**Title:** Mexiletine II Protocol, v5.0, 10 Jul 2017

**NCT Number:** NCT02781454

**IRB Approval Date:** 25 Jan 2016

# CLINICAL STUDY PROTOCOL

| | |
|---|---|
| **STUDY TITLE:** | Effect of Mexiletine on Cortical Hyperexcitability in Sporadic Amyotrophic Lateral Sclerosis (SALS) |
| **STUDY DRUG:** | Mexiletine |
| **VERSION:** | 5.0 |
| **PROTOCOL DATE:** | July 10, 2017 |
| **PROTOCOL NUMBER:** | MX-ALS-002 |

## STATEMENT OF COMPLIANCE

This study will be conducted in compliance with the protocol, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), Good Clinical Practice (GCP), and the applicable regulatory requirements, United States Code of Federal Regulations (CFR) Title 45 CFR Part 46 and Title 21 CFR Parts 50, 56, and 312.

**SIGNATURE PAGE**

I have read the attached protocol entitled, **"Effect of Mexiletine on Cortical Hyperexcitability in Sporadic Amyotrophic Lateral Sclerosis (SALS)"** dated **July 10, 2017 (Version 5.0)** and agree to abide by all described protocol procedures. I agree to comply with the International Conference on Harmonisation Tripartite Guideline on Good Clinical Practice, applicable regulations and guidelines, local Institutional Review Board (IRB) guidelines and policies, and the Health Insurance Portability and Accountability Act (HIPAA).

**Site Investigator (Print Name):**_____

**Signature:** _____ **Date:** _____

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| AE | Adverse Event |
| AM | Morning |
| ALS | Amyotrophic Lateral Sclerosis |
| ALSFRS-R | Amyotrophic Lateral Sclerosis Functional Rating Scale – Revised |
| ALT | Alanine aminotransferase |
| AST | Aspartate aminotransferase |
| APB | Abductor pollicis brevis |
| BID | Twice a day |
| BUN | Blood Urea Nitrogen |
| CBC | Complete Blood Count |
| CHF | Congestive Heart Failure |
| CFR | Code of Federal Regulations |
| CK | Creatine Kinase |
| Cmax | Peak Drug Concentration |
| CRF | Case Report Form |
| CSF | Cerebrospinal Fluid |
| DM | Data Management |
| DPS | Diaphragm Pacing System |
| ECG | Electrocardiogram |
| eCRF | Electronic Case Report Form |
| EDC | Electronic Data Capture |
| FALS | Familial Amyotrophic Lateral Sclerosis |
| GCP | Good Clinical Practice |
| HHD | Hand Held Dynamometry |
| HIPAA | Health Insurance Portability and Accountability Act |
| ICH | International Conference on Harmonisation |
| ICF | Informed Consent Form |
| IRB | Institutional Review Board |
| IS | Information Systems |
| ITT | Intent to Treat |
| MedDRA | Medical Dictionary for Regulatory Activities |
| MGH | Massachusetts General Hospital |
| mITT | Modified Intent to Treat |
| MS | Microsoft |
| NCRI | Neurological Clinical Research Institute |
| NEALS | Northeast ALS Consortium |
| PI | Principal Investigator |
| Q | Once |
| QD | Once a day |
| RBC | Red Blood Cells |
| RR | Relative Risk |
| SAE | Serious Adverse Event |
| SALS | Sporadic Amyotrophic Lateral Sclerosis |
| SAS | Statistical Analysis Software |

| | |
|---|---|
| SICI | Short-interval intracortical inhibition |
| SOD1 | Superoxide dismutase-1 |
| SQL | Structured Query Language |
| SSL | Secure Sockets Layer |
| SUSAR | Serious Unexpected Suspected Adverse Reaction |
| SVC | Slow Vital Capacity |
| Tmax | Time to Peak Serum Concentration |
| TMS | Transcranial Magnetic Stimulation |
| TTNCS | Threshold Tracking Nerve Conduction Study |
| VAS | Visual Analog Scale |
| VC | Vital Capacity |

## PROTOCOL SUMMARY

**Study Title**
Effect of Mexiletine on Cortical Hyperexcitability in Sporadic Amyotrophic Lateral Sclerosis (SALS)

**Version Number**
5

**Study Indication**
Amyotrophic Lateral Sclerosis

**Phase of Development**
2

**Rationale for the Study**
Recent research suggests that hyperexcitability of cortical motor neurons may contribute to the pathogenesis of ALS. Hyperexcitability of both spinal and cortical motor neurons has been demonstrated during the early development of ALS SOD1 mouse mutants prior to signs of disease and may play an important role in causing neuronal injury and cell death in this disorder. Through the use of TMS, an FDA-approved device that involves the use of magnetic fields to determine the physiologic properties of cortical motor neurons, recent studies suggest that stimulus thresholds required to generated a motor evoked potential (motor thresholds) are lower in both sporadic and familial ALS patients, especially early in disease, and may serve as a biomarker for cortical hyperexcitability in ALS. Mexiletine, a cardiac anti-arrhythmic, is a use-dependent sodium channel blocker that has been shown to inhibit neuronal hyperexcitability and prevent cell death in a motor neuron cell line exposed to cultured media from astrocytes engineered to express the human SOD1 gene and to extend survival of hSOD1 transgenic mice and could potentially benefit patients with this disease.

A dose-ranging phase II double-blind randomized controlled trial of 900 mg and 300 mg/day of mexiletine versus placebo was recently completed to determine the safety and tolerability of the drug at these doses and effects on exploratory measures including ALSFRS-R score, slow vital capacity (SVC), and frequency and severity of muscle cramps. The conclusion of the study was that mexiletine was determined safe at both 300 mg and 900 mg / day doses and well tolerated at the lower dose but gastrointestinal side effects led to frequent discontinuation at the higher dose. Mexiletine treatment resulted in large dose-dependent reductions in muscle cramp frequency and severity. An effect on rate of progression was not detected, but clinically important differences could not be excluded in this small and short duration study. An additional early stage study to assess pharmacodynamic effects on cortical excitability, confirm efficacy for cramps, and test intermediate doses is warranted.

**Study Design**
This is a multicenter, randomized, double-blind, placebo-controlled 8-week study evaluating the effect of mexiletine treatment on both cortical motoneuronal and peripheral nerve

hyperexcitability.

**Rationale for Dose and Schedule Selection**

Studies of mexiletine designed to determine the efficacy of the drug in treating neuropathic pain and myotonia have largely demonstrated benefit with minimal side effects at doses between 300 and 600 mg a day. In the first mexiletine study, 300 mg/day was determined to be safe and well tolerated but mexiletine 900 mg/day, while found to be safe, was not well tolerated, largely due to gastrointestinal side effects. Given that the optimal dose of mexiletine is not known for neuroprotection in ALS, the dosage to be use in this study, 300 mg/day, deemed tolerable and safe in the first study, and the intermediate dose of 600 mg/day were chosen to determine whether they reduce motoneuronal and peripheral nerve excitability as well as to determine safety and tolerability of the high dose. The schedule of visits was chosen to ensure that appropriate safety evaluations will be performed at reasonable intervals without being so excessive as to limit subject compliance.

**Study Objectives and Endpoints**

***Primary:***

The primary study objective is to determine whether treatment with mexiletine at doses of 300 mg/day or 600 mg/day suppresses cortical hyperexcitability in sporadic ALS patients relative to placebo, and, thus, may be able to slow progression in ALS. The change in resting motor threshold (RMT), estimated from single pulse transcranial magnetic stimulation (TMS) measurements made before treatment, after 4 weeks of treatment, and then again after a 4 week washout, will be used as the primary pharmacodynamic marker of cortical hyperexcitability.

***Secondary:***

Secondary pharmacodynamics outcome measures will include effects on motor evoked potential (MEP) amplitude (as a % of compound muscle action potential amplitude), cortical silent period (CSP) measured by single pulse TMS, and in short-interval intracortical inhibition (SICI) from dual pulse TMS, as additional pharmacodynamic markers of cortical hyperexcitability, prior to and following treatment with mexiletine or placebo. Additionally, as a pharmacodynamic marker of peripheral motor nerve axonal hyperexcitability, threshold tracking nerve conduction studies (TTNCS) will be performed to assess time constant and recovery cycle analysis. Safety and tolerability and determination of mexiletine concentrations in blood at the time of each of the five TMS studies will also be assessed. Additionally, the effect on frequency and severity of muscle cramps and fasciculations will be assessed using a daily muscle cramps/fasciculations diary and assessment form at Baseline. ALS Functional Rating Scale-Revised (ALSFRS-R) and slow vital capacity (SVC) will be obtained at Screening, Pre-Dose Baseline, Week 4, and Week 8 or the Final Safety Visit, if applicable as exploratory outcomes.

**Study Locations**

Approximately 9 Northeast Consortium (NEALS) centers in the US will participate.

**Number of Planned Subjects**

Approximately 60 subjects will be enrolled.

**Study Population**

This study will be conducted in subjects with a clinical diagnosis of sporadic ALS who meet the El Escorial criteria of possible, laboratory-supported probable, probable, or definite criteria for a diagnosis of ALS. At screening, eligible subjects must be at least 18 years old, must have an SVC $\geq$ 50% of predicted capacity for age, height and gender, and onset of symptoms must be $\leq$ 60 months prior to screening. Subjects using or not using riluzole at the time of screening, and women of child-bearing age at screening are eligible for inclusion as long as they meet specific protocol requirements.

**Treatment Groups**

Subjects will be randomly assigned in a 1:1:1 ratio to 3 treatment groups: oral mexiletine 600 mg/day, oral mexiletine 300 mg/day, or matching placebo.

**Duration of Treatment and Follow-up**

Subjects will remain on randomized, double-blind, placebo-controlled treatment until the Week 4 visit but will return for neurophysiological testing off study drug for the Week 8 visit.

## SCHEDULE OF EVENTS AND STUDY WORKFLOW

### Table 1. Schedule of Events

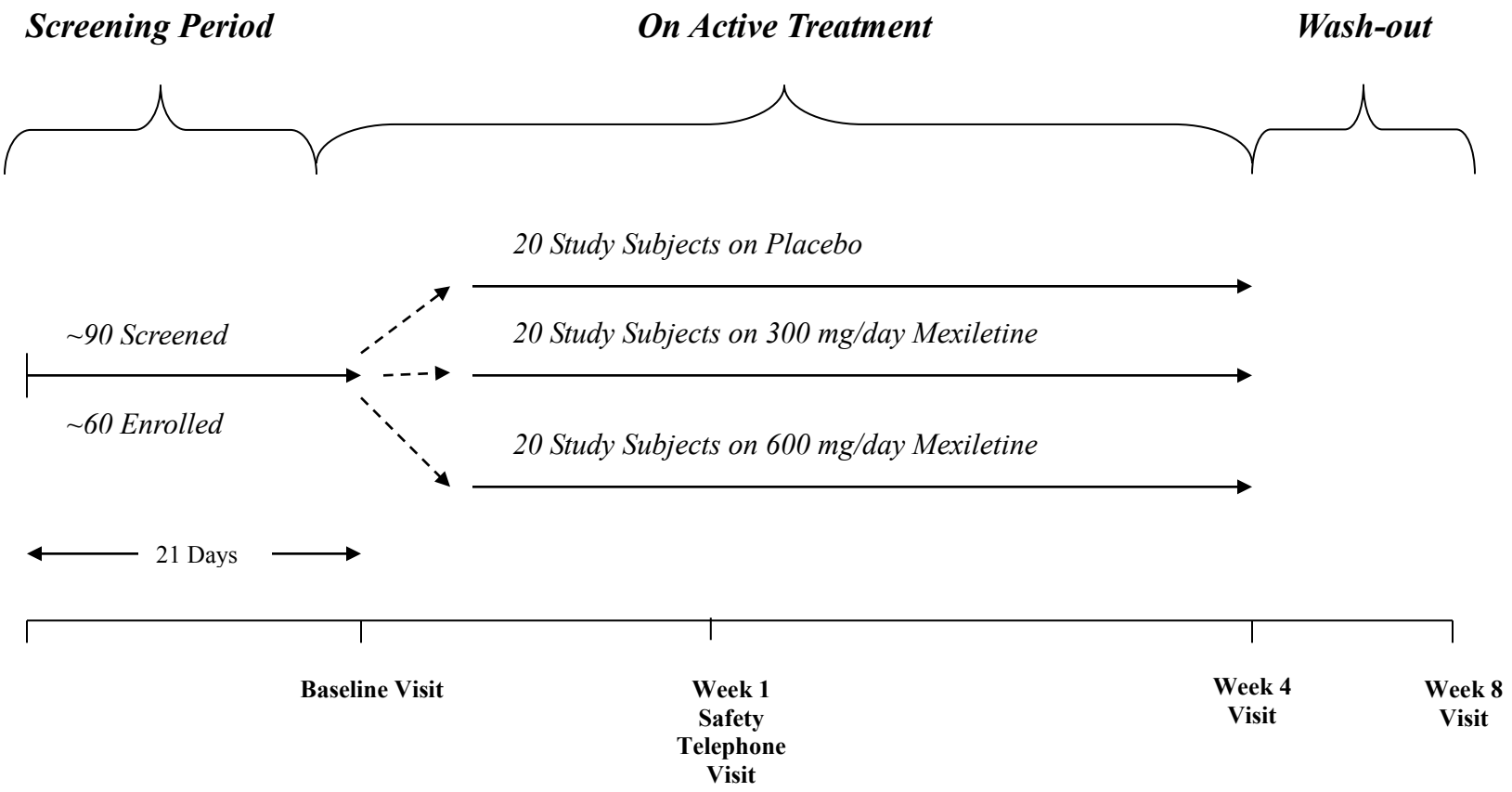
| Activity | Screening Visit[1] | Baseline Visit (Pre-Dose) | Baseline Visit[2] (Post-Dose) | Treatment Period | | | Final Safety Visit (For early termination only) |
|---|---|---|---|---|---|---|---|
| | | | | Week 1 Safety Telephone Visit | Week 4 Visit | Week 8 Visit | |
| Written Informed Consent | X | | | | | | |
| Inclusion/Exclusion Review | X | X | | | | | |
| Medical History / Demographics | X | | | | | | |
| ALS Diagnosis / ALS History | X | | | | | | |
| Physical Examination | X | | | | X | X | X |
| Abbreviated Neurological Examination | X | | | | X | X | X |
| Vital Signs / Height & Weight[3] | X | X | | | X | X | X |
| 12-Lead ECG | X | | X | | X | X | X |
| Safety Labs[4] | X | | | | X | X | X |
| Pregnancy Test[5] | X | X | | | X | X | X |
| Blood sample for Plasma Mexiletine Level[6] | | X | | | X | X | X |
| Blood sample for Plasma (NEALS Biomarker Repository)[7] | | X | | | X | X | X |
| Blood sample for DNA | | X | | | | | |
| ALSFRS-R | X | X | | | X | X | X |
| Transcranial Magnetic Stimulation (TMS) | X | X | | | X | X | X |
| Threshold Tracking Nerve Conduction Studies (TTNCS) | X | X | | | X | X | X |
| Slow Vital Capacity (SVC) | X | X | | | X | X | X |
| Hand Held Dynamometry (HHD) of Abductor pollicis brevis[8] | X | X | | | X | X | X |
| Edinburgh Handedness Inventory Short Form | X | | | | | | |
| RAND-36 | | X | | | X | X | X |
| Concomitant Medication Review | X | X | | X | X | X | X |
| Adverse Event Review[9] | X | X | X | X | X | X | X |
| Randomization[10] | | X | | | | | |
| Administer / Dispense Study Drug | | X | X | | | | |
| Drug Accountability / Compliance | | | | X | X | | X |
| Assessment of Muscle Cramping / Fasciculations | | X | | | | | |
| Summary of Muscle Cramping / Fasciculations Diary[11] | | | | | X | X | X |
| Blindness Questionnaire / Exit Survey | | | | | | X | X |

[1] Screening procedures must be completed within 21 days prior to Baseline Visit.

[2] Baseline Visit (Post-Dose) procedures must be completed on the same day as the Baseline Visit (Pre-Dose).

[3] Vital signs include systolic and diastolic pressure in mmHg, respiratory rate/minute, heart rate/minute, temperature and weight. Height measured at Screening Visit only.
[4] Safety labs includes Hematology, Chemistry, liver function tests (LFTs), urine/serum pregnancy test for women of childbearing potential (WOCBP).
[5] Serum pregnancy test to be completed at screening visit. Urine pregnancy test may be performed in place of serum for Baseline (pre-dose), Week 4 and Week 8 visits.
[6]Blood draw for trough mexiletine level.
[7] Plasma will be stored in the NEALS Biorepository for future analysis. Please refer to the Site Manual of Procedures (MOP) for collection, storage, and shipping information
[8] HHD to be completed prior to TMS
[9]Adverse events that occur AFTER signing consent form will be recorded.
[10] Randomization should occur no more than 48 hours prior to the Baseline Visit, when possible.
[11] Daily summaries of muscle cramping and fasciculations will be completed every morning or evening by the patient in a Muscle Cramping/Fasciculations Diary. Data from the diary will be abstracted by study staff at every visit after the Baseline Visit.
[*] It is strongly encouraged that each visit and the procedures be completed on the same day. However, if this is not possible visits can be split up into 2 consecutive days.

**STUDY WORKFLOW**




Subjects who discontinue from the study early will be asked to return to the study site for Final Safety assessments. Subjects who discontinue study drug early but agree to be followed will be asked to remain in the study and to attend all assigned visits through the Week 8 Visit.

# 1.0. ETHICS

## 1.1. Independent Ethics Committee (IEC) or Institutional Review Board (IRB)

This study will be conducted in compliance with current Good Clinical Practices (GCP) and Title 21 Part 56 of the United States of America Code of Federal Regulations (CFR) relating to IRBs.

## 1.2. Ethical Conduct of Study

The study will be conducted in accordance with GCP defined by the International Conference on Harmonization (ICH) and the ethical principles of the Declaration of Helsinki.

## 1.3. Subject Information and Consent

This study will be conducted in compliance with Title 21 Part 50 of the United States of America Code of Federal Regulations (CFR), Federal Regulations and ICH Guidance Documents pertaining to informed consent. At the first visit, prior to initiation of any study-related procedures, subjects will be informed about the nature and purpose of the study, participation/termination conditions, and risks and benefits. Subjects will be given adequate time to ask questions and become familiar with the study prior to providing consent to participate. Subject will give their written consent to participate in the study and will be provided with a copy of the fully executed consent form for their records.

# 2.0. INTRODUCTION

## 2.1. Background and Significance

### 2.1.1. Clinical Features and Epidemiology of ALS

Amyotrophic lateral sclerosis is a rare degenerative disorder of large motor neurons of the cerebral cortex, brain stem and spinal cord that results in progressive wasting and paralysis of voluntary muscles [1]. The incidence of ALS is currently approximately 2/100,000/year [2, 3] and may be increasing [4]. The lifetime ALS risk is 1 in 600 to 1 in 1000. Fifty percent of people diagnosed with ALS die within three years of onset of symptoms and 90% die within five years [5]. The median age of onset is 55 years. The cause in most cases is unknown. Age and gender are the only risk factors repeatedly documented in epidemiological studies [6]. There is a slight male predominance (3:2 male to female ratio) in sporadic ALS. No treatment prevents, halts or reverses the disease, although riluzole use is associated with a slight prolongation of survival [7, 8].

Essential features of ALS are progressive signs and symptoms of lower motor neuron dysfunction (atrophy, cramps, and fasciculations) associated with corticospinal tract signs (spasticity, enhanced and pathological reflexes) in the absence of sensory findings. There is relative sparing of eye movement muscles and the urinary sphincters. The course is relentless with declines in strength, respiratory function and overall muscle function during the active phase of the disease. Natural history studies have determined that age at onset, site of onset, delay from first symptom to diagnosis, and rate of change in respiratory function are significant predictors of survival.

