the standard therapy Riluzole, which has to be taken as inclusion criterion.

A direct interaction of the two substances Fasudil and Riluzol with adverse effects is not expected due to the different mechanism of action. Fasudil is a substance that is a kinase inhibitor and presumably acts by inhibition of neuronal Rho kinase (ROCK). However, neuroprotection with Fasudil has also been linked to its effects on microglia. The mechanism of action of Riluzole is thought to be related to its stabilizing effect on sodium channels and the resulting reduction of presynaptic glutamate release. The substance could be indicated as an antiglutamatergic drug to prevent the toxic effects of glutamate on motoneurons.

The neuroprotective effect of Riluzole – the standard therapy for patients with ALS – could be increased by the neuroprotective effect of Fasudil. Therefore, a stronger effect on the course of the disease in ALS patients is expected. The investigator will be informed about any relevant or new finding including AEs relating to treatment with the investigational medicinal product.

Hemorrhage, particularly intracerebral bleedings, are described as a side effect of Fasudil. This, however, was assessed in a population of patients with subarachnoid hemorrhage and the incidence of hemorrhage was not significantly different in the placebo-treated group. Thus, hemorrhage it not expected to be a risk for our patient population.

Evaluations, which will be performed at the study visits, comply with the routine assessments for ALS patients, except for a higher frequency in the study setting. Routine assessments for ALS patients are usually performed approximately twice a year.

8.2 Benefits

Irrespective of the actual treatment (Fasudil or placebo), progression longtime benefit is not very likely, yet cannot be ruled out. Furthermore, all patients will contribute to the generation of valid data from a prospective, placebo-controlled, clinical study.

Exploratory assessments will help to identify markers that indicate risk, prognosis, and activity of disease.

8.3 Risk-Benefit Assessment

Taken together, the analysis of risks and benefits suggests that the latter outbalance the risks of participation in this trial. Moreover, a continuous risk-benefit assessment, including the detailed analysis of Suspected Unexpected Serious Adverse Reactions (SUSARs) will be performed by the independent Safety Monitoring Board (SMB) (see section 10.7). In case of significant safety risks, study participation may be terminated for the individual patient or for the entire study (see section 9).

9 Termination and Subsequent Treatment

9.1 Termination of Study Participation for the Individual Patient

The participants in the clinical trial have the right to withdraw participation at any time with no reason given, without risk of being penalized. The investigator, on the other hand, has the right to exclude a patient from the study in the event of concomitant disease, adverse events, therapy failure or any other reason or condition that warrants withdrawal in the interest of the patient.

Every case of premature termination must be evaluated by the investigator and reasons for the termination assessed. The entire documentation must be as complete as possible. The final safety assessments should follow the protocol specifications for the follow-up visit. Any cases of premature termination must be documented in the eCRF, additional information is to be added, if applicable.

The following events are considered as intolerance to the trial medication:

- Significant intolerance of the study medication is assumed if the patient wishes to terminate participation in the trial due to any AE considered to be drug-related.

The following events must cause premature discontinuation of study medication:

- Personal patient wish,
- Severe adverse events / toxicity (grading according to Common Terminology Criteria)
- Significant intolerance of the study medication: assumed if the patient wishes to terminate participation in the trial due to any AE considered to be drug-related
- Significant alterations of clinical or laboratory findings, e.g. persistent increase in ASAT, ALAT or GGT >3 times the upper limit of normal or persistent decrease of GFR <45 ml/min/1,73m² (calculated by MDRD equation)
- Pregnancy
- Significant intercurrent illness or emergency situation requiring cessation of the study
- Circumstances which, according to the study protocol, do not allow the foreseen therapeutic interventions
- Cumulative SAEs that are unexpected and/or life threatening
- Violation of inclusion/exclusion criteria
- Loss of contact
- Significant violation of the study protocol
- Failure to comply with the investigational procedures
- Reasonable wish of the Sponsor

  Any other circumstance that, in the opinion of the investigator, warrants termination of the patient's continuation of study medication

For any patient prematurely discontinuing the study, the investigator must:

- recover all the treatment units
- in as far as possible, carry out all the examinations and tests planned for the final visit after withdrawal of the patient from the study
- fill in the corresponding pages of the case report form, specifying the date and reason for premature discontinuation
- when applicable, ask the patient the reason of his/her Informed Consent withdrawal while fully respecting the patient's rights
- where possible prescribe a visit 2 weeks after withdrawal in order to control and document any change in the patient's clinical status. If a serious adverse event occurs, the Responsible Nominated Safety Contact must be informed according to the procedures described in the full trial protocol.
- All ongoing adverse events and serious adverse events of withdrawn patients have to be followed up until no more signs and symptoms are verifiable or the investigator has assessed its sequelae, even after the end of the trial.

In case of unexpected drug interruptions patient may continue study treatment. Individual cases have to be discussed with CI.

9.2 Premature closure of trial sites

The sponsor has the right to close a trial site due to the following reasons:
- Major protocol violations
- Violations of legal and ethical regulations (GCP)
- Poor recruitment, no patients
- Non-compliance of investigator

9.3 Premature Termination of the Clinical Study

The study will be discontinued if the Coordinating Investigator judges it necessary for medical, safety, regulatory, or other reasons consistent with applicable laws, regulations, and good

clinical practice.

The study may be terminated prematurely in the event of substantial and irreparable deficiencies in data quality, inadequate compliance, or deficient patient recruitment, if a conclusion regarding the study hypothesis can no longer be expected on the basis of the data generated.

Any decision to terminate the study prematurely is to be made by the Sponsor in consultation with the participating Trial Sites and the Coordinating Investigator. The ethics committees and regulatory agencies will be informed about the decision detailing time and reasons for premature termination.

9.4 Continuing Treatment after Termination of the Study

Patients may continue treatment beyond the duration of the trial by potentially participating in an individualized treatment.

10 Safety

10.1 Definition or Adverse Events

During the study, the following definitions apply according to directive 2001/20/EC:

Adverse Event

An adverse event is any new undesirable medical experience or change of an existing condition that occurs during or after administration of an investigational agent, whether or not it is considered agent-related. Abnormal laboratory findings considered clinically relevant by the investigator, e.g. those that are unusual or unusually severe for the population being studied or those that need corrective treatment, will also be considered adverse events. In addition, abnormal laboratory values that cause study discontinuation or constitute in and of itself a serious adverse event (SAE), should be recorded on the “Adverse Events” page of the eCRF.

All Adverse Events, irrespective of seriousness, must be collected from randomization until the follow-up visit/end of trial visit at the time points specified in the trial visits schedule or until all drug-related toxicities are resolved, whichever is later, or until the investigators assess AEs as “chronic” or “stable”.

The investigator should report any Adverse Event occurring after these time period that is believed to be related to study drug or protocol-specified procedure.

Once an AE is detected, it should be followed until its resolution or stabilization, and assessment should be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the study, the interventions required to treat it and the outcome.

Adverse Reaction
An adverse reaction is an untoward and unintended response to an investigational medicinal product related to any dose administered.

Serious Adverse Event
A ‘serious adverse event or serious adverse reaction’ is any untoward medical occurrence or effect that at any dose

- results in death,

- is life-threatening (defined as an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- requires hospitalization or prolongation of existing hospitalization,
- results in persistent or significant disability or incapacity,
- or is a congenital anomaly or birth defect
- is an important medical event, defined as a medical event that may not be immediately life-threatening or result in death or hospitalization but, based on appropriate medical and scientific judgment, may jeopardize the subject or may require intervention (e.g., medical, surgical) to prevent one of the other serious outcomes listed above. Examples of such events include but are not limited to intensive treatment in an emergency department or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.

The following AEs are not recorded as SAEs:

- Hospitalization planned before enrolment (e.g., planned surgery)

Serious Adverse Reaction

Serious Adverse Reaction (SAR) is defined as an adverse drug reaction that is serious and at least possibly related to the investigational medicinal product.
In case of a SAR the investigator has to provide a case narrative.

Suspected Unexpected Serious Adverse Event (SUSAR)

SUSARs are defined as Suspected Unexpected Serious Adverse Reactions, i.e. all suspected adverse reactions related to the tested investigational medicinal product that is both: unexpected and serious.
Since the study medication is used in a new indication, all SAE for which a causal relationship with the administration of study medication may not be ruled out (SAR) need to be defined and reported as SUSARs.

Second Assessment of Serious Adverse Event

All SAE will be subject to a second assessment by a medical expert, who will be independent from the reporting investigator, the trial sponsor and the coordinating investigator.

10.2 Assessment of Adverse Event

10.2.1 Assessment of intensity

Mild:
Symptom(s) barely noticeable to the subject/patient or does not
make the subject/patient uncomfortable. The adverse experience does not
influence performance or functioning. Prescription drugs are not ordinarily needed for relief of symptom(s).
Moderate:
Symptom(s) of a sufficient severity to make the subject/patient
uncomfortable. Performance of daily activities is influenced. Treatment of symptom(s) may be needed.
Severe:
Symptom(s) of a sufficient severity to cause the subject/patient
severe discomfort. Severity may cause cessation of treatment with the study drug. Treatment for symptom(s) may be given.

10.2.2 Assessment of causality

The investigator must make a causality assessment for all AEs. The relationship of an adverse event to the study treatment regimen has to be recorded on the CRF and defined as not related, unlikely, possible related, probable related or highly probable/definite:

Relatedness	Description
Highly probable/definite	An event that follows an established temporal sequence from the study treatment regimen, the re-challenge is positive, or there is a reoccurring pattern of characteristics during onset and cessation of event.
Probably related	An event that follows a reasonable temporal sequence from the study treatment regimen and that is not easily explained by another cause such as known characteristics of the subject´s clinical state or the treatment
Possibly related	An event that follows a reasonable temporal sequence from the study treatment regimen but that may be due to another cause
Unlikely	An event that follows such a temporal sequence from the study treatment regimen that a relationship is not likely, and is likely to be due to cause such as the subject`s clinical state or other treatment
Not related	The event is definitely not associated with the study treatment regimen in the study but is judged clearly and incontrovertibly due to causes other than the study treatment

The categories “Highly probable/definite”, “Probably related” and “Possibly related” will be assumed as related, the categories “Unlikely” and “Not related” are assumed as not related.

10.2.3 Assessment of clinical outcome

recovered / resolved
recovering / resolving
deteriorating
recovered with sequelae
fatal
unknown

10.3 Expedited Reporting

This requirement applies if the adverse event is considered serious, unexpected, and drug related (SUSAR). This type of SAE must be reported by MSZ to the appropriate national health authority and Ethics committees within 15 days; fatal or life-threatening events must be reported within 7 days.
All person-related data will always be transmitted pseudonymized. Before reporting a SUSAR the subject will be unblinded.