### 2.1.2. Overview of ALS Pathogenesis

Many causes of ALS have been proposed including toxicity from excess excitation of the motor neurons by transmitters such as glutamate, free radical-mediated oxidative cytotoxicity, neuroinflammation, mitochondrial dysfunction, autoimmune processes, cytoskeletal abnormalities, and aberrant activation of cyclo-oxygenase [9]. It has also been suggested that atypical viral infections may trigger this disease (e.g. enteroviruses or atypical retroviruses) [10-12]. Whatever the cause, it is evident that there are multiple levels of cellular dysfunction as the disease progresses and that programmed cell death is activated in this disease [13-17]. While the cause of sporadic ALS is not known, about 10% of cases are familial; of these, approximately 70% are caused by mutations in the genes encoding C9orf72, cytosolic SOD1, FUS/TLS and TAR-DNA binding protein; other genes have also been implicated [18, 20-24].

Several lines of investigation are consistent with the view that hyperexcitability is a critical factor in ALS pathogenesis. An extensive literature review documents that reuptake of glutamate at synapses on the motor neuron is impaired, in part by reductions in levels of the critical astroglial glutamate transporter EAAT2. This phenomenon has been demonstrated in human ALS brains and is also evident in transgenic ALS rats that express mutant SOD1 protein [25]. Recent *in vivo* [26-28] and *in vitro* [29-31] studies support a role for hyperexcitability of both peripheral motor nerve axons and cortical motor neurons as a possible pathogenic mechanism of ALS and a plausible mechanistic target for novel therapeutics. Several reports document that motor neurons cultured from transgenic $SOD1^{G93A}$ mice demonstrate hyperexcitability, arising at least in part because of inappropriately elevated levels of inward sodium current [26-28]. It has

been speculated that neuronal hyperexcitability as a direct consequence of persistent inward sodium current results in reverse operation of the Na+-Ca++ exchanger, intracellular and intraxonal Ca++, and motor neuron degeneration by activation of Ca++-dependent enzyme pathways {32]. Increased repetitive firing of cortical motor neurons following injection of current using current clamp conditions in $SOD1^{G93A}$ mice relative to age-matched controls has also been shown [27, 28], correlating with cortical hyperexcitability in the mutant mice. A recent study by Wainger and colleagues also demonstrated that inducible pluripotent stem cell-derived (IPS) motor neurons from ALS patients with superoxide dismutase 1, C9orf72, and fused-in-sarcoma mutations had evidence of hyperexcitability as a consequence of reduced delayed-rectifier potassium current compared to control IPS motor neuron cells from control cells [33]. Additionally, the Kv7 channel activator retigabine was able to block the hyperexcitability and improve survival of the cells *in vitro*, suggesting that it could also be a putative disease modifying therapy [33]. Studies employing TMS have demonstrated that RMT and SICI are reduced and MEP increased in both clinically affected familial ALS and SALS patients when compared to asymptomatic SOD1 mutation carriers and normal controls, consistent with increased cortical hyperexcitability in the patients [31]. Threshold tracking nerve conduction studies have also demonstrated hyperexcitability of peripheral motor nerve axons as a consequence of increased persistent sodium current [29, 30], which could also play a role in the generation of fasciculations and muscle cramps, problematic and poorly treated symptoms in ALS. Additionally, by this technique, there is evidence that measurement of the strength-duration time constant, reflecting axonal persistent sodium currents, inversely correlates with survival in ALS [29].

### 2.1.3. Mexiletine in Neurological Disorders

#### 2.1.3.1. The Neuroprotective Properties of Mexiletine in Animal Models

As a consequence of this body of research, an interest in selective targeting of persistent sodium current and corticomotoneuronal hyperexcitability in ALS has resulted in experiments suggesting a role for mexiletine as a putative disease modifying therapy. Mexiletine is a use-dependent sodium channel blocker that has been shown to have neuroprotective effects on neurons following experimental spinal cord [34] and head injury [35], diabetic oxidative damage [36], and cerebral ischemia paradigms [37-40] in part by acting as an antioxidant, inhibiting caspase 3 activation and preventing apoptosis [34], and by blocking glutamate and inward sodium current and decreasing reverse operation of the $Na^{+}$-$Ca^{++}$ exchanger limiting calcium influx [34]. Swiss albino rats pre-treated with mexiletine were shown in a study of induced head injury to demonstrate significant reduction of malondialdehyde, a marker for lipid peroxidation and therefore brain injury, compared to untreated animals [35]. Under different oxidant conditions *in vitro*, using thiobarbituric acid-reactive substances to measure lipid peroxidation, mexiletine has been found to inhibit iron-ascorbate-$H_2O_2$-induced lipid peroxidation in brain membranes prepared from brain homogenate in a manner that is dose and time-dependent [41]. *In vivo*, compared to untreated animals, mexiletine injected intraperitoneally significantly reduced the levels of malondialdehyde, nitrous oxide, reduced glutathione, and xanthine oxidase, all markers of oxidative stress, in the brain, brain stem, and cervical spinal cord of rats given streptozotocin-induced hyperglycemia, a model for diabetic oxidative damage in the central nervous system [36]. A number of experimental studies have also demonstrated that after spinal cord injury, as in ALS, apoptosis contributes to cellular damage, often in part reflected in increased activity of caspase 3. In one study, Wistar rats that underwent induced traumatic spinal cord injury, treated

immediately thereafter with mexiletine, had an accelerated recovery of hind limb function and an inhibition of caspase 3 up regulation as compared to untreated animals that was comparable to those rats treated with methylprednisolone [34].

Cerebral ischemia in particular shares some of the molecular changes that occur in the central nervous system of ALS patients. Increased levels of glutamate and opening of glutamate operated voltage dependent $Ca^{++}$ channels leading to raised intracellular levels of calcium are thought to promote neuronal cell death in as a consequence of ischemic injury. Mexiletine is believed to prevent neuronal death in cerebral ischemia by blocking glutamate and voltage dependent $Ca^{++}$ channels as well as promoting $Na^{+}$ channel and inward sodium current blockade, thereby inhibiting $Na^{+}$-$Ca^{++}$ exchanger-dependent $Ca^{++}$ overload and $Ca^{++}$-dependent structural injury [38]. In one study, mexiletine given prior to bilateral carotid occlusion and controlled hypotension in Sprague-Dawley rats demonstrated a 50% reduction of the number of ischemic hippocampal neurons compared to animals that received normal saline only [38]. In another study using a similar rat model for cerebral ischemia, the effects of riluzole and mexiletine were compared when given prior to carotid occlusion in the animals. Compared to untreated animals, both drugs had a similar significant effect on promoting the survival of hippocampal neurons [37]. Based on previous studies, mexiletine also appears to penetrate into the central nervous system at concentrations sufficient to confer significant protection [40]. Mexiletine has also recently been demonstrated to inhibit neuronal hyperexcitability and prevent cell death in a motor neuron cell line exposed to cultured media from astrocytes engineered to express the human $SOD1^{G93A}$ mutation [42]. Additionally, compared to untreated $SOD1^{G93A}$ mice (n=75), ALS mutant mice (n=75) treated with mexiletine (100 mg/kg) starting at postnatal day 60 demonstrated prolonged survival (133±10 days versus 128±10 days, Gehan-Breslow-Wilcoxon Test, p=0.0023) with no change in the age of onset (Dr. Robert Brown, University of Massachusetts, personal communication).

Recent studies of the effects of mexiletine on peripheral nerve excitability have shown that a dose of 300 mg reduces persistent sodium current *in vitro* in patients with muscle cramps, as indicated by decreased strength-duration time constant measurements by threshold tracking, in addition to reducing the number of cramps [43]. Mexiletine has also been found to reduce muscle cramps in a small open label study in Machado-Joseph disease, a hereditary spinocerebellar neurodegenerative disorder that also leads to degeneration of anterior horn cells [30]. Muscle cramps are common in ALS and without well-defined therapy [44]. They are thought to be a consequence of peripheral motor nerve axonal hyperexcitability [30]. Often they are more prevalent in the first year of symptoms of ALS and lessen over time, occurring in over 75% of patients [45]. There have been few treatment trials designed to address this often disabling symptom and none have been successful to date. These have included the use of tetrahydrocannabinol, gabapentin, leviteracetam, and vitamin E [44].Though riluzole, like mexiletine, is a use-dependent sodium channel blocker, it has not been shown to have any significant impact on muscle cramps [44].

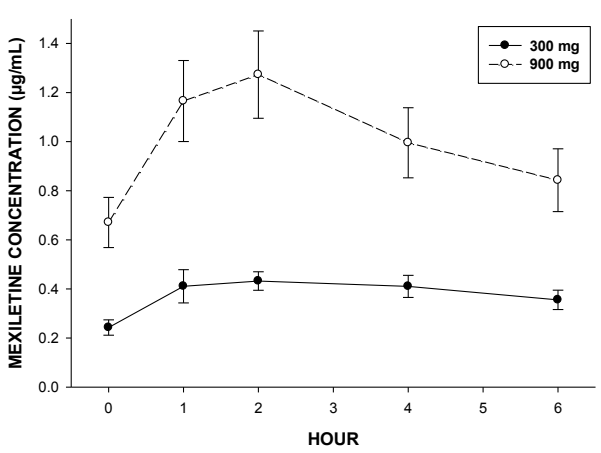
#### 2.1.3.2. Experience with Mexiletine in Neurologic Disease

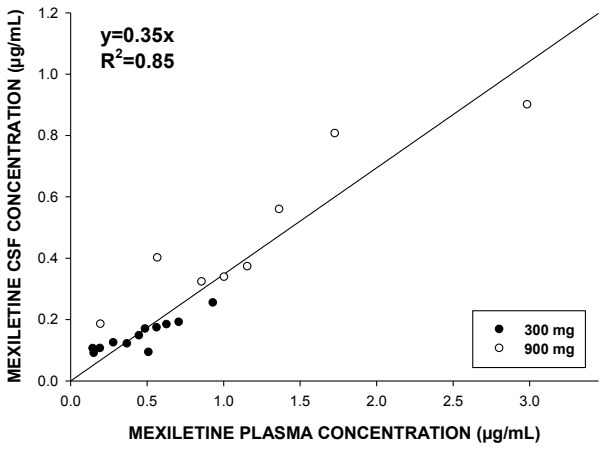
Mexiletine is already approved by the Food and Drug Administration for the treatment of cardiac arrhythmias and has been used in clinical trials for the treatment of clinical myotonia in myotonic dystrophy type 1 [46] and painful diabetic neuropathy [47].

In a recent randomized, double-blind, controlled crossover trial of either 150 mg or 200 mg of mexiletine given three times a day in patients with myotonic dystrophy type 1, only mild side effects were noted for either dose relative to placebo. These included primarily mild upper gastrointestinal distress and lightheadedness. One patient discontinued treatment of the drug at the higher dose due to diarrhea [46]. In a four-arm, randomized, double-blind controlled study of mexiletine for the treatment of diabetic neuropathic pain, 17 out of 95 patients randomized to 225 mg, 450 mg, or 675 mg of mexiletine a day demonstrated a mild increase in gastrointestinal side effects, primarily nausea, diarrhea, and gastric pain, evenly distributed among the groups, relative to placebo. No serious adverse events were noted [47].

Based on these studies and the putative role of cortical and peripheral motor nerve axonal hyperexcitability in the pathogenesis of ALS as well as muscle cramps and fasciculations, we recently completed a 12-week randomized placebo-controlled phase II multicenter study to determine the safety and tolerability of mexiletine on patients with SALS [48]. We decided upon two doses of the medication, 300 and 900 mg, suggested by the known safety profile of this drug for the treatment of cardiac arrhythmias. Effects of the medication on markers of disease progression and muscle cramp frequency and intensity were also determined. Sixty ALS patients from 10 NEALS centers were randomized 1:1:1 to placebo, mexiletine 300 mg, and mexiletine 900 mg per day and followed for 12 weeks. The primary endpoints were safety and tolerability. Secondary endpoints were change on the ALSFRS-R score and SVC and frequency and severity of muscle cramps.

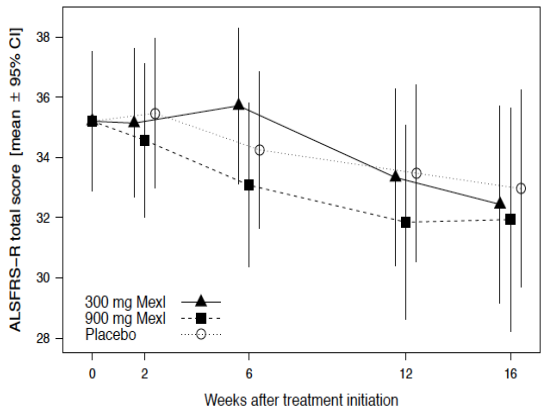
One instance of loss of balance was the only serious adverse event reported among active arm participants, a participant randomized to 900 mg/day. Incidence of nausea and tremor were higher in the mexiletine 900 mg group, with 32% intolerance at that dose vs. 5% at 300 mg, leading to discontinuation in some patients at the higher dose. No significant changes were noted on screening laboratory testing or electrocardiograms performed at each visit. Pharmacokinetic studies showed that at both doses of mexiletine, plasma concentrations reached their peak at 2 hours post treatment (**Fig 1A**). Mean serum concentrations at 2 hours post dose were 3-fold greater with 900 mg/day of mexiletine than with 300 mg/day (1.27 μg/mL versus 0.4 μg/mL) (**Fig 1B**). There was also a strong correlation between plasma and cerebrospinal fluid concentrations at both doses ($R^2$= 0.85 by linear regression analysis, p<0.001). Rates of decline of the ALSFRS-R (**Fig 2A**) did not differ from placebo (300 mg mexiletine vs. placebo = -0.23 / month [95% CI -1.03,0.56], p=0.56; 900 mg vs. placebo = -0.19 / month [1.04,0.66], p=0.66), but given the small size of the study and its short duration, confidence intervals were wide and neither excluded a clinically meaningful slowing of progression. SVC results (**Fig 2B**) were similar (300 mg mexiletine = 1.74 %-predicted/month [-0.72,4.20], p= 0.16; 900 mg = -0.50 %-predicted/month [-3.33,2.32], p=0.72). Analysis of all randomized patients demonstrated significant reductions of muscle cramp frequency (**Fig 3A**; 300 mg mexiletine: 31% of placebo, p=0.047; 900 mg mexiletine: 16% of placebo, p=0.002) and intensity (**Fig 3B**; 300 mg mexiletine: 45% of placebo, p=0.08; 900 mg mexiletine: 25% of placebo, p=0.005). Effects were greater among participants who reported at least 10 cramps over the 30 days prior to baseline.

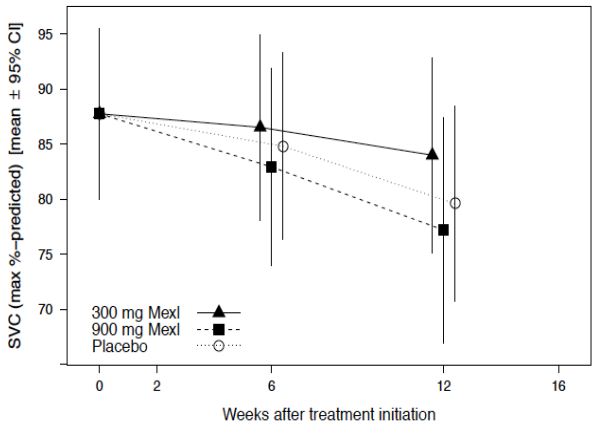



**A.** **B.**

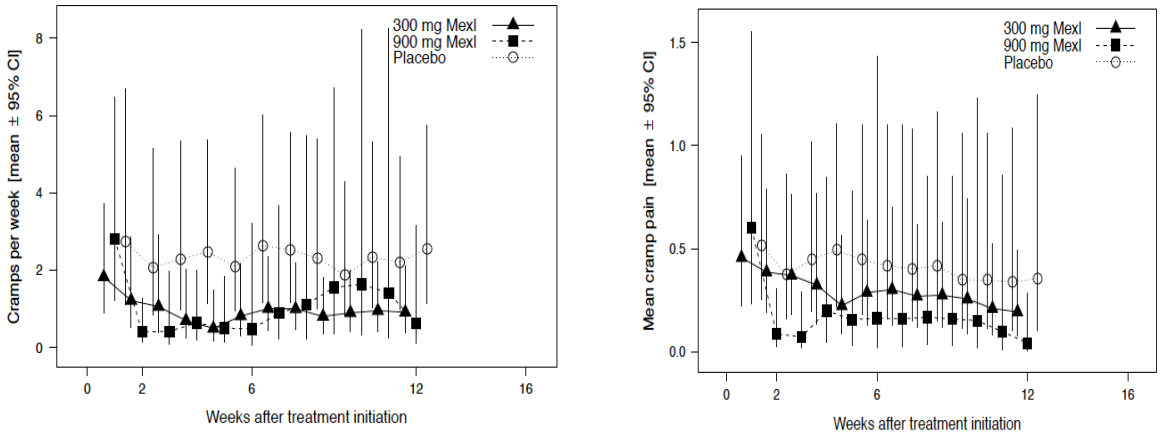
**Figure 1. A. Mean plasma (± SD) concentrations of mexiletine for ALS patients treated with 300 mg and 900 mg a day. B. Comparison of cerebrospinal fluid (CSF) and plasma concentrations.**




**A.** **B.**

**Figure 2. Effects of mexiletine (Mexl) at 300 mg and 900 mg a day versus placebo for changes in ALSFRS-R (Fig 2A) and SVC (Fig 2B).**




**A.** **B.**

**Figure 3. Effects of mexiletine (Mexl) on muscle cramp frequency and intensity (Fig 3A and B, respectively) at 300 mg and 900 mg a day versus placebo.**

Mexiletine was found to be safe at both 300 mg and 900 mg doses and well tolerated at the lower dose. Nausea, imbalance, and dizziness led to discontinuation of the higher dose. The 2-hour peak concentration of mexiletine in serum is similar to studies in non-ALS patients. Mexiletine treatment resulted in large dose-dependent reductions in muscle cramp frequency and severity. While the effects of mexiletine on muscle cramps was not the primary focus of this study, the dose dependent reduction on cramp frequency and intensity, especially on those patients with more frequent cramps at baseline, was dramatic, suggesting that mexiletine could become an important therapy for this symptom of the disease. An effect on rate of progression was not detected, but clinically important differences could not be excluded in this initial study.

The study was limited by the small number of patients and short duration of treatment and was likely insufficiently powered to reliably detect a response on slowing progression of the disease (7% power for a 30% reduction in mean rate of progression). Additionally, a pharmacodynamic marker of cortical hyperexcitability, such as the use of transcranial magnetic stimulation to determine any increase in the RMT, SICI, or other parameters of cortical motor neuronal excitation with treatment, was not performed. However, given that the 95% confidence bounds included a beneficial effect of mexiletine equal to a slowing of progression of the ALSFRS-R by about 0.6 units per month and the SVC by about 4.2% per month for 300 mg and 2.3% per month for 900 mg, clinically important differences could not be excluded.

### 2.1.4. Rationale for Choosing Mexiletine in ALS

Mexiletine has been shown also to be protective of neurons following spinal cord injury, head injury, and cerebral ischemia, largely by blocking neuronal hyperexcitability and excitotoxicity. As a use dependent sodium channel blocker like riluzole, it has a similar potential to reduce persistent inward sodium current and spinal and cortical motor neuron hyperexcitability, which have been implicated as early events in the pathogenesis of ALS. Based on previous studies,

mexiletine appears to penetrate into the central nervous system at concentrations sufficient to confer significant protection. Recent unpublished studies in the laboratory of Dr. Robert Brown at the University of Massachusetts have also demonstrated that mexiletine ingestion in mice genetically engineered to express high levels of mutant cytosolic copper-zinc superoxide dismutase-1 (SOD1) transgene prolong survival in these animals. As mexiletine already has FDA-approval as an anti-arrhythmic agent, much is known about the pharmacology and safety of this drug in non-ALS volunteers. The relatively benign safety profile of mexiletine in the cardiac population and the evidence that it may act as a neuroprotectant suggest that this medication could serve as a potential therapy for ALS. Further study of mexiletine in a randomized, double-blind, placebo-controlled trial of patients suffering from ALS is warranted.

### 2.1.5. Experimental Therapeutics in ALS

Riluzole, a drug that has multiple mechanisms of action including inhibition of glutamate release at pre-synaptic terminals, was reported in two controlled studies to extend survival by three months (about 11%) in subjects with ALS and is the only FDA-approved treatment for ALS. Trials of CNTF, gabapentin, BDNF, xaliproden, topiramate, celebrex, low dose creatine, lithium, and minocycline failed to demonstrate efficacy in ALS clinical trials. Ceftriaxone, arimoclomol, and dexpramipexole are examples of compounds recently tested in clinical trials.

### 2.1.6. Significance

Despite recent critical advances in understanding the pathogenesis of ALS, this remains an untreatable and uniformly lethal disease. The potential of mexiletine in ALS as a disease modifying therapy is unknown, though the previous mexiletine study suggests a beneficial effect on muscle cramps.

## 2.2. Mexiletine

Mexiletine belongs to the Class IB anti-arrhythmic group of medicines. It is used to treat arrhythmias within the heart, or seriously irregular heartbeats. It slows nerve impulses in the heart and makes the heart tissue less sensitive. The FDA approved mexiletine for cardiac arrhythmias in 1985.

### 2.2.1. Pharmacokinetics

#### 2.2.1.1. Absorption, Distribution, Elimination and Metabolism

Mexiletine is well absorbed (~90%) from the gastrointestinal tract. Unlike lidocaine, its first-pass metabolism is low. Peak blood levels are reached in two to three hours. In normal subjects, the plasma elimination half-life of mexiletine is approximately 10 to 12 hours. It is 50 to 60% bound to plasma protein, with a volume of distribution of 5 to 7 liters/kg. Mexiletine is mainly metabolized in the liver, the primary pathway being CYP2D6 metabolism, although it is also a substrate for CYP1A2. With involvement of CYP2D6, there can be either poor or extensive metabolizer phenotypes. Since approximately 90% of mexiletine hydrochloride is metabolized in the liver into inactive metabolites, pathological changes in the liver can restrict hepatic clearance of mexiletine hydrochloride and its metabolites. The metabolic degradation proceeds via various pathways including aromatic and aliphatic hydroxylation, dealkylation, deamination and N-oxidation. Several of the resulting metabolites are submitted to further conjugation with glucuronic acid (phase II metabolism); among these are the major metabolites p-hydroxymexiletine, hydroxy-methylmexiletine and N-hydroxy-mexiletine. Approximately 10%

is excreted unchanged by the kidney. While urinary pH does not normally have much influence on elimination, marked changes in urinary pH influence the rate of excretion: acidification accelerates excretion, while alkalinization retards it.

Several metabolites of mexiletine have shown minimal antiarrhythmics activity in animal models. The most active is the minor metabolite N-methylmexiletine, which is less than 20% as potent as mexiletine. The urinary excretion of N-methylmexiletine in man is less than 0.5%. Thus the therapeutic activity of mexiletine is due to the parent compound.

Hepatic impairment prolongs the elimination half-life of mexiletine. In eight patients with moderate to severe liver disease, the mean half-life was approximately 25 hours.

Consistent with the limited renal elimination of mexiletine, little change in the half-life has been detected in patients with reduced renal function. In eight patients with creatinine clearance less than 10 mL/min, the mean plasma elimination half-life was 15.7 hours; in seven patients with creatinine clearance between 11 to 40 mL/min, the mean half-life was 13.4 hours.

The absorption rate of mexiletine is reduced in clinical situations such as acute myocardial infarction in which gastric emptying time is increased. Narcotics, atropine and magnesium-aluminum hydroxide have also been reported to slow the absorption of mexiletine. Metoclopramide has been reported to accelerate absorption.

Mexiletine plasma levels of at least 0.5 mcg/mL are generally required for therapeutic response. An increase in the frequency of central nervous system adverse effects has been observed when plasma levels exceed 2.0 mcg/mL. Thus the therapeutic range is approximately 0.5 to 2.0 mcg/mL. Plasma levels within the therapeutic range can be attained with either three times daily or twice daily dosing but peak to trough differences are greater with the latter regimen, creating the possibility of adverse effects at peak and arrhythmic escape at trough. Nevertheless, some patients may be transferred successfully to the twice daily regimen.

In the recently completed safety and tolerability study of mexiletine in ALS, the peak concentration in plasma at 2 hours post dose is similar to what has been shown previously in non-ALS patients.

### 2.2.2. Drug Interactions

Since mexiletine hydrochloride is a substrate for the metabolic pathways involving CYP2D6 and CYP1A2 enzymes, inhibition or induction of either of these enzymes would be expected to alter mexiletine plasma concentrations. In a formal, single-dose interaction study (n = 6 males) the clearance of mexiletine was decreased by 38% following the coadministration of fluvoxamine, an inhibitor of CYP1A2. In another formal study (n = 8 extensive and n = 7 poor metabolizers of CYP2D6), coadministration of propafenone did not alter the kinetics of mexiletine in the poor CYP2D6 metabolizer group. However, the metabolic clearance of mexiletine in the extensive metabolizer phenotype decreased by about 70% making the poor and extensive metabolizer groups indistinguishable. In this crossover steady-state study, the pharmacokinetics of propafenone were unaffected in either phenotype by the coadministration of mexiletine. Addition of mexiletine to propafenone did not lead to further electrocardiographic parameters changes of QRS, QTc, RR, and PR intervals than propafenone alone. When concomitant administration of

either of these two drugs is initiated, the dose of mexiletine should be slowly titrated to desired effect.

In a large compassionate-use program, mexiletine has been used concurrently with commonly employed antianginal, antihypertensive, and anticoagulant drugs without observed interactions. A variety of antiarrhythmics such as quinidine or propranolol were also added, sometimes with improved control of ventricular ectopy. When phenytoin or other hepatic enzyme inducers such as rifampin and phenobarbital have been taken concurrently with mexiletine, lowered mexiletine plasma levels have been reported. Monitoring of mexiletine plasma levels is recommended during such concurrent use to avoid ineffective therapy.

In a formal study, benzodiazepines were shown not to affect mexiletine plasma concentrations. ECG intervals (PR, QRS, and QT) were not affected by concurrent mexiletine and digoxin, diuretics, or propranolol.

Concurrent administration of cimetidine and mexiletine has been reported to increase, decrease, or leave unchanged mexiletine plasma levels; therefore patients should be followed carefully during concurrent therapy.

Mexiletine does not alter serum digoxin levels but magnesium-aluminum hydroxide, when used to treat gastrointestinal symptoms due to mexiletine, has been reported to lower serum digoxin levels.

Concurrent use of mexiletine and theophylline may lead to increased plasma theophylline levels. One controlled study in eight normal subjects showed a 72% mean increase (range 35 to 136%) in plasma theophylline levels. This increase was observed at the first test point which was the second day after starting mexiletine. Theophylline plasma levels returned to pre-mexiletine values within 48 hours after discontinuing mexiletine. If mexiletine and theophylline are to be used concurrently, theophylline blood levels should be monitored, particularly when the mexiletine dose is changed. An appropriate adjustment in theophylline dose should be considered.

Additionally, in one controlled study in five normal subjects and seven patients, the clearance of caffeine was decreased 50% following the administration of mexiletine.

### 2.2.3. Mexiletine Adverse Effects

Mexiletine hydrochloride commonly produces reversible gastrointestinal and nervous system adverse reactions but is otherwise well tolerated. Mexiletine has been evaluated in 483 patients in one-month and three-month controlled studies and in over 10,000 patients in a large compassionate use program. Dosages in the controlled studies ranged from 600 to 1200 mg/day; some patients (8%) in the compassionate use program were treated with higher daily doses (1600 to 3200 mg/day). In the three-month controlled trials comparing mexiletine to quinidine, procainamide and disopyramide, the most frequent adverse reactions were upper gastrointestinal distress (41%), lightheadedness (10.5%), tremor (12.6%) and coordination difficulties (10.2%). Similar frequency and incidence were observed in the one-month placebo-controlled trial. Although these reactions were generally not serious, and were dose-related and reversible with a reduction in dosage, by taking the drug with food or antacid or by therapy discontinuation, they

led to therapy discontinuation in 40% of patients in the controlled trials. Table 2 presents the adverse events reported in the one-month placebo-controlled trial.

**Table 2. Comparative Incidence (%) of Adverse Events among Patients Treated with Mexiletine and Placebo in the 4-Week, Double-blind Crossover Trial**

| | Mexiletine N = 53 | Placebo N = 49 |
|---|---|---|
| **Cardiovascular** | | |
| Palpitations | 7.5 | 10.2 |
| Chest Pain | 7.5 | 4.1 |
| Increased Ventricular Arrhythmia / PVCs | 1.9 | - |
| **Digestive** | | |
| Nausea / Vomiting / Heartburn | 39.6 | 6.1 |
| **Central Nervous System** | | |
| Dizziness / Lightheadedness | 26.4 | 14.3 |
| Tremor | 13.2 | - |
| Nervousness | 11.3 | 6.1 |
| Coordination Difficulties | 9.4 | - |
| Changes in Sleep Habits | 7.5 | 16.3 |
| Paresthesias / Numbness | 3.8 | 2.0 |
| Weakness | 1.9 | 4.1 |
| Fatigue | 1.9 | 2.0 |
| Tinnitus | 1.9 | 4.1 |
| Confusion / Clouded Sensorium | 1.9 | 2.0 |
| **Other** | | |
| Headache | 7.5 | 6.1 |
| Blurred Vision / Visual Disturbances | 7.5 | 2.0 |
| Dyspnea / Respiratory | 5.7 | 10.2 |
| Rash | 3.8 | 2.0 |
| Non-specific Edema | 3.8 | - |

Table 3 presents the adverse reactions occurring in one percent or more of patients in the three-month controlled studies.

**Table 3. Comparative Incidence (%) of Adverse Events among Patients Treated with Mexiletine or Control Drugs in the 12-Week Double-blind Trials**

| | Mexiletine N = 430 | Quinidine N = 262 | Procainamide N = 78 | Disopyramide N = 69 |
|---|---|---|---|---|
| **Cardiovascular** | | | | |
| Palpitations | 4.3 | 4.6 | 1.3 | 5.8 |
| Chest Pain | 2.6 | 3.4 | 1.3 | 2.9 |
| Angina / Angina-like Pain | 1.7 | 1.9 | 2.6 | 2.9 |

| | Mexiletine N = 430 | Quinidine N = 262 | Procainamide N = 78 | Disopyramide N = 69 |
|---|---|---|---|---|
| Increased Ventricular Arrhythmias / PVCs | 1.0 | 2.7 | 2.6 | - |
| **Digestive** | | | | |
| Nausea / Vomiting / Heartburn | 39.3 | 21.4 | 33.3 | 14.5 |
| Diarrhea | 5.2 | 33.2 | 2.6 | 8.7 |
| Constipation | 4.0 | - | 6.4 | 11.6 |
| Changes in Appetite | 2.6 | 1.9 | - | - |
| Abdominal Pain / Cramps / Discomfort | 1.2 | 1.5 | - | 1.4 |
| **Central Nervous System** | | | | |
| Dizziness / Lightheadedness | 18.9 | 14.1 | 14.1 | 2.9 |
| Tremor | 13.2 | 2.3 | 3.8 | 1.4 |
| Coordination Difficulties | 9.7 | 1.1 | 1.3 | - |
| Changes in Sleep Habits | 7.1 | 2.7 | 11.5 | 8.7 |
| Weakness | 5.0 | 5.3 | 7.7 | 2.9 |
| Nervousness | 5.0 | 1.9 | 6.4 | 5.8 |
| Fatigue | 3.8 | 5.7 | 5.1 | 1.4 |
| Speech Difficulties | 2.6 | 0.4 | - | - |
| Confusion / Clouded Sensorium | 2.6 | - | 3.8 | - |
| Paresthesias / Numbness | 2.4 | 2.3 | 2.6 | - |
| Tinnitus | 2.4 | 1.5 | - | - |
| Depression | 2.4 | 1.1 | 1.3 | 1.4 |
| **Other** | | | | |
| Blurred Vision / Visual Disturbances | 5.7 | 3.1 | 5.1 | 7.2 |
| Headache | 5.7 | 6.9 | 7.7 | 4.3 |
| Rash | 4.2 | 3.8 | 10.3 | 1.4 |
| Dyspnea / Respiratory | 3.3 | 3.1 | 5.1 | 2.9 |
| Dry Mouth | 2.8 | 1.9 | 5.1 | 14.5 |
| Arthralgia | 1.7 | 2.3 | 5.1 | 1.4 |
| Fever | 1.2 | 3.1 | 2.6 | - |

Less than 1%: Syncope, edema, hot flashes, hypertension, short-term memory loss, loss of consciousness, other psychological changes, diaphoresis, urinary hesitancy/retention, malaise, impotence/decreased libido, pharyngitis, congestive heart failure.

An additional group of over 10,000 patients has been treated in a program allowing administration of mexiletine hydrochloride under compassionate use circumstances. These patients were seriously ill with the large majority on multiple drug therapy. Twenty-four percent of the patients continued in the program for one year or longer. Adverse reactions leading to therapy discontinuation occurred in 15 percent of patients (usually upper gastrointestinal system

or nervous system effects). In general, the more common adverse reactions were similar to those in the controlled trials. Less common adverse events possibly related to mexiletine use include:

*Cardiovascular System:* Syncope and hypotension, each about 6 in 1000; bradycardia, about 4 in 1000; angina/angina-like pain, about 3 in 1000; edema, atrioventricular block/conduction disturbances and hot flashes, each about 2 in 1000; atrial arrhythmias, hypertension and cardiogenic shock, each about 1 in 1000.

*Central Nervous System:* Short-term memory loss, about 9 in 1000 patients; hallucinations and other psychological changes, each about 3 in 1000; psychosis and convulsions/seizures, each about 2 in 1000; loss of consciousness, about 6 in 10,000.

*Digestive:* Dysphagia, about 2 in 1000; peptic ulcer, about 8 in 10,000; upper gastrointestinal bleeding, about 7 in 10,000; esophageal ulceration, about 1 in 10,000. Rare cases of severe hepatitis/acute hepatic necrosis with mexiletine treatment have been reported.

*Skin:* Rare cases of exfoliative dermatitis and Stevens-Johnson syndrome with mexiletine treatment have been reported.

*Laboratory:* Abnormal liver function tests, about 5 in 1000; positive antinuclear antibody (ANA) and thrombocytopenia, each about 2 in 1000; leukopenia (including neutropenia and agranulocytosis), about 1 in 1000; myelofibrosis, about 2 in 10,000 patients.

*Other:* Diaphoresis, about 6 in 1000; altered taste, about 5 in 1000; salivary changes, hair loss and impotence/decreased libido, each about 4 in 1000; malaise, about 3 in 1000; urinary hesitancy/retention, each about 2 in 1000; hiccups, dry skin, laryngeal and pharyngeal changes and changes in oral mucous membranes, each about 1 in 1000; Systemic lupus erythematosus (SLE) syndrome, about 4 in 10,000.

*Hematology*: Blood dyscrasias were not seen in the controlled trials but did occur among 10,867 patients treated with mexiletine in the compassionate use program.

Myelofibrosis was reported in two patients in the compassionate use program; one was receiving long-term thiotepa therapy and the other had pre-treatment myeloid abnormalities.

In postmarketing experience, there have been isolated, spontaneous reports of pulmonary changes including pulmonary infiltration and pulmonary fibrosis during mexiletine therapy with or without other drugs or diseases that are known to produce pulmonary toxicity. A causal relationship to mexiletine therapy has not been established. In addition, there have been isolated reports of drowsiness, nystagmus, ataxia, dyspepsia, hypersensitivity reaction, and exacerbation of congestive heart failure in patients with pre-existing compromised ventricular function. There have been rare reports of pancreatitis associated with mexiletine treatment.

In the recently completed safety and tolerability study in ALS, only nausea and tremor were more commonly seen in treated patients compared to placebo and only in the patients treated with 900 mg /day.

**Table 4. Adverse Events in the Safety and Tolerability Study of Mexiletine in ALS by MedDRA preferred term. [48]**

| | Treatment Groups | | | | | |
|---|---|---|---|---|---|---|
| | Mx300mg n=20 | Mx900mg n=19 | Placebo n=20 | *p* value Mx300mg vs placebo | *p* value Mx900mg vs placebo | *p* value Mx900mg vs Mx300mg |
| Abdominal discomfort | 5% (1) | 11% (2) | 0% (0) | >0.99 | 0.23 | 0.60 |
| Anxiety | 5% (1) | 16% (3) | 0% (0) | >0.99 | 0.11 | 0.34 |
| Asthenia | 5% (1) | 16% (3) | 10% (2) | >0.99 | 0.66 | 0.34 |
| Constipation | 5% (1) | 21% (4) | 10% (2) | >0.99 | 0.41 | 0.18 |
| Depression | 0% (0) | 11% (2) | 10% (2) | 0.49 | >0.99 | 0.23 |
| Dizziness | 15% (3) | 32% (6) | 20% (4) | >0.99 | 0.48 | 0.27 |
| Dysarthria | 0% (0) | 11% (2) | 0% (0) | >0.99 | 0.23 | 0.23 |
| Dysgeusia | 0% (0) | 11% (2) | 0% (0) | >0.99 | 0.23 | 0.23 |
| Affect lability | 0% (0) | 16% (3) | 0% (0) | >0.99 | 0.11 | 0.11 |
| Pain in extremity | 0% (0) | 16% (3) | 0% (0) | >0.99 | 0.11 | 0.11 |
| Fall | 10% (2) | 26% (5) | 20% (4) | 0.66 | 0.72 | 0.24 |
| Fatigue | 10% (2) | 21% (4) | 15% (3) | >0.99 | 0.69 | 0.41 |
| Headache | 10% (2) | 21% (4) | 10% (2) | >0.99 | 0.41 | 0.41 |
| Balance disorder | 0% (0) | 11% (2) | 5% (1) | >0.99 | 0.60 | 0.23 |
| Muscle spasms | 5% (1) | 16% (3) | 10% (2) | >0.99 | 0.66 | 0.34 |
| Muscular weakness | 15% (3) | 16% (3) | 15% (3) | >0.99 | >0.99 | >0.99 |
| Nausea | 5% (1) | 42% (8) | 10% (2) | >0.99 | **0.031** | **0.008** |
| Tremor | 0% (0) | 26% (5) | 5% (1) | >0.99 | 0.091 | **0.020** |

Values are % (N). Significant comparisons ($p<0.05$) are in bold. Mx300mg = 300 mg of mexileine daily. Mx900mg = 900 mg of mexiletine daily.

### 2.2.4. Selection of Dosage in the Study

Studies of mexiletine designed to determine the efficacy of the drug in treating neuropathic pain and myotonia have largely demonstrated benefit with minimal side effects at doses between 300-600 mg a day. However, mexiletine dosing is generally higher for the prevention of cardiac arrhythmias, typically 900 mg to 1200 mg a day, with about 20% of patients developing side effects. Given that the optimal dose of mexiletine is not known for neuroprotection in ALS, though 900 mg/day was not shown to be tolerable in the previous study, the doses for this current mexiletine study, 300 mg and 600 mg, were chosen to be within the spectrum of dosing for these neurologic and non-neurologic disorders with an acceptable side effect profile at the higher dose.

## 2.3 Other Potential Risks and Benefits

Overall, it is believed that the risks of the study are small compared to the devastating impact of the clinical disease and that the potential for improved clinical understanding, treatment and future benefit outweigh the risk to the study subjects of this FDA-approved medication and the associated other risks described here.

### 2.3.1 Potential Risks

Potential risks include those associated with mexiletine treatment, blood draws, neurophysiological studies, risk due to compromise of medical information and psychological risk.

*Blood Draw:* A blood draw may cause pain, bruising and/or bleeding at the needle site. Occasionally, a person feels faint when blood is drawn. Rarely, an infection may develop, which can be treated.

*Slow Vital Capacity (SVC) Testing:* The risks and discomforts associated with the Slow Vital Capacity testing include feeling tired, light-headed or short of breath. These symptoms will disappear with rest.

*Questionnaires:* The ALSFRS-R Questionnaire and the RAND-36 may cause participants to feel sad or upset about how ALS has changed how well they can perform daily activities, and how it has affected their quality of life. Participants may get tired or bored when they are asked questions or completing questionnaires. They do not have to answer any question they do not want to answer.

*Abductor pollicis brevis (APB) Testing via Hand Held Dynamometry (HHD):* The only known risks and side effects associated with the muscle strength testing include fatigue (tiredness) or muscle cramping. These will get better with rest. There may be other risks or side effects that are not known at this time.