10.3.1 Reporting obligation of the investigator

The investigator shall report any serious adverse event (SAE), which occurs in a subject immediately. When an investigator identifies an SAE, he or she must notify the MSZ within 24 hours of discovering the event. Therefore, for documentation purposes it is recommended to the study center to note date and time of awareness.

In case of SAR/SUSAR an extended written record (case narrative) needs to be provided by the investigator.

This announcement will be sent via fax to:

Contract Research Organization (CRO):

Münchner Studienzentrum (MSZ)

Klinikum rechts der Isar

Technische Universität München

Ismaninger Straße 22

81675 München

Tel: 089-4140-6477

→ Fax: +49 89 4140-6480 or

E-Mail: sae-msz@mri.tum.de

Every event will be documented on a record form and will immediately be sent to given address. If at that point all required information is not available, succeeding records will be sent. In the event of death, a copy of the autopsy record should be added if available.

10.3.2 Reporting obligation of the sponsor

MSZ shall keep detailed records of all adverse events relating to a clinical trial, which are reported to him by the investigators for that trial. The Competent Authority may require the sponsor to provide those records. MSZ shall ensure that all relevant information about a SUSAR, which occurs during the course of a clinical trial and **is fatal or life-threatening** is reported as soon as possible to the Competent Authority in which the trial is being conducted, and the relevant ethics committee. This needs to be done **not later than seven days** after the sponsor was first aware of the reaction. Any additional relevant information should be sent within eight days of the report.
MSZ shall ensure that a **SUSAR** which is **not fatal or life-threatening** is reported as soon as possible, and in any event **not later than 15 days** after the sponsor is first aware of the reaction to the competent authorities of any EEA State, in which the trial is being conducted and the relevant ethics committee.

10.4 Other Safety Issues Requiring Expedited Reporting

MSZ will immediately, within 15 days after it becomes known, report all circumstances that require a revision of the risk-benefit analysis to the relevant ethics committees and the federal authorities. This especially includes:

- Singular cases of expected serious adverse events with an unexpected outcome.

- Increased incidence of expected serious adverse events that are judged as being clinically relevant.
- SUSARs which occur after termination of the clinical trial (6 months) after termination or exclusion)
- Events related to study procedures or development of the study medication, which could affect a subject's safety.

10.5 Annual Safety Report (DSUR)

In addition to the expedited reporting required for SUSAR, the sponsor and MSZ will submit a safety report to the Competent Authority and Ethics Committee, once a year throughout the clinical trial or upon request. The annual safety report should take into account all new available safety information received during the reporting period. Serious Adverse Events and Adverse Events will be reported with the DSUR on a yearly basis or upon request.

10.6 Pregnancy

Pregnancies must be reported to the Sponsor and MSZ within 24 hours. Pregnant females will be excluded from the trial immediately. All pregnant subjects will be followed up at least until delivery, whereby delivery includes abortions, miscarriages. Reporting details include labor and birth, congenital abnormalities, and complications in mother or child.

This data on pregnancy will be documented on the pregnancy report form, which will be faxed to the Sponsor. The follow up of the pregnancy will be documented on the same form and will be sent within 24 hours after termination of the pregnancy or within 4 weeks after childbirth.

All pregnancies will be reported to the Trial Office (UMG, Göttingen) and the Contract Research Organization (MSZ, München) in the above-described way.

Abstinence (for site in CH): Specific preventive measures for contraception: Men must use contraceptive methods during the study (e.g. condom).
At the beginning of the study, all women must undergo a pregnancy test. This does not apply to postmenopausal women or those who have been surgically sterilized.
If participating in this study, women must use reliable contraceptive measures. These are birth control pills, contraceptive patches, the hormonal spiral, the mechanical spiral, an operative fallopian tube closure or the sterilization of the partner.

10.7 Safety Monitoring Board (SMB)

The study will be monitored by an independent SMB. The SMB will come together once before the start of the trial, and then hold telephone conferences approximately every 3 months during the trial in order to review trial progress, safety data and adherence to protocol, with the frequency of meetings depending on the rate of recruitment and safety issues.

11 Case Report Forms (CRF)

It is the investigator's responsibility to ensure that the trial is executed in accordance with the GCP guidelines, the applicable national laws and regulations of the participating countries and the study protocol, and that data are entered into the database for this study in a correct and complete way. Data are entered directly into the eCRF, whenever possible.

All patient related data will be recorded under a pseudonym. The Investigator will compile a confidential list, which relates these patient numbers to the patient's full name. This list will only be accessible to the study team and the monitor. Original patient files may be viewed by monitors, auditors and inspectors.

12 Investigator Site File

All essential documents will be kept in the Investigator Site File (ISF), which will be stored at the Trial Site in accordance with ICH GCP.

13 Quality Control and Quality Management

13.1 Monitoring, Data Quality Assurance

Monitoring activities are performed to ensure that the trial is conducted in accordance with the trial protocol, the principles of GCP and local legislation. A monitoring manual describing the scope of the monitoring activities in detail will be prepared.

The responsible monitor will contact the investigator and will be allowed, on request, to inspect the various records of the trial (eCRF and other pertinent data) provided that patient confidentiality is maintained in accord with local requirements.

The monitor should have access to patient records, any information needed to verify the entries in the eCRF and all necessary information and essential study documents. The investigator agrees to cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits are resolved. A monitoring visit report is prepared for each visit describing the progress of the clinical trial and all identified problems

13.1.1 Audits / Inspections

Authorized representatives of the Sponsor, a regulatory authority) may visit the Trial Sites to perform audits or inspections, including source data verification. The purpose of a Sponsor audit or inspection is to systematically and independently examine all study related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, Good Clinical Practice (GCP), guidelines of the International Conference on Harmonization (ICH), and any applicable regulatory requirements.

Auditors and inspectors are bound by professional confidentiality and may not pass on any personal information that comes to their knowledge. In the course of audits or inspections, data in the case report forms will be compared with the data for medical records. All the documentation held by the investigators within the scope of the clinical trial, as well as the drug logs of the study medications will be verified.

14 Clinical Data Management

14.1 Data collection

The documentation of the study data in adherence to the GCP-guidelines and the clinical trial protocol is the responsibility of the investigator. Original data (source documents) remain in hospital medical record and information on the eCRF must be traceable and consistent with

the original data. Source documents are e.g. laboratory results, ALSFRS-R measurements, vital capacity measurements and quality of life questionnaire. Original written informed consent signed by the patient is kept by the investigator and a signed copy will be given to the patient. No information in source documents about the identity of the patients will be disclosed. All data collected in this study must be entered in an eCRF which has to be completed by the investigator or authorized trial personnel and signed by the principle investigator. This also applies for those patients who do not complete the study. If a patient withdraws from the study, the reason must be recorded on the eCRF. The investigator is responsible for ensuring the accuracy, completeness, and timeliness of all data reported to the sponsor in the eCRFs and in all required reports.

After database lock, the principle investigator will receive data on electronic device of the investigational site for archiving in the Investigator Site File (ISF).

14.2 Database management

Data are administered and processed by data management of the MSZ with the support of a study database (eCRF) according to the SOPs of the MSZ.

A description of the study specific processes is given in the Data Management Plan that details the key planning and control elements for the data management component of the study.

The evaluation of the data takes place by programmed validity- and consistency checks. In addition, a manual/visual evaluation of plausibility is performed in accordance to the requirements of GCP. Queries may occur, which will be visualized on the study database. The investigator has to resolve all data discrepancies in the study database.

After entry of all collected data and clarification of all queries, the database will be closed at the completion of the study. The database closure has to be documented.

Data and results electronically recorded will be archived according to legal guidelines at least 10 years after study termination.

15 Statistical Parameters

15.1 Sample Size

A sample size of 102 patients (i.e. 34 patients per treatment group) yields sufficiently narrow confidence intervals for the difference in proportions between the placebo group and the treatment groups for both primary endpoints, the proportion of patients without drug-related SAEs and the proportion of patients without treatment intolerability. Under the assumption of no difference between the treatment groups and the placebo group the half-width of the 95% confidence interval for the difference in proportions is at most 0.24. Expected are high proportions of tolerability and safety in which case the confidence interval becomes narrower. If the proportion is for instance 0.9 the half-width reduces to 0.14, which is considered sufficiently narrow. Calculations were done using nQuery 4.0. Adjusting for dropout of 15% we aim to recruit a total number of 120 patients (i.e. 40 patients per treatment group).

15.2 Randomization

This trial has three parallel groups (i.e. Fasudil 2 x 15 mg/d, Fasudil 2 x 30 mg/d, and matching placebo). Patients are randomly allocated to treatment using an allocation ratio of 1:1:1. The randomization list will be centrally generated using a computerized system stratified by region and type of onset (bulbar, spinal). At the screening visit (V0), each patient will receive the next consecutive screening number. At the randomization, each patient eligible for study participation will receive the next consecutive randomization/patient number according to his stratum (bulbar onset vs. spinal onset) from a block of randomization numbers per region. Patients will be assigned to the bulbar or spinal stratum according to the location of the earliest experienced ALS symptom (defined by the first muscle weakness, or in the case of bulbar onset, by the presence of dysarthria and/or dysphagia). In the case of a patient with simultaneous onset of spinal and bulbar symptoms, onset will be defined as bulbar. Cervical and respiratory onsets are stratified to the spinal-onset stratum. This stratum assignment must be consistent with the diagnosis and clinical assessment (i.e., maximum score on bulbar scale and low score on manual muscle testing would contradict the assignment to bulbar stratum). The randomization list will be generated by the Biometry and Bioinformatics Core using a pseudo-random number generator to ensure that the resulting treatment sequence will be both reproducible and non-predictable. Study medication will be packed and blinded by the Universitätsklinikum Leipzig AöR, Apotheke, Germany, according to the randomization list. Each patient medication package will be sent together with the sealed unblinding codes to the sites. The investigator at the site takes care that each patient will be provided with the study medication box of the correct randomization number. The randomization list will be kept in safe and confidential custody at Universitätsklinikum Leipzig AöR, Apotheke, Germany.

16 Statistical Analysis

16.1 General Statistical Considerations

A specific Statistical Analysis Plan (SAP) will be finalized prior to database lock and unblinding. The SAP will be in accordance with the study protocol and will describe the analysis at an operational level. The development, respectively the validation of the statistical analysis programs will be defined along with the responsible staff. Further exploratory analysis will be given in the SAP as well. Additional exploratory analyses of the data will be conducted as deemed appropriate. Analyses will be fully detailed in the SAP. Changes to the SAP will be documented. The statistical analysis will be conducted with SAS® or R.