*TMS/TTNCS:* There are small risks associated with neurophysiological tests. TMS is associated with risk of headache (common), neck pain (uncommon), tinnitus and transient decreased hearing (made minimal by wearing ear plugs), seizure (rare), skin irritation (common), changes in memory (theoretical), attention (theoretical) or other cognitive function (theoretical) or other

unknown risk. The threshold tracking NCS (TTNCS) tests can be associated with mild to moderate discomfort and transient skin irritation due to either electrodes or stimulation.

*Other Risks:* Reviewing health related information might be stressful or make participants feel uncomfortable. Participants do not have to answer any questions they do not want to, and may stop the interview at any time if it is too uncomfortable.

Despite the Genetic Information Nondiscrimination Act (GINA) protections and the best efforts of the research team, there may still be a risk if information about participants were to become known to people outside of this study.

Genetic information is unique to the participant, even without their name or other identifiers. For this reason, genetic information like DNA may be used to identify participants and possibly their family members. The main risk of allowing us to store and use participant samples and certain limited health information for research is a potential loss of privacy. We will protect participant privacy by labeling participant samples and information only with a code, and keeping the key to the code in a password protected database. However, there is the risk this can happen as new ways of tracing genetic information are being developed that may make re-identification of genetic information possible.

Genetic information that results from this study does not have medical or treatment importance at this time. However, there is a risk that information about taking part in a genetic study may influence insurance companies and/or employers regarding participant health, or have a negative impact on family or other relationships. To further safeguard privacy, genetic information obtained in this study will not be placed in the medical record. Samples will be coded.

There may be other side effects and discomforts that are not yet known.

### 2.3.2. Potential Benefits

There are no specific anticipated potential benefits to subjects for participating in this study, although subjects may benefit from the close follow-up and detailed medical care associated with study participation. It is not known whether treatment with mexiletine will be helpful for ALS subjects. It is possible that results from the study may yield benefits to future ALS patients.

# 3.0. STUDY OBJECTIVES

## 3.1. Primary

The primary study objective is to determine whether treatment with mexiletine at doses of 300 mg or 600 mg/day suppresses cortical hyperexcitability in sporadic ALS patients relative to placebo, and, thus, may be able to slow progression in ALS. The change in RMT, estimated from single pulse TMS measurements made before treatment, after 4 weeks of treatment, and then again after a 4 week washout, will be used as the primary pharmacodynamic marker of cortical hyperexcitability.

## 3.2. Secondary

Secondary pharmacodynamics outcome measures will include effects on motor evoked potential (MEP) amplitude (as a % of CMAP) and cortical silent period (CSP) measured by single pulse TMS, and SICI measured by dual pulse TMS, as additional pharmacodynamic markers of cortical hyperexcitability, prior to and following treatment with mexiletine or placebo. Additionally, as a pharmacodynamic marker of peripheral motor nerve axonal hyperexcitability, threshold tracking nerve conduction studies (TTNCS) will be performed to assess strength duration time constant and recovery cycle analysis. Safety and tolerability and determination of mexiletine concentrations in blood at the time of each of the four TMS studies will also be assessed. Additionally, the effect on frequency and severity of muscle cramps and fasciculations will be assessed using a daily muscle cramps/fasciculations diary from the Baseline Visit through the Week 8 Visit and using an assessment form at the Baseline Visit. ALS Functional Rating Scale-Revised (ALSFRS-R) and slow vital capacity (SVC) will be obtained at Screening, Pre-Dose Baseline, Week 4, and Week 8 or the Final Safety Visit, if applicable as exploratory outcomes.

# 4.0. STUDY DESIGN

## 4.1. Overall Study Design and Plan

During the enrollment period, approximately 90 sporadic ALS (sALS) patients will be screened at approximately 9 Northeast ALS Consortium (NEALS) centers in the US. Approximately 60 eligible sALS subjects will be randomly assigned in a 1:1:1 ratio to oral mexiletine 300 mg/day, 600 mg/day or matching placebo. After randomization, subjects will undergo the Baseline Visit and take their first dose of study drug in clinic at that visit. All visit windows are consecutive calendar days and are calculated from the day the participant has their Baseline Visit (Day 1).

In the phase II study, within three weeks following screening, RMT will be assessed by single pulse TMS prior to initiating study drug (baseline), four weeks after initiating placebo or mexiletine at either 300 mg or 600 mg per day, and then again after a four week washout period. Therefore, there will be a total of four visits (screening, baseline, week 4, and week 8). To ensure tolerance, the medication dose will be escalated over the course of one week. The RMT will serve as a pharmacodynamic marker of the effects on cortical excitability in ALS using this medication. The goal of these studies is to determine if targeting this putative mechanism of motor neuron injury in ALS reduces this excitability (significantly increases the RMT), which may serve as a pathogenic mechanism for the disease, potentially slowing progression in ALS.

Secondary outcome measures will include effects on MEP amplitude % and CSP measured by single pulse TMS, and SICI by dual pulse TMS, as additional pharmacodynamics markers of cortical hyperexcitability and TTNCS as a pharmacodynamic marker of peripheral motor axonal hyperexcitability (and therefore a potential marker for the therapeutic effect of mexiletine on muscle cramps and fasciculations), prior to and following treatment with mexiletine or placebo and then again after withdrawal of treatment. Safety and tolerability and determination of mexiletine concentrations in blood at the time of each of the two post-baseline TMS studies will also be assessed. Additionally, the effects on muscle cramps and fasciculations will be determined utilizing an Assessment Form at Baseline, and by employing a daily muscle cramping and fasciculations diary describing both frequency and severity of these symptoms from the Baseline Visit through the Week 8 Visit. Finally, ALSFRS-R, APB testing via HHD, and SVC will be obtained at Screening, Pre-Dose Baseline, Week 4, and Week 8 or the Final Safety Visit, if applicable.

## 4.2 Informed Consent

At the screening visit, prior to initiation of any study-related procedures, research participants will give their written consent to participate in the study after having been informed about the nature and purpose of the study, participation/termination conditions, and risks and benefits.

The appropriate ICF will be presented to the research participant and reviewed by the Site Investigator or study staff designee. The original signed and dated consent form will be maintained in the participant's research file. One copy of the signed informed consent form will be given to the research participant and one will remain in the research participant's medical/ research record per site standards. The Site Investigator will determine study eligibility as determined by the inclusion and exclusion criteria.

Informed consent can only be obtained by the Site Investigator for the purposes of this research study.

### 4.3. Study Centers

This study will be conducted at approximately 9 NEALS centers in the US selected because of their excellent record of recruitment, compliance with study protocols and regulations, clinical research expertise and resources.

### 4.4. Study Duration

There will be an enrollment period of approximately 40 weeks (10 months) during which time subjects will be screened and randomized. Subjects will receive study medication for a total of 4 weeks (1 month).

### 4.5. Protocol Adherence

Each Site Investigator must adhere to the protocol detailed in this document and agree that any changes to the protocol must be approved by the Coordination Center or their representative prior to seeking approval from the site IRB. Each Site Investigator will be responsible for enrolling only those study subjects who have met protocol eligibility criteria.

# 5.0. STUDY ENROLLEMENT AND WITHDRAWAL

## 5.1. Number of Study Subjects

Approximately 60 subjects will be enrolled and equally randomized 1:1:1 to receive 300 mg/day oral mexiletine or 600 mg/day oral mexiletine or placebo.

## 5.2. Inclusion and Exclusion Criteria

### 5.2.1. Inclusion Criteria

Volunteers will be eligible if they meet the following criteria:

1. Sporadic ALS diagnosed as possible, laboratory-supported probable, probable, or definite ALS as defined by revised El Escorial criteria.
2. Age 18 years or older.
3. Symptom onset of weakness or spasticity due to ALS ≤ 60 months prior to Screening Visit.
4. Slow vital capacity (SVC) measure ≥50% of predicted for gender, height, and age at the screening visit.
5. Must be able to swallow capsules throughout the course of the study, according to Site Investigator judgment.
6. Capable of providing informed consent and following trial procedures.
7. For TMS: a resting motor threshold, defined as 50% of pulses eliciting a motor evoked potential (MEP) of amplitude ≥ 50 μV.
8. For TTNCS: Median CMAP ≥ 1.5 mV.
9. Subjects must not have taken riluzole for at least 30 days or be on a stable dose of riluzole for at least 30 days prior to the Screening Visit and continue on the stable dose throughout the course of the study (riluzole-naïve subjects are permitted in the study).
10. Subjects must not have taken medication for muscle cramping such as cyclobenzaprine, baclofen, carisoprodol, or methacarbamol, for at least 30 days prior to screening or be on a stable dose for at least 60 days prior to screening.
11. Geographic accessibility to the site.
12. Women must not become pregnant for the duration of the study and must be willing to use two contraceptive therapies and have a negative pregnancy test throughout the course of the study.
13. Use of medications known to affect the neurophysiology measures in the study must be scheduled, not as needed (pro re nata, PRN). A subject must have been on a fixed dose for 30 days prior to the Screening Visit, and there must be no reason to believe that a subsequent change would be necessary during the course of the study. These medications include: benzodiazepines, muscle relaxants, tricyclic antidepressants, selective serotonin reuptake inhibitors, non-selective serotonin reuptake inhibitors, hypnotics (including anti-histamines) and anti-cholinergics.

### 5.2.2. Exclusion Criteria

Volunteers who meet any of the following criteria are not eligible:

1. Invasive ventilator dependence, such as tracheostomy.
2. Creatinine level greater than 1.5 mg/dL at screening.

3. SGOT (AST) / SGPT (ALT) greater than 3 times the upper limit of normal at screening.
4. History of known sensitivity or intolerability to mexiletine or lidocaine.
5. Any history of either substance abuse within the past year, unstable psychiatric disease, cognitive impairment, or dementia.
6. Clinically significant conduction abnormalities on electrocardiogram or a known history of cardiac arrhythmia.
7. Known history of epilepsy.
8. Known history of congestive heart failure (CHF) or history of myocardial infarction within the past 24 months.
9. Use of mexiletine for 30 days prior to Screening Visit.
10. Exposure to any other experimental agent (off-label use or investigational) including high dose creatine (>10 grams a day) within 30 days prior to Screening Visit.
11. Metal in the head and neck region, cardiac pacemaker or brain stimulator, cochlear implants, implanted infusion device or personal history of epilepsy.
12. Use of amiodarone, flecainide, duloxetine, tizanidine, or clozapine.
13. Pregnant women or women currently breastfeeding.
14. Placement of Diaphragm Pacing System (DPS) device < 60 days prior to Screening Visit.
15. Planned DPS device implantation during study participation.

***Riluzole.*** Subjects taking concomitant riluzole at study entry must be on a stable dose for 30 days prior to the Screening Visit and must continue taking the same dosage throughout the study, unless the Site Investigator determines that riluzole should be discontinued for medical reasons.

***Date of ALS Symptom Onset.*** For the purposes of this study, the date of symptom onset will be defined as the date the subject first had symptoms of their disease, i.e., weakness. To be eligible for this study, the date of symptom onset must be no greater than exactly 60 months prior to the Screening Visit date.

### 5.3. Re-screening

Participants who are determine ineligible may be re-screened a single time if their ineligibility was due to one of the following time-limited or modifiable criteria: inclusion criterion 2, 8, 9, 10, 11, or 13 or exclusion criterion 9, 10, 12, 13, 14, or 15. Re-screened participants will be issued a new screening identifier that is linked to their original screening identifier.

### 5.4 Randomization Procedures

A permuted-block randomization schedule, stratified by site and use of riluzole at screening, will be developed by the study statistician with assignments in a 1:1:1 ratio among 300 mg/day oral mexiletine, 600 mg/day oral mexiletine, and placebo. If a randomized subject withdraws from the study prior to initiating study drug, their assignment will be released and re-used.

### 5.5. Reasons for Withdrawal

A subject will be withdrawn from the study if:

- Any clinical AE, laboratory abnormality, concurrent illness, or other medical condition or situation occurs such that continued participation in the study would not be in the best

interest of the subject. Note, however, that any subject who has initiated treatment should only discontinue study drug and remain on study if the safety concern relates to study drug and not to study procedures.

- The subject no longer meets eligibility criteria at any time prior to initiating study drug or is discovered at anytime to have been enrolled in error.

Subjects are free to withdraw from participation in the study at any time upon request.

## 5.6. Handling of Withdrawals

The medical monitor should be notified in all instances a subject may choose to discontinue their participation, start a prohibited medication, or wishes to withdraw consent.

A subject may choose to discontinue participation in the study at any time. However, the SI or designee will encourage subjects to continue with follow-up, regardless of their compliance with study drug. If a subject who initiated study drug permanently discontinues study drug, the SI or designee should still encourage subjects to follow the study protocol under the modified intent-to-treat principle (ITT). Subjects unable or unwilling to return for clinic visits should be encouraged to receive follow-up telephone calls per the study visit schedule up to Week 8. These calls will include administration of the ALSFRS-R and review of adverse events and concomitant medications. Loss to follow-up should be prevented whenever possible

Any subject who is on study drug and needs to begin the use of any prohibited medication, must immediately discontinue use of study drug and should not begin use of the prohibited medication before an appropriate wash-out period occurs. Subjects who permanently discontinue study drug should return any unused study drug and complete early study drug discontinuation procedures without any study drug unblinding, if possible. Subjects who must permanently discontinue study drug may continue in the ITT portion of the study, per protocol.

If a subject wishes to withdraw consent, i.e., withdraw his or her participation in future study procedures, the subject will be asked to delay consent withdrawal to allow for a final safety visit and final safety telephone call. The subject will be asked to return to the study site for a final safety visit within 14 days of asking to withdraw consent. The subject will also be asked to have a final telephone call no sooner than 28 days after taking their last dose of study drug to monitor their safety and to permit review of their medical records at the end of the study to document their vital status.

Subjects who discontinue study drug due to an AE will be followed for outcome measures under the ITT protocol as noted above.

## 5.7. Termination of Study

This study may be prematurely terminated if, in the opinion of the PI or sponsor, there is sufficient reasonable cause. Written notification, documenting the reason for study termination, will be provided to the PI or sponsor by the terminating party.

Circumstances that may warrant termination include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to subjects.

- Enrollment is unsatisfactory.
- Insufficient adherence to protocol requirements.
- Data that is not sufficiently complete and/or evaluable.
- Plans to modify, suspend or discontinue the development of the study drug.

If the study is prematurely terminated or suspended, the sponsor will promptly inform the investigators/institutions, of the termination or suspension and the reason(s) for the termination or suspension. The site IRBs will also be informed promptly and provided the reason(s) for the termination or suspension by the sponsor or by the investigator/institution. Study subjects will be called to explain the decision to terminate the trial and asked to return study drug at a final safety visit scheduled to occur within 14 days after last known dose of study drug. If the study was prematurely terminated due to safety concerns, they will be provided with relevant safety information to share with their primary care provider.

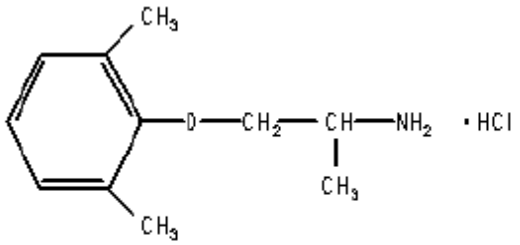
# 6.0. TREATMENT OF SUBJECTS

## 6.1. Treatments

### 6.1.1. Mexitil

Mexitil (Mexiletine hydrochloride [mexiletine]) is an orally active anti-arrhythmic agent available as 150 mg, 200 mg and 250 mg capsules. One-hundred milligrams (100 mg) of mexiletine is equivalent to 83.31 mg of mexiletine base. It is a white to off-white crystalline powder with slightly bitter taste, freely soluble in water and in alcohol. Mexiletine has a pKa of 9.2.

Chemically, mexiletine 1-methyl-2-(2, 6-xylyloxy) ethylamine hydrochloride has the following structural formula:



$C_{11}H_{17}NO \cdot HCl$ mexiletine hydrochloride, USP
Mol. Wt. 215.73 (MEXITIL)

Mexiletine capsules contain the following excipients: corn starch, NF. The capsule shell contains: FD&C Yellow #6, gelatin and titanium dioxide. The 150 mg capsule also contains D&C Red #28 and FD&C Blue #1. The imprinting ink contains ammonium hydroxide, black iron oxide, D&C Yellow #10, ethyl alcohol, FD&C Blue #1, FD&C Blue #2, FD&C Red #40, isopropyl alcohol, n-butyl alcohol, propylene glycol and shellac.

The mexiletine capsules will be over-encapsulated in #00 Swedish Orange Opaque (4188) capsules and will be overfilled with corn starch, NF.

### 6.1.2. Placebo

A matched placebo will be used to maintain the dosage-blind. The placebo capsules for this study will match the corresponding mexiletine capsules in size, color, and presentation.

The placebo capsules are #00 Swedish Orange Opaque (4188) capsules and will be overfilled with corn starch, NF.

Administration of matching placebo will be the same as for subjects in the two mexiletine treatment groups.

## 6.2. Treatments Administered

The three treatment arms include placebo, mexiletine 300 mg/day, and mexiletine 600 mg/day. Mexiletine or the matching placebo will be taken orally as a capsule. Subjects must be able to

swallow oral medication at the Baseline Visit and expected to be able to swallow the capsule throughout the course of the study to be eligible for enrollment. The capsules must not be opened and mixed with food/drink or any gastrostomy feedings. Study drug capsules must be taken with solid food or antacids. It may not be taken with a liquid diet.

Capsules will contain 150 mg of either placebo or mexiletine and will be administered concurrently from two bottles containing 60 capsules each. Study subjects will be titrated up to the appropriate dosage level over the course of 7 days (see Table 5. Study Drug Titration Schedule). After the titration period, the subject will continue at the target dosage level of 2 capsules twice a day until the Week 4 Visit. Mexiletine and placebo will be packaged in kits. Each subject is expected to utilize one kit during study participation. Additional drug kits will be shipped to each site, as needed based on enrollment.

Mexiletine or placebo will be started at the Baseline Visit and continue treatment until the Week 4 Visit. The day that a subject starts treatment with study drug will be designated as Day 1. All subsequent visits must be scheduled from the Baseline Visit, not the date of their last assessment. The first dose of study treatment will be administered in the clinic by authorized site personnel and will be observed in clinic for 1-2 hours post-dose.

### 6.2.1. Dosage Initiation/Escalation

Given the gastrointestinal (GI) side effects to taking mexiletine, a titration approach was chosen for this study. Mexiletine will be supplied as 150 mg capsules in bottles containing 60 capsules. Study drug kits will contain two bottles labeled 1 and 2. Each participant will start with 1 capsule a day, the first dose taken at the Baseline Visit and the second taken in the am from bottle 1 for 2 days (Day 1 – 2). Subjects will follow the titration schedule illustrated below in Table 5 until the full study dosage level is reached at Day 7. After initial titration, subjects will take 2 capsules of study drug in the morning and in the evening. One capsule will be taken from each bottle labeled with a 1 in the morning and evening until the Week 4 Visit. Each bottle is good for 4 weeks of dosing.

**Table 5. Study Drug Titration Schedule**

**Number of capsules per bottle per day – Bottle 1 and 2**

| | Day 1-2 | Day 3-4 | Day 5-6 | Day 7 |
|---|---|---|---|---|
| **Bottle #1** | 1 QD | 1 BID | 1 BID | 1 BID |
| **Bottle #2** | - | - | 1 Q AM | 1 BID |

## 6.3. Method of Assigning Study Subjects to Treatment Groups (Randomization)

Each subject who meets all eligibility criteria, is accepted for the study, and signs an informed consent form will be randomized to receive mexiletine 300 mg/day or mexiletine 600 mg/day or placebo on a 1:1:1 basis. Randomization will be stratified by site and use of riluzole at screening.

The randomization scheme will be independently developed by the Biostatistics Center and will indicate the treatment assignment and the subject numbers to be used by each site. A programmer in the Biostatistics Center will develop the plan under the guidance of the chief biostatistician.