It is planned, that the data will be used for meta-analysis with data of a corresponding twin trial, ROCK-ALS-US, which will be conducted in parallel in centers in the United States of America. The precise procedure of meta-analysis will be specified elsewhere.

16.2 Study Participant Disposition

All patients who discontinue from the study will be identified, and the extent of their participation in the study will be reported. If known, a reason for their discontinuation will be given.

16.3 Study Participant Characteristics

The patient's age, sex, weight, height, and other demographic characteristics will be recorded and may be used in the efficacy and safety analyses as quantitative or classification variables.

16.4 Definition of Population for Analysis

Analyses of safety, tolerability, and efficacy will be performed using the full analysis set, i.e. the intention-to-treat (ITT) population. The ITT population consists of all randomized patients, whether or not they confirm all protocol requirements. The following circumstances lead to exclusion of randomized patients from the full analysis set:

- Violation of inclusion/exclusion criteria
- Absence of data post randomization (e.g. no IMP received)

Subjects who fail to satisfy an entry criterion may be excluded without introducing bias only if:

- the entry criterion was measured prior to randomization;
- the detection of relevant eligibility violations can be made completely objective;
- all subjects receive equal scrutiny for eligibility violations;
- all detected violations of the particular entry criterion are excluded.

Potential biases arising from these specific exclusions, or any others, must be addressed.

Efficacy analysis will be performed for the ITT population, and supporting analyses will be conducted on the per-protocol (PP) population, which is a subset of the ITT population that will be treated according to trial schedule. Additional exploratory analyses of the data will be conducted as deemed appropriate. Analyses will be fully detailed in the SAP.

16.5 Clinical Evaluation of Safety

A Safety Monitoring Board (SMB) will be established. Also, a possible relationship to the study drug will be carefully evaluated for every occurring Serious Adverse Event (SAE) by the responsible clinician. All study drug and protocol procedure AEs will be listed, and if the frequency of events allows, safety data may be summarized using descriptive methodology.

The incidence of symptoms for each treatment will be presented by severity and by association with study drug as perceived by the investigator. Symptoms reported to occur prior to enrolment will be distinguished from those reported as new or increased in severity during the study. Each symptom will be classified by the most suitable term from the medical regulatory dictionary.

16.6 Statistical Analysis of Primary Endpoints

The study will yield information about safety, tolerability, and efficacy. Main focus of the primary analysis is to determine whether either one or both doses are *safe*, and *tolerable*. Each patient will be treated with Fasudil or placebo over 45 minutes twice a day. Significant drug intolerance (criteria in Section 9.1) will result in stop of infusion, and termination of the trial participation for this patient.

The treatment with Fasudil is considered *safe* for an individual patient if no drug-related Serious Adverse Event (SAE) is recorded through to visit V23. The treatment with Fasudil is considered *tolerable* if no significant drug intolerance during the treatment period is recorded. The proportions of patients for whom the treatment is tolerable / safe are derived for each treatment group. For both active treatment groups and for both, safety and tolerability, separately the difference in proportions to the placebo group will be calculated with its 95% confidence interval. Subsequent analyses will model tolerability and safety in logistic regressions adjusting for randomization stratification factors and important prognostic factors assessed at baseline. Treatment group differences will be reported in terms of odds ratios with 95% confidence intervals.

Primary analyses of safety and tolerability will be carried out on the ITT population. For the purpose of the tolerability analyses subjects who discontinue during the treatment period will be considered as worst case, i.e. no drug tolerability. If a number of patients withdraw from the study following completion of the treatment period, there observations will be dealt with as independent right censoring and the time to first drug-related SAE will be considered as primary endpoint. The difference is proportions free of any drug-related SAE at V23 will be assessed using Kaplan-Meier estimates and corresponding 95% confidence intervals for their differences. Subsequent analyses will model time to first drug-related SAE in Cox proportional hazard regressions adjusting for randomization stratification factors and important prognostic factors assessed at baseline. Treatment group differences will be reported in terms of hazard ratios with 95% confidence intervals. The ITT analyses will be complemented with a per-protocol analysis. Differences between both analyses will be reported and evaluated in detail.

16.7 Statistical Analysis of Secondary Endpoints

Efficacy: Efficacy outcomes including ALSFRS-R, ALSAQ-5, vital capacity and MUNIX through to visit V23 will be analyzed by means of Gaussian linear model for repeated measures (so-called MMRM) with treatment group, time (visits V20, V22, V23), treatment-by-time interaction, region and stratum as factors and baseline measurements of the outcome as covariate. The error terms are assumed to follow a multivariate normal distribution with unstructured covariance. Least squares mean changes from baseline will be reported for the treatment groups with 95% confidence interval (CI) as well as the difference between the least squares treatment group means with 95% CI and p-value testing the null hypothesis of no treatment

effect. The analysis will be primarily performed on the ITT population, but complemented by per-protocol analysis.

Survival time will be used as a secondary endpoint. The Kaplan-Meier method will be applied to estimate the survival time in each group. The 95% CIs will be calculated with the variance derived from Greenwoods' formula. Subjects will be right-censored at end of their follow-up. Pairwise group comparisons against placebo will be analyzed using exact log rank tests. Cox proportional hazards regression will be carried out if there is a sufficient number of events.

16.8 Statistical Analysis of Exploratory Endpoints

The analyses for the exploratory endpoints will be specified in the SAP prior to data base lock.

16.9 Interim Analyses

No interim analyses are planned during this study.

17 Reporting

17.1 Statistical Report

The Department of Medical Statistics, Universitätsmedizin Göttingen, will produce the statistical analysis and biometrical report in cooperation with the Sponsor and the Coordinating Investigator. All data in this report are confidential.

17.2 Final Report

All data regarding this clinical trial are to be handled confidentially. The composition of a final integrated report will be conducted in accordance with ICH E3: Structure and Contents of Clinical Study Reports. After completion of the biometrical analysis the Coordinating Investigator will compose an integrated report. This report will contain a clinical record, a statistical record, single value tables and conclusions. It will be signed by the Coordinating Investigator, the Sponsor, and the Project Statistician.

Due to his location at the Department of Medical Statistics, Universitätsmedizin Göttingen, it is guaranteed that the Project Statistician named in the protocol (see list of responsibilities) will be able to supervise the statistical analysis.

17.3 Publications

The study results will be published irrespective of study outcome. The results will be published on www.clinicaltrials.gov and in a peer-reviewed, international journal.

18 Ethical, Legal and Regulatory Aspects

18.1 ICH-GCP-guidelines

This trial will be conducted in accordance with the current ICH-GCP-guidelines. Good Clinical Practice (GCP) is an international ethical and scientific quality standard for designing, conducting, recording and reporting trials that involve the participation of human subjects. Compliance with this standard provides public assurance that the rights, safety and well-being of trial subjects are protected, consistent with the principles that have their origin in the Declaration of Helsinki, and that the clinical trial data are credible.

18.2 Legal Requirements of the Study

This study is conducted in compliance with all applicable laws and regulations and in accordance with the applicable local requirements. The study will start only after the following documents have been obtained:

- Approval of ethics committee
- Approval of competent authority
- Notification to applicable regional authorities
- Informed Consent
- Insurance
- Data privacy and confidentiality

As for Switzerland, competent authority means both swissmedic and swissethic, please take into account that wherever “competent authority” is mentioned in the Trial Protocol, for Switzerland in this case only swissmedic is meant, not additionally swissethics.

18.2.1 Responsibilities of the Sponsor and the Investigator

The Sponsor of the clinical trial, the Universitätsmedizin Göttingen, is responsible for the initiation, organization, and financing of this clinical trial. Sponsor and investigator ensure that the clinical trial is executed in accordance with the existing laws and regulations, according to the ICH-GCP guidelines, the Declaration of Helsinki (1996), and the applicable national and international regulations. The investigator accepts the requirements of the signed study protocol.

The investigator’s responsibilities include:

- to understand the nature of the study drug as described in the Summary of Product Characteristics,
- to understand and execute the study in accordance to the study plan,
- to ensure that sufficient time and capacities are available for the execution of the trial,
- to ensure the correct collection and documentation of data and reporting,
- to prepare and provide all data for Sponsor, monitoring, or relevant authorities for audits and/ or inspections,
- to ensure that all information on participating persons and all information obtained from the Sponsor is handled confidentially by all persons involved in the trial,
- to declare the non-inclusion of persons possibly dependent on the Sponsor or the investigator,
- to declare any possible financial or other interests of the investigator in the context of the study medication.

The respective investigator accepts the responsibilities for the execution of the clinical trial in the Trial Site according to applicable national and international regulations.

18.2.2 Approval from Ethics Committee and Competent Authorities

The study will be applied with the ethics committee of the Universitätsmedizin Göttingen, Georg-August-Universität as well as other competent ethics committees and respective national competent authorities. The trial will only start after approvals have been granted.

18.2.3 Patient Information and Informed Consent

Patient Information: Before enrolment, every patient will receive full oral and written information about the nature, purpose, expected advantages and possible risks of the trial.

Consent to Participation in the Trial: The patient will agree to participation in the trial by signing the informed consent form. See section 7.1 for details on the methods of obtaining informed consent.

The final versions of patient information and consent forms will be presented to the ethics committee.

18.2.4 Patient Insurance

For all participating patients, an insurance has been established with the following company:

Versicherung: CNA Hardy
Versicherungsmakler: Marsh Medical Consulting GmbH
Bismarckstraße 2
32756 Detmold
Germany

For German sites the insurance is limited to a maximum of 500.000 Euro per patient, for sites in France it is limited to 1.000.000 EUR per patient and for site in Switzerland it is limited to 1.000.000 CHF. The insurance covers all possible damages that the patients may suffer directly or indirectly through administration of the investigational product or assessments within the clinical trial.

In order not to risk the insurance cover, all patients participating in this trial must follow strictly the instructions from the study personnel. Participants must not undergo another medical treatment without prior consent from the investigator (exception: emergency treatment). The investigator must be notified immediately of any emergency treatment. Damage to health that may be the result of this clinical trial must be communicated immediately to the investigator and the insurance company. The participating patients are to take any appropriate measure that help to detect the cause and to minimize the extent of the damage.

The participant will receive one original of the informed consent document and the terms and conditions of the insurance.