## 6.4. Study Drug Adjustments, Dosage Reductions and Suspensions

Any dosage adjustment including the reason for and dates of adjustment will be documented in the CRF for each subject requiring this manipulation. The Site Investigator or licensed physician Sub-Investigator has the option of reducing or temporarily stopping the study drug for AEs thought to be related to the study drug or any other reason during the trial (the reason for, and dates of suspension or dose reduction must be documented). If the adverse event is mild or moderate, the dosage may be reduced until the event improves (See Table 6. Study Drug Reduction Schedule). The Site Investigator may then choose to resume the higher dosage or maintain the subject at a reduced dosage. If the event is serious or life threatening, and deemed to be drug related, the study drug should be discontinued immediately. Study subjects may remain off the study drug permanently, until the condition improves or they may resume on a reduced dosage, as advised by the Site Investigator. Subjects may not resume study drug after a suspension that is longer than 10 days. Any resumption of drug after a suspension requires approval from the Medical Monitor. All adverse events will be followed for resolution or 28+5 days after stopping study drug.

**Table 6. Study Drug Reduction Schedule: Number of capsules per bottle per day**

| | Day 1-2 Dose Reduction | Day 3-4 Dose Reduction | Day 5-6 Dose Reduction |
|---|---|---|---|
| **Bottle #1** | 1 BID | 1 BID | 1 Q AM |
| **Bottle #2** | 1 Q AM | - | - |

### 6.4.4. Dosage Re-Challenge

The Site Investigator or licensed physician Sub-Investigator may choose to re-challenge a subject after approval from the Medical Monitor if the adverse event resolves or it is felt that the study subject can continue safely. Subjects may not resume study drug after a suspension that is longer than 10 days.

### 6.4.5. Dosage Discontinuation

Reasons for discontinuation of study medication may include an adverse event, Medical Monitor or Site Investigator recommendation, Sponsor termination, protocol violation, loss to follow-up, patient request, or death. All serious adverse events (SAEs) that occur in a subject who has terminated early must be recorded and reported within 24 hours of awareness.

Study subjects who discontinue study drug prematurely should remain on study as described in Section 5.5.

## 6.5. Blinding

The Randomization ID will be used to identify the subject's electronic case report forms (eCRFs), laboratory tests, study medication and all communications. Study Subjects, Investigators, Coordinators, Clinical Evaluators (and all other study site staff), Study Monitors, Project Management, Clinical Laboratory and Data Management personnel, and the Sponsor will be blinded to treatment group assignment throughout the study. Only the Biostatistician developing the randomization schedule, the Research Pharmacists at the Central Pharmacy and the Site Pharmacist storing and distributing the study drug at each site will be unblinded to the individual drug assignments in this study.

### 6.5.1. Emergency Unblinding

An emergency unblinding procedure will allow the Site Investigator the option of learning the treatment assignment for an individual subject if clinical circumstances require it. For unblinding, the Site Investigator must contact the Medical Monitor. The Site Investigator should attempt to contact the NCRI Coordination Center prior to completing emergency unblinding if time allows. If this is not possible, the site must inform the NCRI Coordination Center as soon as possible regarding the emergency unblinding and the circumstances surrounding it. The Site Investigator must document the reason for unblinding in the subject's source documents.

Rarely should such an extreme action be taken. Experimental medications can usually be withdrawn without the need for unblinding. Sites should take care to unblind only those study staff member(s) and other persons whose knowledge of the treatment assignment is necessary for the clinical safety of the subject. Sites should take all reasonable measures to keep study staff, NCRI Coordination Center staff and the subject blinded to treatment assignment, especially site staff completing any Early Termination Visit procedures, if possible.

In the event that emergency disclosure of treatment assignment is necessary, the study subject will be withdrawn from further participation in the trial. All adverse events (AEs) resulting in emergency unblinding will be followed for resolution.

## 6.6. Excluded, Prior and Concomitant Medications

Throughout the study, Site Investigators or licensed physician Sub-Investigators may prescribe concomitant medications or treatments deemed necessary to provide adequate supportive care provided that the medications are licensed in the US and not prohibited.

Study subjects should not receive other experimental therapy during the study. This includes marketed agents at experimental dosages that are being tested for the treatment of ALS. Subjects and/or primary caregivers should consult with the site Principal Investigator or licensed physician Sub-Investigator prior to initiating any new medication, including non-prescription compounds or any other non-drug therapy.

The following medications are prohibited when given on an as needed (PRN) basis. A subject must therefore be on a stable dose for 30 days prior to the Screening Visit, and the dose must not change during the course of the study, unless there is a safety risk to the subject, in which case the change must be documented at the subsequent clinical encounter.

* Benzodiazepines

* Muscle relaxants
* Tricyclic antidepressants
* Selective serotonin reuptake inhibitors
* Non-selective serotonin reuptake inhibitors
* Sedating antihistamines including the following:
  - Alimemazine
  - Chlorphenamine
  - Clemastine
  - Cyproheptadine
  - Hydroxyzine
  - Ketotifen
  - Promethazine
* Anti-cholinergics.

Any investigational therapy being used or evaluated for the treatment of ALS is prohibited beginning 30 days prior to Screening Visit and throughout the study. This includes, but is not limited to, the following:

- Pioglitazone
- Arimoclomol
- Olanzapine
- Tamoxifen
- Mexiletine
- NP001
- Ezogabine (Retigabine)
- Tocilizumab (Actemra)
- Tirasemtiv
- Ibudilast
- Fingolimod
- Ozanezumab (GSK 1223249)
- Rasagiline
- Memantine
- CK-2017357
- CK-2127107
- RNS60 (Revalesio)
- Masitinib
- Immune Modulating Treatments
- Anti-sense oligonucleotide (ASO) or other gene experimental therapies targeting SOD1 or C9orf72

The Site Investigator should contact the medical monitor with any questions regarding prohibited concomitant medication usage.

### 6.6.1. Drug Interactions

Due to increased risk of prolonged QT and cardiac arrhythmias, study subjects will not be able to take concomitantly the anti-arrhythmics amiodarone and flecainide. The use of duloxetine,

tizanidine, and clozapine will also be prohibited in this study due to their ability to inhibit hepatic metabolism and alter mexiletine levels. Site investigators should contact the medical monitor for concerns about potential interactions with other medications.

## 6.7. Treatment Compliance

Subjects will be instructed to return their empty and unused study medication bottle at the Week 4 Visit or the Final Safety Visit, whichever occurs first. Site personnel will review returned and unused study medication to determine compliance.

Non-compliance will be otherwise defined as taking less than 80% or more than 120% of study medication as determined by capsule counts. If a study subject is non-compliant with study medication, the Site Investigator and staff should re-educate and train the subject in administration of study drug. Data indicating non-compliance will be used in the end of study analysis.

## 6.8. Study Drug Storage

The Site Investigator must ensure that all investigational drug supplies are kept in a locked, safe area at room temperature (68-77°F) with access limited to those directly involved in the study. Investigational drug supplies should not be repackaged in any way.

## 6.9. Warnings/Precautions

Mexiletine should be used cautiously in patients with known hypersensitivity to other class IB anti-arrhythmics. For this reason, subjects who have had a previous reaction to lidocaine will be excluded from this study. Mexiletine can uncommonly cause seizures, especially in patients with a known history of seizures and should be used cautiously in this setting. Cigarette smoking can diminish the efficacy of mexiletine and should be restricted in patients taking mexiletine. Mexiletine can also prolong the stimulant effects of caffeine and its intake should also be limited.

### 6.9.1. Death or Heart Attack

Antiarrhythmic drugs, similar to mexiletine, have been reported to increase the risk of death or heart attack, especially in people who have had a heart attack within the past 2 years. Mexiletine may increase the chance of having arrhythmias (irregular heartbeats) and has not been proven to help people without life-threatening arrhythmias to live longer.

### 6.9.2. Acute Liver Injury

In post-marketing experience abnormal liver function tests have been reported, some in the first few weeks of therapy with mexiletine. Most of these have been observed in the setting of congestive heart failure or ischemia and their relationship to mexiletine has not been established.

### 6.9.3. General

If a ventricular pacemaker is operative, patients with second or third degree heart block may be treated with mexiletine if continuously monitored. A limited number of patients (45 of 475 in controlled clinical trials) with pre-existing first degree AV block were treated with mexiletine; none of these patients developed second or third degree AV block. Caution should be exercised when it is used in such patients or in patients with pre-existing sinus node dysfunction or intraventricular conduction abnormalities.

Like other antiarrhythmics, mexiletine can cause worsening of arrhythmias. This has been uncommon in patients with less serious arrhythmias (frequent premature beats or non-sustained ventricular tachycardia), but is of greater concern in patients with life-threatening arrhythmias such as sustained ventricular tachycardia. In patients with such arrhythmias subjected to programmed electrical stimulation or to exercise provocation, 10-15% of patients had exacerbation of the arrhythmia, a rate not greater than that of other agents.

Mexiletine should be used with caution in patients with hypotension and severe congestive heart failure (CHF) because of the potential for aggravating these conditions.

Since mexiletine is metabolized in the liver, and hepatic impairment has been reported to prolong the elimination half-life of mexiletine, patients with liver disease should be followed carefully while receiving mexiletine. The same caution should be observed in patients with hepatic dysfunction secondary to congestive heart failure.

Concurrent drug therapy or dietary regimens which may markedly alter urinary pH should be avoided during mexiletine therapy. The minor fluctuations in urinary pH associated with normal diet do not affect the excretion of mexiletine.

### 6.9.4. Pregnancy & Nursing Mothers

**Subjects or partners of male subjects should not become pregnant during the study.** If a female subject becomes pregnant, study treatment must be discontinued immediately.

Reproduction studies performed with mexiletine hydrochloride in rats, mice and rabbits at doses up to four times the maximum human oral dose (24 mg/kg in a 50 kg patient) revealed no evidence of teratogenicity or impaired fertility but did show an increase in fetal resorption.

Mexiletine appears in human milk in concentrations similar to those observed in plasma. Therefore, no subject should nurse their infant while participating in this study.

### 6.9.5. Diaphragm Pacing System (DPS) Device

Subjects should not undergo DPS implantation surgery during the study. If a subject does undergo DPS implantation surgery during the study, study treatment must be discontinued immediately.

# 7.0. SAFETY VARIABLES AND OUTCOME MEASURES

## 7.1. Safety Variables

The safety of mexiletine will be evaluated using vital signs, weight, clinical laboratory determinations, physical examinations, AEs (including deaths and other SAEs), use of concomitant medications and treatment discontinuations (tolerability).

### 7.1.1. Vital Signs, Height and Weight

Vital signs will be obtained after the subject has been in a seated position for at least 3 minutes. Systolic and diastolic blood pressure, and pulse rate (radial artery) will be obtained at specified visits. Height will be measured and recorded at the Screening Visit only. Weight and vital signs will be measured and recorded at Screening, Pre-Dose and Post-Dose Baseline, Week 4, and Week 8 or the Final Safety Visit, if applicable.

### 7.1.2. Clinical Laboratory Assessments

The following laboratory tests will be performed to assess the safety profile of mexiletine:

- **Hematology and differential panel:** complete blood count with differential (hematocrit, hemoglobin, mean corpuscular hemoglobin [MCH], mean corpuscular hemoglobin concentration [MCHC], mean corpuscular volume [MCV], platelets, red blood cell count [RBC], white blood cell count [WBC], RBC morphology, basophils, eosinophils, lymphocytes, monocytes, neutrophils)
- **Blood chemistry panel:** ALT (SGPT), AST (SGOT), albumin, calcium, chloride, alkaline phosphatase, bicarbonate, cholesterol, creatine kinase (CK), creatinine, direct bilirubin, Gamma glutamyl transpeptidase (GGT), glucose, phosphorus, potassium, sodium, total bilirubin, total protein, triglycerides, urea nitrogen
- Serum human chorionic gonadotrophin (hCG) for women of childbearing potential (WOCBP) to be completed at screening visit
- Serum or urine human chorionic gonadotrophin (hCG) for women of childbearing potential (WOCBP) to be completed at Baseline (pre-dose), Week 4 and Week 8 visits

All subjects will have safety laboratory tests as above at the Screening Visit and Weeks 4 and 8, or the Final Safety Visit, if applicable. These samples will be analyzed locally at the site's institutional laboratory. If a subject is unable to go to the clinic for the testing, other arrangements may be made with the approval of the NCRI Coordination Center. The Site Investigator may order additional testing, if needed, to further assess an adverse event, or if there is any suspicion that a subject may be pregnant.

### 7.1.3. Mexiletine Levels/Biomarker Samples

Subjects will have blood drawn to assess a random mexiletine plasma concentration at the Pre-Dose Baseline Visit and Weeks 4, and 8 or the Final Safety Visit, if applicable. Levels will be assayed in a blinded fashion by an experienced clinical pharmacologist at Tufts University

Public Health in Boston, MA. Tufts University will be analyzing plasma for mexiletine trough levels.

Additionally, subjects will have blood drawn for storage and use though the New York Genome Center (NYGC) and the NEALS Biorepository.

#### 7.1.3.1. DNA Collection

Consented patients will have blood drawn for deoxyribonucleic acid (DNA) extraction for genome sequencing at the Baseline Visit. Blood samples will be stored at the New York Genome Center (NYGC) in New York City, NY.

The NYGC will be conducting the sequencing of the de-identified samples, doing the analysis of the sequencing and sharing the results of such sequencing and analysis with researchers pursuant to this protocol, as well as uploading the data to data repositories such as the National Institutes of Health (NIH) Database of Genotypes and Phenotypes (dbGaP).

#### 7.1.3.2. Plasma Collection

Consented patients will have blood drawn for plasma processing, storage and use in the NEALS Biorepository at the Pre-Dose Baseline, Weeks 4, and 8 Visits or the Final Safety Visit, if applicable. These samples will be used for future research in motor neuron diseases.

### 7.1.4. 12-Lead Electrocardiogram (ECG)

A standard 12-lead ECG will be performed at the Screening Visit, Post-Dose Baseline Visit and Weeks 4, and 8 or the Final Safety Visit, if applicable. A copy of the tracings will be kept on site as part of the source documents. The following measures will be obtained: heart rate, RR interval, PR interval, QRS interval, QT interval, and either Bazett-corrected QT interval or Fridericia-corrected QT interval, and any clinical abnormalities. The ECG will be reviewed same day by the site PI. The following conditions will be brought to the attention of the reviewing cardiologist and medical monitor. The medical monitor will determine if the subject should discontinue study medication.

- any significant change from the screening ECG noted by the reviewing cardiologist
- a PR interval > 200 ms
- a QRS complex > 120 ms
- a QTc interval > 450 ms in males or > 470 ms in females

### 7.1.5. Physical and Neurological Examinations

A physical examination will be performed and recorded at Screening Visit, Week 4, and Week 8 or the Final Safety Visit, if applicable and will include the following systems: head/neck, eyes, ears, nose/throat, cardiovascular, lungs, abdomen, musculoskeletal, central nervous system, extremities, and skin. In addition, an abbreviated neurological examination will be performed at the Screening Visit, Week 4, Week 8, and the Final Safety Visit, if applicable. The examination will include assessment of mental status, cranial nerves, motor and sensory function, reflexes, coordination, and stance/gait.

### 7.1.6. Adverse Events

Adverse events will be documented at each study visit, including the Screening Visit once the informed consent form has been signed by the subject. Information on adverse effects of study medication and on inter-current events will be determined at each visit by direct questioning of the subjects, clinical examination, review of concomitant medications, vital signs and laboratory test results. Tolerability will be determined by the ability to complete the study on the assigned treatment at the assigned dose. Subjects on a dose reduction are considered intolerant. Side effects that would not be tolerable in a drug chronically administered are of particular interest. Given the severity of the disease, some significant side effects might be tolerated.

## 7.2. Outcome Measures

### 7.2.1. Transcranial Magnetic Stimulation and Threshold Tracking Nerve Conduction Studies

As a *primary outcome measure*, the RMT will be recorded from the abductor pollicis brevis muscle. The RMT is defined as the minimum stimulus intensity required to elicit a 50 μV MEP in at least 5/10 trials. RMT is thought to reflect the density of corticomotoneuronal projections onto the spinal motor neuron. It is also a biomarker of corticomotor neuronal excitability, in part correlating with persistent sodium current [49].

As *secondary outcome measures*, the slope of the input/output curve of the relationship between stimulus strength and MEP output and the maximum MEP amplitude will also be recorded. The maximum MEP amplitude reflects density of corticomotoneuronal projections onto motor neurons and is affected by cortical hyperexcitability early in ALS and axonal degeneration later in the disease [49]. It is defined as a percentage of the maximum compound muscle action potential (CMAP), to account for lower motor neuron pathology, as measured by standard motor nerve conduction study, which will be performed on the median motor nerve recoding over the APB muscle contralateral to the side of cortical stimulation and just prior to TMS [49]. Maximum MEP amplitudes have been shown to increase in ALS, especially early, reflecting cortical hyperexcitability [49].

Another *secondary outcome measure* to be assessed by TMS, CSP, is recorded as a duration from the onset of the MEP response to resumption of voluntary electromyography activity. The CSP is induced by having patients perform a voluntary contraction, set to 30% of maximal voluntary contraction. The level of contraction will be monitored by recording muscle activity by surface electromyography of the abductor pollicis brevis muscle. The CSP duration is measured from the beginning of MEP to the return of EMG activity. The CSP is thought to be mediated by spinal mechanisms and cortical inhibitory neurons and is a measure of cortical inhibition. Absent or reduced CSP duration is seen in both sporadic and familial ALS patients, most prominently early in the disease [49].

As another key *secondary outcome measure*, changes in SICI, will be measured by dual pulse TMS at the Pre-Dose Baseline Visit, Weeks 4, and 8, and the Final Safety visit, if applicable with conditioned (80% of RMT) and test pulses (120% of RMT) to generate a stable MEP amplitude of 0.2 mV, averaged over interstimulus intervals of 1-7 ms. SICI is thought to reflect refractory cortical axons and subsequent resynchronization of cortico-cortical and corticomotoneuronal

volleys or activation of non-GABAergic cortical inhibitory circuits (initial phase) and synaptic neurotransmission through $GABA_A$ receptors (second phase) [49].

To determine the effects of mexiletine on muscle cramps and fasciculations, 14-day history of fasciculations and a 7 and 30 day history of muscle cramps will be assessed at Baseline and daily summaries of muscle cramping and fasciculations will be completed every morning or evening by participants in a Muscle Cramping/Fasciculations Diary (see Section 7.2.8). Data from the diary will be abstracted by study staff at every visit after the Baseline Visit.

As another *secondary outcome measure* and pharmacodynamic marker, peripheral nerve axonal excitability will be assessed by TTNCS performed at the same visits as TMS using a Digitimer DS5 stimulator. CMAPs will be recorded by standard motor nerve conduction study (NCS) on electromyography machines at each site. Determinations will be made of the strength duration time constant using pulses of varying stimulus duration as this correlates with persistent sodium current [29], have been shown previously to be reduced in patients with muscle cramps using mexiletine [30, 43], and have been shown to predict survival in ALS [29].

There may be up to two TMS physiologists and up to two TTNCS physiologists at each site.

After the screening visits and randomization, ALS subjects will undergo two study visits over the course of 8 weeks with TMS and TTNCS studies at each visit. For these visits, the time of the TMS study (i.e. morning or afternoon) should be kept as consistent as possible. The same TMS and TTNCS physiologist should perform tests at both visits for an individual subject.

As part of the inclusion criteria, a study subject must meet TMS inclusion criteria (resting motor threshold, defined as 50% of pulses eliciting a motor evoked potential (MEP) of amplitude $\geq 50$ μV). A study subject must meet TTNCS inclusion criteria (CMAP $\geq 1.5$ mV). Specific attention must be paid to exclusion criteria related to TMS, namely metal in the head and neck region, cardiac pacemaker or brain stimulator, cochlear implants, implanted infusion device or personal history of epilepsy.

All recordings will use the abductor pollicis brevis (APB) muscle on a single hand for the duration of the study. The hand to be recorded will be determined during the neurological exam with the goal of selecting a modestly affected muscle.