18.2.5 Data Privacy and Confidentiality

The collection, transmission, storage, and evaluation of personal data within this clinical study will be conducted in accordance with respective national legal requirements. Precondition for this is the voluntary consent of the participating patients within the information- and consent forms provided, to be signed before participation in the clinical trial. Participants in this trial will be informed of the following:

1. Within this clinical trial, data will be collected on paper or electronically and will be treated strictly confidential in a pseudonymized form and will be transmitted only to:
    - the Sponsor of the trial for scientific evaluation of undesired events,
    - the relevant surveillance authorities (regional councils and the competent national authorities, the ethics committee of the Universitätsmedizin Göttingen and the European Database for the surveillance of the proper execution of the trial, as well as for the evaluation of study results and of undesired events.

2. As far as required for the examination of the study, delegates of the Sponsor (monitoring, auditing) and/or of the relevant surveillance authority, who are authorized and sworn to secrecy, may gain insight into personal data. For these purposes, the investigator will be released from their medical confidentiality.
3. Consent to collection and handling of personal data within this study is irrevocable. The participating persons will be informed that they can withdraw consent at any time without the need to give reasons and without disadvantages. In case of withdrawal of consent, all data collected until this time will be used, without the name of the participant, as far as required for the evaluation of study objectives and to ensure that none of the participant's rights worthy of protection have been violated.

18.3 Changes to the Protocol

To ensure that comparable conditions are achieved as far as possible at individual trial sites and in the interests of a consistent and valid data analysis, changes to the provisions of this trial protocol are not planned. In exceptional cases, however, changes may be made to the trial protocol. Such changes can only be made if agreed by the sponsor, sponsor's representative, the CI and biometrician. Any changes to the trial procedures must be made in writing and must be documented with reasons and signed by all Authors of the original trial protocol.

Amendments made in accordance with § 10 Secs. 1 and 4 GCP Regulations that require approval are submitted to the ethics committee and the supreme federal authority and will not be implemented until approved. Exceptions to this are amendments made to avoid immediate dangers.

18.4 Archiving of Data / Access to Records

The investigator must keep the ISF, CRFs and all information related to the clinical trial archived for at least 10 years after completion or discontinuation of the study in accordance with GCP Regulations. The sponsor will also archive all study related report forms for at least 10 years after completion of the study. After that period of time the documents may be destroyed, subject to local regulations.

18.5 Financing

The Sponsor is responsible for financing the clinical trial. It will be funded by the national public funding bodies delineated within the 2016 E-Rare Joint Transnational Call for "Clinical research for new therapeutic uses of already existing molecules (repurposing) in rare diseases". Additional funding may be applied for.

19 References

1. Bensimon G, Lacomblez L, Meininger V, Group TARS. A Controlled Trial of Riluzole in Amyotrophic Lateral Sclerosis. N Engl J Med. 1994 Mar 2;330(9):585–91.

2. Lacomblez L, Bensimon G, Leigh PN, Guillet P, Meininger V. Dose-ranging study of riluzole in amyotrophic lateral sclerosis. Amyotrophic Lateral Sclerosis/Riluzole Study Group II. Lancet. Sciencedirect; 1996 May 24;347(9013):1425–31.

3. Komagome R, Kimura K, Saito M. Postnatal changes in Rho and Rho-related proteins in the mouse brain. Jpn J Vet Res. 2000 Feb;47(3-4):127–33.

4. Dong M, Yan BP, Liao JK, Lam Y-Y, Yip GWK, Yu C-M. Rho-kinase inhibition: a novel therapeutic target for the treatment of cardiovascular diseases. Drug Discov Today. 2010 Aug;15(15-16):622–9.

5. Tönges L, Frank T, Tatenhorst L, Saal KA, Koch JC, Szego EM, et al. Inhibition of rho kinase enhances survival of dopaminergic neurons and attenuates axonal loss in a mouse model of Parkinson's disease. Brain. 2012 Oct 31;135(Pt 11):3355–70.

6. Takata M, Tanaka H, Kimura M, Nagahara Y, Tanaka K, Kawasaki K, et al. Fasudil, a rho kinase inhibitor, limits motor neuron loss in experimental models of amyotrophic lateral sclerosis. British Journal of Pharmacology. 2013 Aug 31;170(2):341–51.

7. Tönges L, Koch J-C, Bähr M, Lingor P. ROCKing Regeneration: Rho Kinase Inhibition as Molecular Target for Neurorestoration. Front Mol Neurosci. 2011;4:1–11.

8. Shibuya M, Hirai S, Seto M, Satoh S-I, Ohtomo E. Effects of fasudil in acute ischemic stroke: Results of a prospective placebo-controlled double-blind trial. Journal of the Neurological Sciences. 2005 Nov;238(1-2):31–9.

9. Taniguchi J, Seki C, Takuwa H, Kawaguchi H, Ikoma Y, Fujinaga M, et al. Evaluation of Rho-Kinase Activity in Mice Brain Using N-[11C]Methyl-hydroxyfasudil with Positron Emission Tomography. Mol Imaging Biol [Internet]. Springer US; 2013 Oct 24;16(3):395–402. Available from: http://link.springer.com/10.1007/s11307-013-0695-y

10. Vicari RM, Chaitman B, Keefe D, Smith WB, Chrysant SG, Tonkon MJ, et al. Efficacy and Safety of Fasudil in Patients With Stable Angina. Journal of the American College of Cardiology. 2005 Nov;46(10):1803–11.