- if both sides have MMT score of 5/5, choose the right side
- if both sides are <5, choose the stronger side
- if one side is 5/5 and the other side is ≥3, choose the weaker side
- if one side is 5/5 and the weak side is <3, choose the stronger side

**TMS Protocol Summary**

For TMS recording, motor evoked potentials (MEPs) will be recorded using the Powerlab 26T with LabChart V8 software. The TMS protocol will consist of four components:

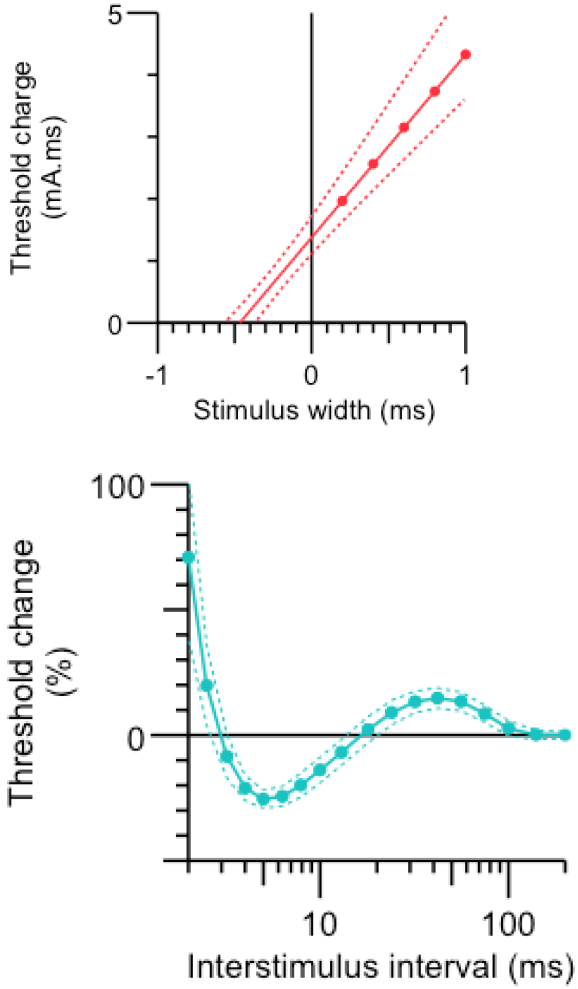
1) Identification of hot spot and measurement of RMT and maximum MEP amplitude. Both RMT and MEP amplitude must be manually entered into the LabChart software data file.
2) Paired testing. This consists of pseudorandomized blocks of:

- Single unconditioned pulses with stimulator output 120% of RMT
- Conditioned pulses (prepulse 80% RMT and test pulse 120% RMT) and interstimulus interval 3 ms for determination of short intracortical inhibition (SICI)
- Single unconditioned pulses with stimulator output 80% of RMT as a control.

3) Input output curves to determine relationship between stimulus strength and MEP output. These are performed over a range from 25% to 100% of maximum stimulus output.
4) Cortical silent period. This will consist of pulses of 120% RMT while subject is activating muscle.

File naming convention and data handling will be specified once all TMS physiologists are confirmed.

**TTNCS Measurements (Hugh Bostock)**
Top, determination of strength duration time constant using pulses of different stimulus widths.. Bottom, recovery cycle analysis




**TTNCS Protocol Summary**

TTNCS will be performed using a Digitimer DS5 stimulator, which is controlled by Qtracs software through a National Instruments Data Acquisition Board. Compound muscle action potentials (CMAPs) will be recorded using the site EMG/NCS machine at almost all sites (requires analog output on EMG machines).

The entire protocol is controlled via the Qtracs software and consists of:

1) Setup. It is essential that the physiologist enters his/her unique code, as this is part of the file name. The codes will be assigned once all TTNCS physiologists are confirmed.
2) Determination of suprathreshold CMAP amplitude
3) Measurement of CMAP response/stimulus relationship
4) Determination of strength duration time constant using pulses of varying stimulus duration.
5) Recovery cycle analysis. Figure shows threshold change following a single prepulse, illustrating periods of refractoriness, superexcitability and late subexcitability.
6) The procedure concludes with recording of final temperature generation of a MEM data file and visual display of the collected data.

File naming convention and data handling will be specified once all TTNCS physiologists are confirmed.

To ensure reproducibility, each neurophysiologist at each site will need to perform TMS and TTNCS on a single unpaid volunteer over three separate days, preferably at the same time of day, and receive approval after review of the data.

### 7.2.2. ALSFRS-R

The ALSFRS-R is a quickly administered (5 min) ordinal rating scale (ratings 0-4) used to determine subjects' assessment of their capability and independence in 12 functional activities. All 12 activities are relevant in ALS. Initial validity was established by documenting that in ALS patients, change in ALSFRS-R scores correlated with change in strength over time, was closely associated with quality of life measures, and predicted survival. The test-retest reliability is greater than 0.88 for all test items. The advantages of the ALSFRS-R are that the categories are relevant to ALS, it is a sensitive and reliable tool for assessing activities of daily living function in those with ALS, and it is quickly administered. With appropriate training the ALSFRS-R can be administered with high inter-rater reliability and test-retest reliability. The ALSFRS-R can be administered by phone, again with good inter-rater and test-retest reliability. The equivalency of phone versus in-person testing, and the equivalency of study subject versus caregiver responses have also recently been established. Therefore, if necessary, the ALSFRS-R may be given to the study subject over the phone. The ALSFRS-R will be administered at the Screening, Pre-Dose Baseline, Weeks 4 and 8 Visits, or the Final Safety Visit, if applicable. All ALSFRS-R evaluators must be NEALS certified.

### 7.2.3. Slow Vital Capacity (Pulmonary Function Testing/Spirometry)

The vital capacity (VC) (percent of predicted normal) will be determined, using the slow VC (SVC) method with normalization to percent predicted based on age, height, and gender. The SVC can be measured using conventional spirometers that have had a calibration check prior to subject testing. A printout from the spirometer of all SVC trials will be retained. All SVC evaluators must be NEALS certified. Three SVC trials are required for each testing session, however up to 5 trials may be performed if the variability between the highest and second highest SVC is 10% or greater for the first 3 trials. Only the 3 best trials are recorded on the CRF. The highest SVC recorded is utilized for eligibility. The SVC will be completed at the Screening, Pre-Dose Baseline, Weeks 4 and 8 Visits, or the Final Safety Visit, if applicable.

### 7.2.4. Hand Held Dynamometry (HHD)

The abductor pollicis brevis (APB) muscle will be measured by hand held dynamometry (HHD). The APB will be tested at the Screening, Pre-Dose Baseline, Weeks 4, and 8 Visits, or the Final Safety Visit, if applicable. The APB testing via HHD must always be completed prior to TMS.

### 7.2.5. Edinburgh Handedness Inventory Short Form

The Edinburgh Handedness Inventory Short Form is a common instrument used to assess handedness and is important in interpreting the neurophysiological data. This will be completed at the Screening Visit.

### 7.2.6. Blindedness Questionnaire and Exit Survey

The Blindedness Questionnaire will be used to determine whether there is effective unblinding due to treatment effects, as assessed by the subject and a member of the study team. The Exit Survey will assess subject tolerance of components of the study, including TMS and TTNCS. The exit survey will be completed at the Week 8 Visit or the Final Safety Visit, if applicable.

### 7.2.7. RAND-36

The RAND 36-Item Health Survey taps eight health concepts: physical functioning, bodily pain, role limitations due to physical health problems, role limitations due to personal or emotional problems, emotional well-being, social functioning, energy/fatigue, and general health perceptions. It also includes a single item that provides an indication of perceived change in health.

Scoring the RAND 36-Item Health Survey is a two-step process. First, numeric values are recoded so that a high score defines a more favorable health state and then scaled to a 0 to 100 range so that the lowest and highest possible scores are 0 and 100, respectively. Scores represent the percentage of total possible score achieved. Second, items in the same scale are averaged together to create 8 scale scores. Hence, scale scores represent the average for all items in the scale that the respondent answered.

### 7.2.8. Assessment of Muscle Cramps and Fasciculations

The effects of mexiletine on muscle cramps will also be assessed as a secondary endpoint. Cramps in SALS patients are common and are often debilitating. Various medications including quinine sulfate, magnesium, lioresal, dantrolene, clonazepam, diphenylhydantoin and gabapentin, have been used in the treatment of cramps in ALS, though few have been studied in the ALS population and none have clearly shown efficacy. Mexiletine has been shown to have a beneficial impact on muscle cramps in a small non-randomized study of patients with Machado-Joseph disease [45] but has never been studied in ALS patients until the recent safety and tolerable study using mexiletine.

The visual analog scale (VAS) is a scale that measures pain associated with muscle cramping. It will be used to measure muscle cramping in this study. The scale rating is from 0-10; 0 equals no symptoms, 10 equals most severe symptoms. The VAS will be explained at the Baseline visit to all subjects. It will then be completed each day by the subject and entered on a muscle cramps and fasciculations diary. At baseline, subjects will be asked to recount the number and intensity on VAS of muscle cramps experienced in the previous 7 and 30 days. At each visit (beginning at the Week 4 Visit), the coordinator will review the Muscle Cramp/Fasciculations Diary and complete a Muscle Cramp CRF based on this data and/or subject's verbal self-report. Cramp intensity as assessed by a VAS is the most common measure used in cramp trials [44].

Fasciculations will also be recorded in the diary on a daily basis, graded in the following manner: 0. no fasciculations on this day; 1. fasciculations after exertion only, not substantially interfering with daily activity; 2. fasciculations at rest or after exertion, not substantially interfering with daily activity; and 3. fasciculations at rest or after exertion and substantially interfering with daily activity. At baseline, subjects will be asked several questions related to their fasciculations experienced in the previous 14 days. Subjects will be provided with a muscle cramps and fasciculations diary to record muscle cramp frequency and intensity and severity of

fasciculations at home daily. At each subsequent visit, the coordinators will review the diary and record the daily frequency and maximal intensity of muscle cramps and fasciculations before returning the diary or providing the subject with a new diary.

### 7.2.9. Training and Validation

All Evaluators must be NEALS certified for ALSFRS-R and SVC; specific certification requirements are outlined in the study manual of operations. Repeat NEALS certification will be required on bi-annual basis for all outcome measures. It is strongly preferred that a single Evaluator performs all measures throughout the study. NEALS certification is required for all Evaluators prior to performing any study tests. Additionally, Evaluators will receive separate certification following a TMS and TTNCS training session immediately after the site investigator meeting.

## 8.0. STUDY PROCEDURES

No study procedures or obtainment of biospecimens will be performed prior to the signing of the informed consent form. All subjects will sign an informed consent form prior to undergoing any study tests or procedures. The order of testing will be at the discretion of each Site Investigator with the exception of APB testing via HHD. That must always be completed prior to TMS.

It is strongly encouraged that each visit and the procedures be completed on the same day. However, if this is not possible visits can be split up into 2 consecutive days. For the baseline visit, all measurements and procedures must be completed prior to initiating treatment. For the week 4 visits, all measurements and procedures must be completed after study drug is finished. Visit windows are consecutive calendar days and the target visit dates are calculated from the day study drug is started (i.e. the day of the Baseline Visit – Post-Dose). Study visits have the following visit windows:

**Table 7. Visit Windows**

| | Baseline Visit (Pre-Dose) | Baseline Visit (Post-Dose) | Week 1 Safety Telephone Visit | Week 4 Visit | Week 8 Visit |
|---|---|---|---|---|---|
| **Visit Window** | Within 21 days of Screening Visit | 1-2 hours after administration of study drug | 7 ± 3 days from Baseline | 29 + 3 days from Baseline | 56 ± 3 days from Baseline |

Subjects who withdraw consent will be asked to come in for a Final Safety Visit.

### 8.1. Schedule of Study Procedures

#### 8.1.1. Screening Visit

The following procedures will be performed at the Screening Visit:

- Obtain written informed consent from subject
- Assess inclusion and exclusion criteria
- Obtain medical history and demographics
- Obtain ALS diagnosis and ALS history
- Perform physical and neurological examinations
- Measure vital signs including height & weight
- Perform APB testing by HHD
- Perform 12-lead ECG
- Collect blood samples for clinical laboratory assessments including:
  - Hematology and differential panel
  - Blood chemistry panel
  - Serum pregnancy test (for woman of childbearing potential [WOCBP])
- Perform TMS and NCS to determine if subject meets inclusion criteria

- Administer ALSFRS-R questionnaire
- Edinburgh Handedness Inventory Short Form
- Perform slow vital capacity (SVC)
- Review and document concomitant medications and therapies
- Assess and document AEs after subject signs informed consent form
- Schedule the Baseline Visit within 21 days

### 8.1.2. Pre-dose Baseline Visit

This visit will take place within 21 days of the Screening Visit. The following procedures will be performed:

- Assess inclusion and exclusion criteria (Note: Baseline Visit SVC is not exclusionary, even if below 50%)
- Measure vital signs and weight
- Administer ALSFRS-R questionnaire
- Perform APB testing by HHD
- Perform SVC
- Administer RAND-36
- Muscle Cramps/Fasciculations Assessment
- Perform TMS/TTNCS
- Review and document concomitant medications and therapies
- Collect blood for plasma mexiletine level, DNA and plasma for NEALS biorepository
- Urine pregnancy test (for woman of childbearing potential [WOCBP])
- Assess and document AEs
- Distribute Muscle Cramps/Fasciculations Diary
- Randomize subject*
- Administer first dose of the study drug on site. The subject will be observed at the site for 60-120 minutes by an appropriate healthcare staff member according to the site's institutional/state regulations to assess any immediate reaction to the study drug.

**Randomization should occur as close to the Baseline Visit as possible to give enough time for the site pharmacy to distribute the study drug kits (usually 24-48 hours in advance of the Baseline Visit).*

### 8.1.3. Post-dose Baseline Visit

This visit will take place the same day as the Baseline Visit (1-2 hours after administering first dose of the study drug). The following procedures will be performed:

- Perform 12 Lead ECG
- Assess and document AEs
- Dispense study medication and dosing diary
- Schedule the Week 1 Safety Telephone Visit

### 8.1.4. Week 1 Safety Telephone Visit

This visit will take place 7 ± 3 days after the Baseline Visit. The following procedures will be performed:

- Review and document concomitant medications and therapies
- Assess and document AEs
- Check study drug compliance and dosing diary
- Schedule the Week 4 Visit

### 8.1.5. Week 4 Visit

This visit will take place 29 + 3 days after the Baseline Visit. **Please note this visit must occur on Day 29, Day 30, Day 31, or Day 32. The visit cannot occur before Day 29**. **Study drug is to be taken for at least 28 days. The last dose will be the night before the scheduled visit.** The following procedures will be performed:

- Perform physical and neurological examinations
- Measure vital signs and weight
- Perform 12-lead ECG
- Collect blood samples for clinical laboratory assessments including:
  - Hematology and differential panel
  - Blood chemistry panel
  - Urine pregnancy test (for woman of childbearing potential [WOCBP])
- Collect blood for plasma mexiletine level and plasma for NEALS biorepository
- Administer ALSFRS-R questionnaire
- Administer RAND-36
- Perform APB testing by HHD
- Perform SVC
- Perform TMS/TTNCS
- Review and document concomitant medications and therapies
- Assess and document AEs
- Check study drug compliance and dosing diary
- Collect Muscle Cramp/Fasciculations Diary and dispense new Diary to subject
- Perform study drug accountability and collect all unused study drug and empty containers
- Schedule the Week 8 Visit

### 8.1.6. Week 8 Visit

This visit will take place 56 ± 3 days after the Baseline Visit. The following procedures will be performed:

- Perform physical and neurological examinations
- Measure vital signs and weight
- Perform 12-lead ECG
- Collect blood samples for clinical laboratory assessments including:
  - Hematology and differential panel
  - Blood chemistry panel
  - Urine pregnancy test (for woman of childbearing potential [WOCBP])
- Collect blood for plasma mexiletine level and plasma for NEALS biorepository
- Administer ALSFRS-R questionnaire
- Administer RAND-36
- Perform APB testing by HHD

- Perform SVC
- Perform TMS/TTNCS
- Review and document concomitant medications and therapies
- Assess and document AEs
- Review returned Muscle Cramping/Fasciculations Diary
- Perform Blindness Questionnaire and Exit Survey

### 8.1.7. Final Safety Visit

Subjects who discontinue study drug early are encouraged to come in for a Final Safety Visit. The following will be performed:

- Perform physical and neurological examinations
- Measure vital signs and weight
- Perform 12-lead ECG
- Collect blood samples for clinical laboratory assessments including:
  - Hematology and differential panel
  - Blood chemistry panel
  - Urine pregnancy test (for woman of childbearing potential [WOCBP])
- Collect blood for plasma mexiletine level and plasma for NEALS biorepository
- Administer ALSFRS-R questionnaire
- Administer RAND-36
- Perform APB testing by HHD
- Perform SVC
- Perform TMS/TTNCS
- Review returned Muscle Cramping/Fasciculations Diary
- Review and document concomitant medications and therapies
- Assess and document AEs
- Check study drug compliance and collect dosing diary
- Perform study drug accountability and collect all unused study drug and empty containers
- Perform Blindness Questionnaire and Exit Survey

All subjects who discontinue study drug early will be encouraged to complete follow-up per study protocol under the intent-to-treat (ITT) principle. If they are unable to return for in-person visits, the scheduled in-person visits can be replaced by Telephone Interviews. Telephone Interviews will occur at the same intervals as regularly-scheduled Study Visits (Weeks 4 and 8). The first Follow-up Telephone Call will occur at the next scheduled study visit. Subjects who withdraw consent will also be asked to come in for a Final Safety Visit.

## 8.2. Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol. The noncompliance may be either on the part of the subject, the SI, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

All deviations from the protocol must be addressed in the subject's source documents. Protocol deviations must be sent to the local IRB per their guidelines and entered in the Protocol Deviations Log in the EDC System.

## 8.3. Missed Visits and Procedures

Missed visits and any procedures not performed (not attempted) for reasons other than illness, injury or progressive disability (i.e., subject is physically unable to perform test) will be reported as protocol deviations.

Procedures or visits not performed due to illness, injury or disability, including procedures that were attempted but failed (i.e., blood samples unable to be drawn after multiple attempts, or weight unable to be obtained due to subject immobility) will not be reported as protocol deviations.

Study drug compliance that is outside the limits set in the protocol will be reported as a protocol deviation.

Details and specific instructions regarding protocol deviations, including any exceptions to this standard procedure, are found in the Site Manual of Operations.

# 9.0. SAMPLE PROCESSING

## 9.1. Sample Processing for Repository

The participating sites will label and process the samples collected from participants in a standardized manner. The purpose of standardization is to both minimize collection and processing variability and to maximize the quality and continuity of the samples. Specific instructions for site personnel regarding the collection and processing of these samples will be provided in the Study Manual for this study. All supplies including blood collection tubes and the vials used for blood, urine, and aliquots for freezing, will be standardized either by delineation of supplies in the Study Manual or by providing supplies to the study sites from the study coordination center.

## 9.2. Sample Storage and Shipping

Specific instructions for site personnel regarding the storage and shipment of these samples will be provide in the Study Manual.

Using a bar coding and scanner system, each sample aliquot will be catalogued for future reference. In the electronic data capture system, sample number and location will be cross-referenced to the clinical information. All samples will be linked to GUID and may be stored using the Global Unique Identifier (GUID) to identify the sample.

Samples may be stored locally at the site of collection, and/or in the NEALS Biorepository.

## 10.0 SAMPLE SHARING

Samples collected will become a part of the Northeast ALS (NEALS) Consortium Biorepository operated by the Neurological Clinical Research Institute (NCRI) located at the Massachusetts General Hospital, Boston, MA under IRB protocol (2006P000982). The NCRI has extensive experience in handling multicenter ALS clinical research studies and currently manages a large infrastructure for the storage, cataloging, and sharing of biofluid samples collected in prior studies. The mission of this resource is to provide essential, valuable research samples to researchers that may help them to develop new diagnostic tests, new treatments and a new understanding of the pathogenesis of ALS and other medical diseases. The NEALS Sample Repository Committee will review all requests for samples and, based on the merits and scientific validity of the application, will decide who the samples will be shared with. Samples and clinical and demographic data will be available to academic researchers at hospitals, universities, and commercial organizations. Global Unique Identifier (GUID) will be provided to collaborators on samples and accompanied by clinical information. The information that will be provided with the sample will include, but will not be limited to, current age, gender, diagnosis, race/ethnicity, and information about the disease and diagnostic and general medical history information.

### 10.1 Global Unique Identifier (GUID)

The GUID is generated on a secure website that utilizes 128-bit Secure Socket Layer (SSL). Of note, this website is not linked to NeuroBANK™. The GUID is generated using an irreversible encryption algorithm – it accepts twelve identifying data elements, (e.g. last name at birth, first name at birth, gender at birth, day, month and year of birth, city and country of birth, etc.), and produces a unique random-generated character string, or GUID. No identifying information is stored in the system; it is simply used to generate the GUID. If the same information is entered again, the same GUID will be returned.

## 11.0 SAFEGUARDS TO PROTECT PARTICIPANT CONFIDENTIALITY

For the subject's safety, certain information may be included in the participant's medical records. This could include the participant's participation status, the procedures performed, and any complications arising from the procedure, treatment associated with the complications and results of routine clinical tests. Results of genetic research testing from this study will not go into the medical record and will not be used to make healthcare decisions; however, results of any standard clinical testing that is performed as a part of this study (e.g., vital capacity or ALSFRS-R) may go into the participant's medical record. Organizations that make rules and policy about how research is conducted have the right to review the study records. Agencies that help to fund the study have the right to review the study records. We will make every effort to use only the linked code and not the participants' names, when these agencies are reviewing records. Participants will not be identified by name should the data from this study lead to publications.

All clinical information, study procedures performed, and the code linking the participant's information to the samples will be placed in a research database. The only individuals who will be able to use the study code to link a participant's identity with the clinical information, procedures and samples are the study staff at the research site.

# 12.0. DATA SAFETY MONITORING

## 12.1. Medical Monitoring

The designated Medical Monitor for the study is identified in the Site Manual of Procedures. Site personnel should contact the Medical Monitor for assistance with the following:

- Medically-related protocol questions
- Safety concerns, including AEs and SAEs
- Protocol deviations
- Protocol eligibility questions

The Medical Monitor can decide whether a specific subject must discontinue study drug due to concerns about recurrent or persistent serious side effects such as cardiac events.

# 13.0. STATISTICAL CONSIDERATIONS

## 13.1. Safety and Tolerability Variables

The primary safety and tolerability variables include:

- Adverse Events (AEs) and Serious Adverse Events (SAEs)
- Treatment discontinuations due to AEs/SAEs (tolerability)
- Treatment discontinuations due to loss to follow-up or other reasons not related to AEs/SAEs (tolerability)

The secondary safety variables include:

- Vital signs
- Clinical laboratory test results
- Physical and neurological examinations
- Concomitant medication requirements

## 13.2. Efficacy Variables

The efficacy variables include:

- TMS/TTNCS Parameters
- ALS Functional Rating Scale – Revised (ALSFRS-R)
- Slow Vital Capacity (SVC)
- Visual Analog Scale (VAS) for Muscle Cramps/Fasciculation Assessment
- Pharmacokinetic analysis of the study drug

## 13.3. Analysis Populations

The modified intent-to-treat (mITT) population will include all study subjects who are randomized and receive at least one dose of study drug. The mITT population will be used for all safety and tolerability analyses, with participants classified according to the treatment actually received. The mITT population will be used for primary efficacy analyses, with participants classified according to their randomized treatment assignment regardless of which treatment was actually received.

## 13.4. Missing Data

The trial will be intent-to-treat. We will get follow-up outcome measure information for all subjects whether or not they continue on treatment. If a subject is lost to follow-up, their data will be included in the primary analysis. Data from unobserved visits will be assumed missing at random conditional on the observed visits. If loss to follow-up appears to be associated with treatment arm, then sensitivity analyses will be pursued to investigate the possible influence of non-ignorable missingness.

## 13.5. Baseline and Demographic Characteristics

Demographic and baseline data will be presented according to randomized treatment in tabular form. Summary statistics will be presented for each assessment.

## 13.6. Analysis of Primary Outcome Measure

The effect of mexiletine on RMT will be estimated from a modified intention-to-treat sample (all participants receiving at least one dose of study drug, classified according to randomization) using a shared-baseline repeated-measures two-way ANOVA with fixed effects of visit (4 levels) and treatment x post-baseline visit interaction (2 x 2 = 4 levels) and unstructured covariance among repeated measures (10 terms). Use of a shared baseline reflects the true state of the sample prior to randomization and has the benefit of adjusting for chance baseline differences in RMT across the three treatment groups and improved power similar to ANCOVA. Treatment-dependent differences in the 4-week change in RMT and any sustained benefit at 8 weeks will be estimated using linear contrasts of the least-square means. Not knowing whether a linear dose effect will be present, the primary test will compare each active arm to placebo, using Dunnett's method to control for multiple comparisons. A secondary analysis will test for linear dose response. The proposed mixed effects model makes efficient use of observed data and is unbiased in the presence of loss to follow-up if the probability of loss to follow-up is predictable from observed RMT results. Additional covariates may be included in the primary analysis if rates of loss to follow-up are higher among participants randomized to mexiletine. Wilcoxon rank sum tests of changes from baseline for each active arm vs. placebo and each follow-up time point, imputing the worst observed outcome for missing data, will be evaluated as a sensitivity analysis. The final primary model will be specified prior to unblinding. An additional secondary analysis will look at the effect of mexiletine dosing as achieved, using the estimated serum mexiletine level in place of treatment group. Subgroup differences will be tested by including subgroup, subgroup x visit, and subgroup x treatment x post-baseline visit terms. Subgroups of interest will be pre-specified prior to unblinding.

## 13.7. Analysis of Secondary Efficacy Variable

Equivalent models will be used to analyze other pharmacodynamic markers obtained by TMS and NCS and clinical measures of progression. Frequency of muscle cramping will use a similar generalized linear mixed model assuming that weekly counts follow a negative binomial distribution and including participant-specific random slopes with unstructured covariance of random intercepts and slopes. Pain from cramping and interference from fasciculations will be analyzed in similar models assuming a normal distribution and identity link for pain and a multinomial distribution and cumulative logit link for interference from fasciculations.

Analyses of safety will include frequency of AEs compared by negative binomial regression, proportion of participants experiencing a given AE or SAE classified by MedDRA system organ class or preferred term by Fisher's exact test, occurrence of clinically significant clinical laboratory abnormalities by Fisher's exact, and trends in vital signs and ECG parameters by linear mixed models. Participants will be analyzed according to the treatment actually received. With a plan of 40 participants exposed to mexiletine, the study will have an 80% probability of observing at least one instance of any safety outcome expected to occur in at least 4% of exposed patients, or 8% for events unique to a single dose. Comparisons of event rates across doses will be sensitive only to relatively large differences. The study will have 80% power to detect treatment differences in event rates if the rates in the placebo arm are moderately frequent (20% to 50%) and mexiletine exposure increases the odds at least 6-fold.

## 13.8. Selection Criteria

The best dosage level to carry forward in future trials will be based on safety and tolerability as estimated from the rates of AEs, SAEs, mortality, clinical abnormalities, and early termination of study drug or withdrawal from the study. Given the uncertain nature of possible differences in safety and tolerability, absolute criteria cannot be pre-specified.

## 13.9. Determination of Sample Size

Steve Vucic (personal communication) reports a mean 4-week change in RMT among 18 ALS patients of 6.2% and a SD of 5.1%. A sample size of 60 participants randomized 1:1:1 with up to 10% loss to follow-up will provide 80% power to detect an effect of a given dose of mexiletine on RMT if the increase in RMT relative to placebo is at least 5.3% based on a simple one-way ANOVA and two-sided alpha = 0.027 for each of the two active arms. This estimate of power is conservative relative to our proposed shared-baseline mixed model analysis. This effect is roughly 85% of the natural variation in RMT over 4 weeks. Power will also be greater if a linear dose-response is present (a secondary analysis), with 80% power to detect a slope of 2.4% / 300 mg dose. Selection of the active dose exhibiting greater increase in RMT in our sample, irrespective of compliance with assigned dose and thus reflecting variation in tolerance, will correctly identify the dose with greater true ITT efficacy with at least 80% probability if the difference in efficacy is at least 1.4%.

## 14.0. SAFETY AND ADVERSE EVENTS

The AE definitions and reporting procedures provided in this protocol comply with all applicable International Conference on Harmonization (ICH) guidelines. The SI will carefully monitor each subject throughout the study for possible AEs. All AEs will be documented on CRFs designed specifically for this purpose. It is also important to report all AEs, especially those that result in permanent discontinuation of the investigational product being studied, whether serious or non-serious.

### 14.1. Definitions of AEs, Suspected Adverse Drug Reactions & SAEs

#### 14.1.1. Adverse Event and Suspected Adverse Drug Reactions

An AE is any unfavorable and unintended sign (including a clinically significant abnormal laboratory finding, for example), symptom, or disease temporally associated with a study, use of a drug product or device whether or not considered related to the drug product or device.

Adverse drug reactions (ADR) are all noxious and unintended responses to a medicinal product related to any dose. The phrase "responses to a medicinal product" means that a causal relationship between a medicinal product and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out. Therefore, a subset of AEs can be classified as suspected ADRs, if there is a causal relationship to the medicinal product.

Examples of AEs include: new conditions, worsening of pre-existing conditions, clinically significant abnormal physical examination signs (e.g., skin rash, peripheral edema), or clinically significant abnormal test results (e.g., lab values or vital signs), with the exception of outcome measure results, which are not being recorded as AEs in this trial (they are being collected, but analyzed separately). Stable chronic conditions (e.g., diabetes, arthritis) that are present prior to the start of the study and do not worsen during the trial are NOT considered AEs. Chronic conditions that occur more frequently (for intermittent conditions) or with greater severity, would be considered as worsened and therefore would be recorded as AEs.

AEs are generally detected in two ways:

Clinical → symptoms reported by the subject or signs detected on examination.

Ancillary Tests → abnormalities of vital signs, laboratory tests, and other diagnostic procedures (other than the outcome measures, the results of which are not being captured as AEs).

For the purposes of this study, symptoms of progression/worsening of ALS, including 'normal' progression, will be recorded as AEs.

The following measures of disease progression will not be recorded as AEs even if they worsen (they are being recorded and analyzed separately): vital capacity results and ALSFRS-R results.

If discernible at the time of completing the AE log, a specific disease or syndrome rather than individual associated signs and symptoms should be identified by the SI and recorded on the AE

log. However, if an observed or reported sign, symptom, or clinically significant laboratory anomaly is not considered by the SI to be a component of a specific disease or syndrome, then it should be recorded as a separate AE on the AE log. Clinically significant laboratory abnormalities, such as those that require intervention, are those that are identified as such by the SI.

Subjects will be monitored for AEs from the time they sign consent until completion of their participation in the study (defined as death, consent withdrawal, loss to follow up, early study termination for other reasons or following completion of the entire study).

An unexpected AE is any AE, the specificity or severity of which is not consistent with the current IB. An unexpected, suspected ADR is any unexpected AE that, in the opinion of the SI or Sponsor, there is a reasonable possibility that the investigational product caused the event.

### 14.1.2. Serious Adverse Events

An SAE is defined as an AE that meets any of the following criteria:

1. Results in death.
2. Is life threatening: that is, poses an immediate risk of death as the event occurred.
    a. This serious criterion applies if the study subject, in the view of the SI or Sponsor, is at immediate risk of death from the AE as it occurs. It does not apply if an AE hypothetically might have caused death if it were more severe.
3. Requires inpatient hospitalization for 24 hours or more or prolongation of existing hospitalization.
    a. Hospitalization for an elective procedure (including elective PEG tube/g-tube/feeding tube placement) or a routinely scheduled treatment is not an SAE by this criterion because an elective or scheduled "procedure" or a "treatment" is not an untoward medical occurrence.
4. Results in persistent or significant disability or incapacity.
    a. This serious criterion applies if the "disability" caused by the reported AE results in a substantial disruption of the subject's ability to carry out normal life functions.
5. Results in congenital anomaly or birth defect in the offspring of the subject (whether the subject is male or female).
6. Necessitates medical or surgical intervention to preclude permanent impairment of a body function or permanent damage to a body structure.
7. Important medical events that may not result in death, are not life-threatening, or do not require hospitalization may also be considered SAEs when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

An inpatient hospital admission in the absence of a precipitating, treatment-emergent, clinical AE may meet criteria for "seriousness" but is not an adverse experience, and will therefore, not be considered an SAE. An example of this would include a social admission (subject admitted for other reasons than medical, e.g., lives far from the hospital, has no place to sleep).

A serious, suspected ADR is an SAE for which, in the opinion of the SI or Sponsor, there is a reasonable possibility that the investigational product caused the event.

The SI is responsible for classifying AEs as serious or non-serious.

### 14.2. Assessment and Recording of Adverse Events

The SI will carefully monitor each subject throughout the study for possible AEs. All AEs will be documented on CRFs designed specifically for this purpose. All AEs will be collected and reported in the EDC system and compiled into reports for monthly reviewing by the Medical Monitor. The Medical Monitor shall promptly review all information relevant to the safety of the investigational product, including all SAEs. Special attention will be paid to those that result in permanent discontinuation of the investigational product being studied, whether serious or non-serious.

#### 14.2.1. Assessment of Adverse Events

At each visit (including telephone interviews), the subject will be asked if they have had any problems or symptoms since their last visit in order to determine the occurrence of AEs. If the subject reports an AE, the SI will probe further to determine:

1. Type of event
2. Date of onset and resolution (duration)
3. Severity (mild, moderate, severe)
4. Seriousness (does the event meet the above definition for an SAE)
5. Causality, relation to investigational product and disease
6. Action taken regarding investigational product
7. Outcome

#### 14.2.2. Relatedness of Adverse Event to Investigational Product

The relationship of the AE to the investigational product should be specified by the SI using the following definitions:

1. Not Related: Concomitant illness, accident or event with no reasonable association with treatment.

2. Unlikely: The reaction has little or no temporal sequence from administration of the investigational product, or a more likely alternative etiology exists.

3. Possibly Related: The reaction follows a reasonably temporal sequence from administration of the investigational product and follows a known response pattern to the suspected investigational product; the

reaction could have been produced by the investigational product or could have been produced by the subject's clinical state or by other modes of therapy administered to the subject. (Suspected ADR)

4. Probably Related: The reaction follows a reasonably temporal sequence from administration of investigational product; is confirmed by discontinuation of the investigational product or by re-challenge; and cannot be reasonably explained by the known characteristics of the subject's clinical state. (Suspected ADR)

5. Definitely Related: The reaction follows a reasonable temporal sequence from administration of investigational product; that follows a known or expected response pattern to the investigational product; and that is confirmed by improvement on stopping or reducing the dosage of the investigational product, and reappearance of the reaction on repeated exposure (Suspected ADR).

### 14.2.3. Recording of Adverse Events

All clinical AEs are recorded in the AE Log in the subject's study binder. The site should fill out the AE Log and enter the AE information into the EDC system within 48 hours of the site learning of a new AE or receiving an update on an existing AE.

**Please Note:** SAEs must be reported to the NCRI CC within 24 hours of the site learning of the SAE. This applies regardless of whether the subject is taking study drug or not. Any events of drug-induced liver injury whether classified as SAEs or not, must also be reported within 24 hours.

Entries on the AE Log (and into the EDC) will include the following: name and severity of the event, the date of onset, the date of resolution, relationship to investigational product, action taken, and primary outcome of event.

## 14.3 Adverse Events and Serious Adverse Events - Reportable Events

The following are considered reportable events and must be reported to the NCRI CC within 24 hours of the site being notified of the event.

- All events that meet the above criteria for Serious Adverse Events (SAEs)
- Dosage Changes (Dose Management)
  - Investigational Product Suspension, Reduction or Re-challenge
  - Investigational Product Discontinuation

- Key Study Events:
  - Subject Final Disposition
  - Feeding Tube Placement
  - Permanent Assisted Ventilation (PAV)*
  - Tracheostomy

- Mortality
- Pregnancy
- Diaphragm Pacing System (DPS) device implantation

* Permanent Assisted Ventilation (PAV) is defined as more than 22 hours daily of non-invasive mechanical ventilation for more than one week. The date of onset of PAV is the first day of the seven days.

# 15.0. DATA COLLECTION, MANAGEMENT AND MONITORING

## 15.1. Role of Data Management

Data Management is the development, execution and supervision of plans, policies, programs, and practices that control, protect, deliver, and enhance the value of data and information assets.

All data will be managed in compliance with NEALS policies, and applicable Sponsor and regulatory requirements. Site personnel will collect, transcribe, correct, and transmit the data onto source documents, Case Report Forms (CRFs), and other forms used to report, track and record clinical research data. Clinical sites will be monitored to ensure compliance with data management requirements and Good Clinical Practices. NCRI Data Management is responsible for developing, testing, and managing clinical data management activities.

### 15.1.1. Data Entry and Checks

The site personnel are instructed to enter information into the EDC System within 5 days of a visit. Data collection is the responsibility of the staff at the site under the supervision of the SI. During the study, the SI must maintain complete and accurate documentation for the study.

The EDC includes password protection. An edit checking and data clarification process will be put in place to ensure accuracy and completeness of the database. Logic and range checks as well as more sophisticated rules will be built into the EDC to provide immediate error checking of the data entered. The system has the capability to automatically create electronic queries for forms that contain data that are out of range, out of window, missing or not calculated correctly. The sites will only have access to the queries concerning their subjects.

### 15.1.2. Database Security

The MS SQL Server database is located on a secure database server. This server is located in a restricted area of the Partners Healthcare server farm and physical access to it is limited to authorized personnel only. Both database and Web servers are located on the Partners Healthcare network behind the firewall. Access to the data at the clinical site will be restricted and monitored through the system's software with its required log-on, security procedures and audit trail. The data will not be altered, browsed, queried, or reported via external software applications that do not enter through the protective system software. There will be a cumulative record that indicates, for any point in time, the names of authorized personnel, their titles, and a description of their access privileges. The record will be in the study documentation accessible at the site. Controls will be in place to prevent, detect, and mitigate effects of computer viruses on study data and software. The application utilizes SSL (Secure Sockets Layer) technology and 128-bit encryption to comply with requirements of 21 CFR Part 11 for Open Systems. Backups of the database will be performed nightly using the services provided by the MGH network. All PCs run virus protection software full-time and are updated with the latest virus detection strings regularly; the Windows NT server does this as well and has the additional security of scanning all e-mail for viruses before a user can even access them. All accounts are password protected and passwords must be changed on a regular basis.

In addition, the EDC system will have an extra level of password security. At study initiation, the Data Manager will set default passwords for the relevant study personnel at the study sites. When a new user logs in with the assigned username and default password for the first time, he or she will be forced to change the password to a unique one (at least six characters long), known only to the user. An ongoing paper log will be kept showing when usernames and passwords are set up, for whom, in what user capacity and when usernames are disabled. In case an employee forgets her/his password, they will submit a password request form via email to the Data Manager, who will issue a new default password. They must then go through the Change Password process. The passwords will expire every 90 days, users will then be required to go through the Change Password process. To avoid password-based software attacks, the system will lock a user for one minute if an incorrect password is provided three times in a row. A user will also be able to change the password at will if he or she feels that it may have been compromised.

### 15.1.3. Data Lock Process

The application will have the ability to lock the database to prevent any modification of data once the study is closed. Once this option is activated, every user will have Read-Only access to the data. The database can only be locked after each SI has signed off on their subjects and all queries have been resolved.

### 15.1.4. Quality Assurance

Protocol procedures are reviewed with the SI and associated personnel prior to the study to ensure the accuracy and reliability of data. Each SI must adhere to the protocol detailed in this document and agree that any changes to the protocol must be approved by the NCRI Coordination Center prior to seeking approval from the site IRB. Each SI will be responsible for enrolling only those study subjects who have met protocol eligibility criteria.

## 15.2. Study Monitoring

Study Monitors will visit each study site to review source documentation materials, ICFs, and confirm entered data and that data queries have been accurately completed, and again at a study close-out visit. Study Monitors will also verify that SAEs and protocol deviations have been reported appropriately, as required. The Study Monitors will also review clinical facilities, resources and procedures for evaluating study subjects and study drug dispensing. Subsequently, the Study Monitors will provide monitoring reports to the Project Manager and, if requested, will provide reports of protocol compliance to the Study PI and the Steering Committee. Completed ICFs from each subject must be available in the subject's file and verified for proper documentation. A document outlining the monitoring plan is provided to each Study Monitor.