11. Fukumoto Y, Yamada N, Matsubara H, Mizoguchi M, Uchino K, Yao A, et al. Double-Blind, Placebo-Controlled Clinical Trial With a Rho-Kinase Inhibitor in Pulmonary Arterial Hypertension. Circulation Journal [Internet]. 2013;77(10):2619–25. Available from: http://jlc.jst.go.jp/DN/JST.JSTAGE/circj/CJ-13-0443?lang=en&from=CrossRef&type=abstract

12. Tönges L, Günther R, Suhr M, Jansen J, Balck A, Saal K-A, et al. Rho kinase inhibition modulates microglia activation and improves survival in a model of amyotrophic lateral sclerosis. Glia. 2014 Feb;62(2):217–32.

13. Zhang H, Li Y, Yu J, Guo M, Meng J, Liu C, et al. Rho kinase inhibitor fasudil regulates microglia polarization and function. Neuroimmunomodulation [Internet]. Karger Publishers; 2013;20(6):313–22. Available from: http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=23941863&retmode=ref&cmd=prlinks

14. Borrajo A, Rodriguez-Perez AI, Villar-Cheda B, Guerra MJ, Labandeira-Garcia JL.

Inhibition of the microglial response is essential for the neuroprotective effects of Rho-kinase inhibitors on MPTP-induced dopaminergic cell death. Neuropharmacology. Elsevier Ltd; 2014 May 29;:1–8.

15. Bowerman MM, Murray LML, Boyer JGJ, Anderson CLC, Kothary RR. Fasudil improves survival and promotes skeletal muscle development in a mouse model of spinal muscular atrophy. BMC Med. 2011 Dec 31;10:24–4.

16. Asano T, Ikegaki I, Satoh S, Seto M, Sasaki Y. A protein kinase inhibitor, fasudil (AT-877): A novel approach to signal transduction therapy. Cardiovascular Drug Reviews. 1998;16(1):76–87.

17. Hinderling PH, Karara AH, Tao B, Pawula M, Wilding I, Lu M. Systemic Availability of the Active Metabolite Hydroxy-Fasudil After Administration of Fasudil to Different Sites of the Human Gastrointestinal Tract. The Journal of Clinical Pharmacology. 2007 Jan 1;47(1):19–25.

18. Suzuki Y, Shibuya M, Satoh S, Sugimoto Y. A postmarketing surveillance study of fasudil treatment after aneurysmal subarachnoid hemorrhage. Surgical neurology. 2007.

19. Hanada Y, Kudo H, Kohmura E. Chronologic changes of fasudil hydrochloride and hydroxyfasudil in cerebrospinal fluid of patients with aneurysmal subarachnoid hemorrhage. J Stroke Cerebrovasc Dis. 2004 Dec 31;14(2):47–9.

20. Inhibition of Rho kinase (ROCK) increases neurite outgrowth on chondroitin sulphate proteoglycan in vitro and axonal regeneration in the adult optic nerve in vivo. 2007 Oct;103(1):181–9.

21. Cedarbaum JM, Stambler N, Malta E, Fuller C, Hilt D, Thurmond B, et al. The ALSFRS-R: a revised ALS functional rating scale that incorporates assessments of respiratory function. BDNF ALS Study Group (Phase III). Journal of the Neurological Sciences. 1999 Oct 31;169(1-2):13–21.

22. Maier A, Holm T, Wicks P, Steinfurth L, Linke P, Münch C, et al. Online assessment of ALS functional rating scale compares well to in-clinic evaluation: a prospective trial. Amyotroph Lateral Scler. Informa Healthcare Stockholm; 2012 Feb;13(2):210–6.

23. Shefner J, Cedarbaum JM, Cudkowicz ME, Maragakis N, Lee J, Jones D, et al. Safety, tolerability and pharmacodynamics of a skeletal muscle activator in amyotrophic lateral sclerosis. Amyotroph Lateral Scler. Informa Healthcare Stockholm; 2012 Sep;13(5):430–8.

24. Jenkinson C, Fitzpatrick R. Reduced item set for the amyotrophic lateral sclerosis assessment questionnaire: development and validation of the ALSAQ-5. Journal of Neurology, Neurosurgery & Psychiatry. 2001 Jan;70(1):70–3.

25. Neuwirth C, Nandedkar S, Stålberg E, Barkhaus PE, Carvalho M de, Furtula J, et al. Motor Unit Number Index (MUNIX): A novel neurophysiological marker for neuromuscular disorders; test–retest reliability in healthy volunteers. Clinical Neurophysiology. 2011 Sep;122(9):1867–72.

26. Neuwirth C, Barkhaus PE, Burkhardt C, Castro J, Czell D, de Carvalho M, et al. Tracking motor neuron loss in a set of six muscles in amyotrophic lateral sclerosis using the Motor Unit Number Index (MUNIX): a 15-month longitudinal multicentre trial. Journal of Neurology, Neurosurgery & Psychiatry. 2015 Oct 14;86(11):1172–9.

27. Abrahams S, Newton J, Niven E, Foley J, Bak TH. Screening for cognition and behaviour changes in ALS. Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration. Taylor & Francis; 2014 Mar;15(1-2):9–14.

Appendices

I. Safety and Monitoring Board (SMB)

A current list of Safety and Monitoring Board members is kept by the CI.