## 15.3. Steering Committee

The Steering Committee (SC) is composed of the Principal Investigator of the study (serving as SC Chair), the biostatistician, and independent Investigator members of the NEALS consortium with expertise in ALS and study-related medical (e.g., renal and cardiac) conditions and priorities (trial recruitment and drug supply). The SC is responsible, along with the Study Principal Investigator, for the design of the study protocol and analysis plan, and oversees the clinical trial from protocol development to study analysis and publication.

## 15.4. Data Handling and Record Keeping

The SI is responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Dark ink is required to ensure clarity of reproduced copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. Do not erase, overwrite, or use correction fluid or tape on the original.

Source document templates (SDTs) will be provided for use and maintained for recording data for each subject enrolled in the study. Data reported in the eCRF derived from source documents should be consistent with the source documents and discrepancies should be explained. The NCRI CC will provide guidance to SIs on making corrections to the source documents and eCRFs.

### 15.4.1. Confidentiality of Data

Study subject medical information obtained by this study is confidential, and disclosure to third parties other than those noted below is prohibited. Upon the subject's permission, medical information may be given to his or her personal physician or other appropriate medical personnel responsible for his or her welfare. All local and federal guidelines and regulations regarding maintaining study subject confidentiality of data will be adhered to.

Data generated by this study must be available for inspection by representatives of the Office for Human Research Protections (OHRP), the sponsor, all pertinent local health and regulatory authorities, the NCRI CC or their representative, Study Monitoring personnel, and the IRBs.

### 15.4.3. Retention of Records

Research records will be retained in accordance with site IRB policies.

### 15.4.4. Publications

The PI, Dr. Michael Weiss, will be responsible for publications of results from this trial. This responsibility will include the following:

- Analyze and interpret data gathered in this study, and write publications from these data.
- Submit manuscripts to selected journals and address peer reviewers' comments.
- Submit abstracts to selected meetings and present data at the meetings.
- Determine authorship on the basis of the Uniform Requirements for Manuscripts.

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## 17.0. APPENDICES

Appendix 1 Revised El Escorial Criteria (ECC)
Appendix 2 Revised ALS Functional Rating Scale (ALSFRS-R)
Appendix 3 NEALS Sample Repository Banking Policy
Appendix 4 Visual Analog Scale
Appendix 5 Edinburgh Handedness Inventory Short Form

## Appendix 1 Revised El Escorial Criteria (ECC)

### El Escorial Criteria

The diagnosis of Amyotrophic Lateral Sclerosis [ALS] requires:

A - the presence of:

(A:1) evidence of lower motor neuron (LMN) degeneration by clinical, electrophysiological or neuropathologic examination,

(A:2) evidence of upper motor neuron (UMN) degeneration by clinical examination, and

(A:3) progressive spread of symptoms or signs within a region or to other regions, as determined by history or examination, together with

B - the absence of:

(B:1) electrophysiological and pathological evidence of other disease processes that might explain the signs of LMN and/or UMN degeneration, and

(B:2) neuroimaging evidence of other disease processes that might explain the observed clinical and electrophysiological signs.

**CLINICAL STUDIES IN THE DIAGNOSIS OF ALS**

A careful history, physical and neurological examination must search for clinical evidence of UMN and LMN signs in four regions [brainstem, cervical, thoracic, or lumbosacral spinal cord] (see Table 1) of the central nervous system [CNS]. Ancillary tests should be reasonably applied, as clinically indicated, to exclude other disease processes. These should include electrodiagnostic, neurophysiological, neuroimaging and clinical laboratory studies. Clinical evidence of LMN and UMN degeneration is required for the diagnosis of ALS. The clinical diagnosis of ALS, without pathological confirmation, may be categorized into various levels of certainty by clinical assessment alone depending on the presence of UMN and LMN signs together in the same topographical anatomic region in either the brainstem [bulbar cranial motor neurons], cervical, thoracic, or lumbosacral spinal cord [anterior horn motor neurons]. The terms Clinical Definite ALS and Clinically Probable ALS are used to describe these categories of clinical diagnostic certainty on clinical criteria alone:

A. Clinically Definite ALS is defined on clinical evidence alone by the presence of UMN, as well as LMN signs, in three regions.

B. Clinically Probable ALS is defined on clinical evidence alone by UMN and LMN signs in at least two regions with some UMN signs necessarily rostral to (above) the LMN signs.

C. Clinically Probable ALS - Laboratory-supported is defined when clinical signs of UMN and LMN dysfunction are in only one region, or when UMN signs alone are present in one region, and LMN signs defined by EMG criteria are present in at least two limbs, with proper application of neuroimaging and clinical laboratory protocols to exclude other causes.

D. Clinically Possible ALS is defined when clinical signs of UMN and LMN dysfunction are found together in only one region or UMN signs are found alone in two or more regions; or LMN signs are found rostral to UMN signs and the diagnosis of Clinically Probably-Laboratory supported ALS cannot be proven by evidence on clinical grounds in conjunction with electrodiagnostic, neurophysiologic, neuroimaging or clinical laboratory studies. Other diagnoses must have been excluded to accept a diagnosis of clinically possible ALS.

**Table 1**

| | **Brainstem** | **Cervical** | **Thoracic** | **Lumbosacral** |
|---|---|---|---|---|
| **Lower motor neuron signs** weakness, atrophy, fasciculations | jaw, face, palate, tongue, larynx | neck, arm, hand, diaphragm | back, abdomen | back, abdomen, leg, foot |
| **Upper motor neuron signs** pathologic spread of reflexes, clonus, etc. | clonic jaw<br>gag reflex exaggerated<br>snout reflex<br>pseudobulbar features<br>forced yawning<br>pathologic DTRs<br>spastic tone | clonic DTRs<br>Hoffman reflex<br>pathologic DTRs<br>spastic tone<br><br>preserved reflex in weak wasted limb | loss of superficial abdominal reflexes<br>pathologic DTRs<br>spastic tone | clonic DTRs - extensor plantar response<br>pathologic DTRs<br>spastic tone<br><br>preserved reflex in weak wasted limb |

## Appendix 2 ALSFRS-R

**QUESTIONS:** **SCORE:**

**1.Speech**
4 = Normal speech processes
3 = Detectable speech disturbances
2 = Intelligible with repeating
1 = Speech combined with nonvocal communication
0 = Loss of useful speech

**2. Salivation**
4 = Normal
3 = Slight but definite excess of saliva in mouth; may have nighttime drooling
2 = Moderately excessive saliva; may have minimal drooling
1 = Marked excess of saliva with some drooling
0 = Marked drooling; requires constant tissue or handkerchief

**3. Swallowing**
4 = Normal eating habits
3 = Early eating problems – occasional choking
2 = Dietary consistency changes
1 = Needs supplemental tube feeding
0 = NPO (exclusively parenteral or enteral feeding)

**4. Handwriting**
4 = Normal
3 = Slow or sloppy; all words are legible
2 = Not all words are legible
1 = No words are legible but can still grip a pen
0 = Unable to grip pen

**5a. Cutting Food and Handling Utensils (patients without gastrostomy)**
4 = Normal
3 = Somewhat slow and clumsy, but no help needed
2 = Can cut most foods, although clumsy and slow; some help needed
1 = Food must be cut by someone, but can still feed slowly
0 = Needs to be fed

**5b. Cutting Food and Handling Utensils (alternate scale for patients with gastrostomy)**
4 = Normal
3 = Clumsy, but able to perform all manipulations independently
2 = Some help needed with closures and fasteners
1 = Provides minimal assistance to caregivers
0 = Unable to perform any aspect of task

**6. Dressing and Hygiene**
4 = Normal function
3 = Independent, can complete self-care with effort or decreased efficiency
2 = Intermittent assistance or substitute methods

1 = Needs attendant for self-care
0 = Total dependence

**7. Turning in Bed and Adjusting Bed Clothes** ☐
4 = Normal function
3 = Somewhat slow and clumsy, but no help needed
2 = Can turn alone, or adjust sheets, but with great difficulty
1 = Can initiate, but not turn or adjust sheets alone
0 = Helpless

**8. Walking** ☐
4 = Normal
3 = Early ambulation difficulties
2 = Walks with assistance
1 = Nonambulatory functional movement only
0 = No purposeful leg movement

**9. Climbing Stairs** ☐
4 = Normal
3 = Slow
2 = Mild unsteadiness or fatigue
1 = Needs assistance
0 = Cannot do

**R-1. Dyspnea** ☐
4 = None
3 = Occurs when walking
2 = Occurs with one or more of the following: eating, bathing, dressing
1 = Occurs at rest, difficulty breathing when either sitting or lying
0 = Significant difficulty, considering using mechanical respiratory support

**R-2 Orthopnea** ☐
4 = None
3 = Some difficulty sleeping at night due to shortness of breath, does not routinely use more than two pillows
2 = Needs extra pillow in order to sleep (more than two)
1 = Can only sleep sitting up
0 = Unable to sleep without mechanical assistance

**R-3 Respiratory Insufficiency** ☐
4 = None
3 = Intermittent use of BiPAP
2 = Continuous use of BiPAP during the night
1 = Continuous use of BiPAP during the night and day
0 = Invasive mechanical ventilation by intubation or tracheostomy

**Total Score: _____**

## Appendix 3 NEALS BioRepository Committee Policies and Procedures

**October 2nd, 2013**

**Mission of the Committee**

Our mission is to provide investigators with patient derived samples linked to clinical information that can be used to advance our understanding of disease mechanisms and therapies for ALS. However, there are a limited number of samples and to safeguard their distribution NEALS established a scientific review committee with specific review criteria to evaluate requests. The BioRepository Committee of the Northeast ALS Consortium reviews all applications. Applications are judged on potential impact of study, research design and investigator.

**Applications**

The application requires a brief description and scientific justification for the use of the samples. A NIH biosketch for the PI should also be provided. The investigator can request samples from specific studies. A list of studies, sample processing, age ranges/gender of subjects and dates collected is provided with the application form.

There are a very limited number of samples and to help evaluate their distribution NEALS established a scientific review committee and specific review criteria to evaluate requests. The BioRepository Committee of the Northeast ALS Consortium reviews and approves applications. Applications will be accepted at any time, but the committee meets every other month to review applications. Applications must be submitted 2 weeks prior to the meeting date. Applicants can expect a response from the committee within 7 days after a committee meeting.

Priority will be given to members of NEALS and investigators from sites that participated in the collection of samples. Investigators must provide IRB approval from their institution.

NEALS will make every effort to send approved samples approximately three weeks after the request has been accepted. Should the request come from a commercial application, samples will be sent pending a Material Transfer Agreement (MTA) with Massachusetts General Hospital, where NEALS/NCRI samples are stored.

**Application Scoring:**

Each application will be scored during its review by the BioRepository Committee based on the strengths and weaknesses of the following criteria:

- **Impact**: *The significance of developing biomarkers and potential for understanding disease mechanisms is understood. Reviewers should use this category to best judge the feasibility and likelihood of success of the proposed project as well as its potential to make a difference and move the field forward.*

- **Research Approach**: *Evaluation of approach- Is there appropriate use of techniques and technology? Does the experimental design include appropriate controls? Is the ability to interpret data present?*

- **Investigator:** *Reviewers should consider the experience, expertise and past productivity of the research team. Additional consideration is given to whether the applicant is a*

*member of NEALS and participated and contributed to the collection of samples for the repository.*

**Administrative Processes and Fees**
NEALS collects an administrative fee of $1,000 at the time of application submission to offset processing costs. Applications will not be reviewed until payment is received. If an application for samples is denied, 80% of the administrative fee will be returned. The administrative fee is waived for NEALS members. Checks may be made payable to: The Northeast ALS Consortium. Non-academic requests will require a Material Transfer Agreement (MTA) with Massachusetts General Hospital, where NEALS/NCRI samples are stored. Academic request will require a transfer letter. All investigators, including NEALS members, are required to cover any shipping costs associated with the sample request.

**Research and Publications**
NEALS requires that all journal publications that result from the use of NEALS samples acknowledge the "NEALS BioRepository" as their sample source. The committee also strongly encourages that researchers using NEALS samples contact the Sponsor/PI(s) who initially collected the samples and invite them to be co-authors/collaborators on the research. You will find study Sponsor/PI information at the end of this document.

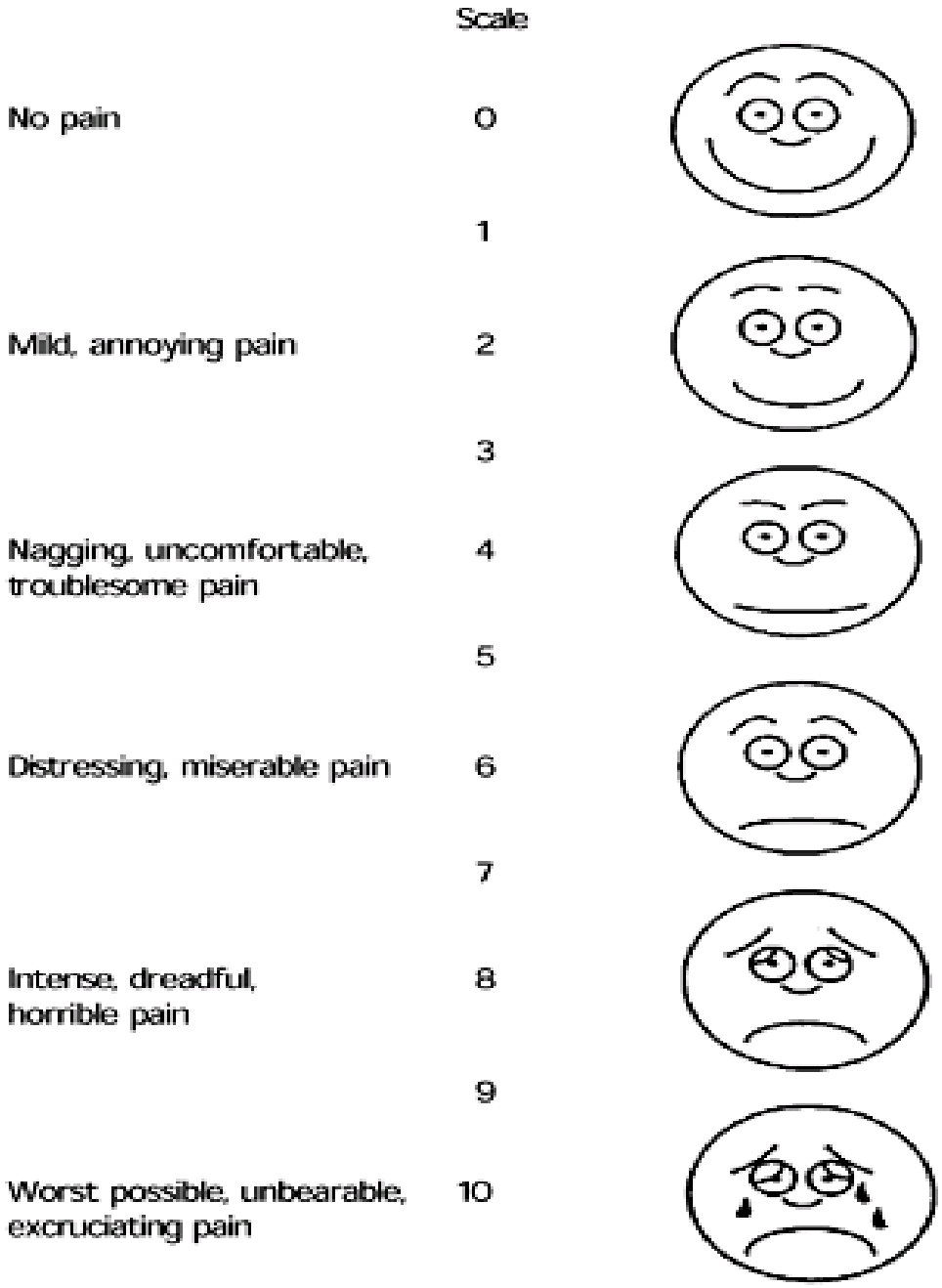
**Follow-Up to NEALS Sample Repository Requests**
The NEALS BioRepository Committee will send all applicants whose requests have been approved a simple form to fill out five months after they have received their requested samples. This form will serve as a progress report and should be completed and sent to the Committee **six months** after the initial request is approved.

**NEALS BioRepository Policy to Confirm Reproducibility of Approaches**
Before samples are sent from the NEALS repository for an assay/lab combination that has not been used before or has insufficient preliminary data regarding assay characteristics, special samples are sent for 'pilot studies' to test the accuracy and reproducibility of the measurement at a particular lab. These studies will be small; the total number of samples is generally less than 30, and the samples generally do not come from the main pool of participant samples. Duplicate samples from the same individual will be sent blinded to the outside lab for analysis, in order to assess the measurement error, or reproducibility of the lab's assay.

**NEALS BioRepository Policy to Send Blinded-Samples to Investigators**
It is NEALS policy that all samples are sent blinded to investigators. Once the experiments are complete, a data set should be generated and sent to NEALS. NEALS in turn will unblind the samples and return the information to the investigator. It is not NEALS intent to interpret or evaluate the investigator's data, but to insure consistency with how samples are released.

| Trial Name | Study Principle Investigator |
|---|---|
| A Clinical Trial of Topiramate in ALS | Merit Cudkowicz (mcudkowicz@partners.org) |
| A Clinical Trial of Celebrex in Subjects with ALS | |
| A Clinical Trial of Coenzyme Q10 in Patients with Amyotrophic Lateral Sclerosis | Merit Cudkowicz (mcudkowicz@partners.org) |
| A Multicenter, Dose-Ranging, Safety & Pharmacokinetics Study of Arimoclomol in ALS – Phase IIa | Merit Cudkowicz (mcudkowicz@partners.org)<br>Jeremy Shefner (shefnerj@upstate.edu)<br>Robert Brown (robert.brown@umassmed.edu) |
| A Multicenter Study for the Validation of ALS Biomarkers | Merit Cudkowicz (mcudkowicz@partners.org)<br>Robert Bowser (robert.bowser@dignityhealth.org) |
| Determination of Biological Markers in Cerebrospinal Fluid of Subjects with Amyotrophic Lateral Sclerosis | Merit Cudkowicz (mcudkowicz@partners.org)<br>Robert Bowser (robert.bowser@dignityhealth.org) |
| Determinants of Disease Severity in Amyotrophic Lateral Sclerosis | Merit Cudkowicz (mcudkowicz@partners.org) |
| Application of a product enhanced reverse transcriptase (PERT) assay to search for evidence of retroviral involvement in amyotrophic lateral sclerosis (ALS) | Merit Cudkowicz (mcudkowicz@partners.org) |
| Metabolomic Signatures in Amyotrophic Lateral Sclerosis | Merit Cudkowicz (mcudkowicz@partners.org) |
| Identification of Diagnostic Biomarkers and Therapeutic Targets for Amyotrophic Lateral Sclerosis | Merit Cudkowicz (mcudkowicz@partners.org)<br>Robert Bowser (robert.bowser@dignityhealth.org) |
| Validation of ALS Metabolomic Biomarkers and Development of ALS Diagnostics | Merit Cudkowicz (mcudkowicz@partners.org) |
| Clinical Trial of Ceftriaxone in Subjects with ALS | Merit Cudkowicz (mcudkowicz@partners.org) |

## Appendix 4 Visual Analog Scale (Pain Scale)



## Appendix 5 Edinburgh Handedness Inventory- Short Form

### Edinburgh Handedness Inventory - Short Form

Please indicate your preferences in the use of hands in the following activities or objects:

| | Always right | Usually right | Both equally | Usually left | Always left |
|---|---|---|---|---|---|
| Writing | ☐ | ☐ | ☐ | ☐ | ☐ |
| Throwing | ☐ | ☐ | ☐ | ☐ | ☐ |
| Toothbrush | ☐ | ☐ | ☐ | ☐ | ☐ |
| Spoon | ☐ | ☐ | ☐ | ☐ | ☐ |

**Scoring:**

For each item: Always right = 100; Usually right = 50; Both equally = 0; Usually left = -50; Always left = -100

To calculate the Laterality Quotient add the scores for the four items in the scale and divide this by four:

| | |
|---|---|
| Writing score | |
| Throwing score | |
| Toothbrush score | |
| Spoon score | |
| Total | |
| Total ÷ 4 (Laterality Quotient) | |

| Classification: | Laterality Quotient score: |
|---|---|
| Left handers | -100 to -61 |
| Mixed handers | -60 to 60 |
| Right handers | 61 to 100 |