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1. Introduction

This Statistical Analysis Plan (SAP) defines the outcome measures and analysis samples and specifies the planned analyses of data from Dr. Michael Weiss's Mexiletine II trial. This SAP is specific to analysis of data related to the ALS participants in the randomized intervention trial. The SAP supplements the clinical protocol. Please refer to the clinical protocol for details on the rationale for the intervention, eligibility criteria, conduct of the trial, clinical assessments and the timing of their use in the trial, definitions and reporting of adverse events, data management conventions, and regulatory oversight and compliance procedures. In case of discrepancies between the SAP and the clinical protocol concerning matters of analysis, the SAP is authoritative. On all other matters, the clinical protocol is authoritative.

2. Study Design

2.1 Overview

This is a multicenter, double-blind, placebo-controlled, three-arm, parallel-group, phase 2 randomized controlled trial to examine the effect of two dosages of mexiletine on neuronal excitability in ALS patients. Trial participation includes a screening visit, randomization to placebo, 300 mg/day oral mexiletine, or 600 mg/day oral mexiletine, 4 weeks of follow-up on study drug, and 4 weeks of follow-up after last dose of study drug. The trial is registered at Clinicaltrials.gov as study NCT02781454 (see <https://clinicaltrials.gov/ct2/show/NCT02781454>).

2.2 Study Objectives

The primary objective of the trial 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 as assessed by change in resting motor threshold (RMT) estimated from single-pulse transcranial magnetic stimulation (TMS).

Secondary objectives include evaluating the effect of mexiletine on additional pharmacodynamic markers of cortical hyperexcitability, pharmacodynamic markers of peripheral motor nerve axonal hyperexcitability by threshold tracking nerve conduction studies (TTNCS), and frequency and severity of muscle cramps and fasciculations and evaluating the safety, tolerability, and blood concentration of mexiletine.

Exploratory objectives include evaluating the effect of mexiletine on measures of clinical progression and quality of life.

2.3 Study Population

Individuals eligible for trial participation are men or women at least 18 years old who meet the El Escorial criteria of possible, laboratory-supported probable, probable, or definite criteria for a diagnosis of sporadic ALS received within 60 months of screening who have a slow vital capacity (SVC) at least 50% of that expected based on age, sex, and height, a measurable RMT of the abductor pollicis brevis (APB) by TMS, and a median nerve compound motor action potential (CMAP) by TTNCS of at least 1.5 mV. Detailed inclusion and exclusion criteria are specified in the clinical protocol.

Participants will be recruited from approximately 9 clinical sites located through the US that are part of the Northeast ALS (NEALS) Consortium.

2.4 Participant Flow

After a site is activated for recruitment of ALS participants and after each ALS patient provides informed consent and is determined eligible, approximately 60 participants will be randomized to receive placebo, 300 mg/day oral mexiletine, or 600 mg/day oral mexiletine in a 1:1:1 ratio, stratified by clinical site and use of riluzole at screening. Participants are first dose of study drug is administered at the baseline visit and are intended to remain on study drug for 4 weeks. After baseline, participants receive a telephone call at week 1 and complete in-clinic evaluations at weeks 4 and 8. Participants who discontinue study drug should remain on study following the normal schedule of assessments. Patients withdrawing consent are asked to delay withdrawing consent until after they return for a Final Safety Visit within 2 weeks after their last dose of study drug and after they complete a final telephone call 4 weeks after their last dose of study drug.

Detailed descriptions of study procedures and timing are specified in the clinical protocol.

2.5 Treatment Allocation

Prior to the baseline visit, eligible participants are randomly allocated in equal proportions to one of three treatment groups (placebo, 300 mg/day oral mexiletine, or 600 mg/day oral mexiletine) according to a permuted-block randomization schedule, stratified by site and use of riluzole at screening. The randomization schedule was prepared by computer program by the unblinded study statistician.

2.6 Treatment Administration

Study drug will be orally self-administered from 2 bottles containing either 150 mg capsules of mexiletine or placebo capsules matched for size, color, and presentation. All participants will take one capsule for bottle #1 at their baseline visit (day 1) and on the morning of day 2, one capsule from bottle #1 BID from day 3 until their week 4 visit, one capsule from bottle #2 on the morning of days 5 and 6, and one capsule from bottle #2 BID from day 7 until their week 4 visit.

In the placebo arm, bottles #1 and #2 will contain placebo capsules, and the dosage of mexiletine will be 0 mg/day throughout their participation. In the 300 mg/day arm, bottle #1 will contain 150 mg capsules of mexiletine and bottle #2 will contain placebo. Dosage of mexiletine will be titrated as follows: 150 mg at their baseline visit, 150 mg on the morning of day 2, and 150 mg BID (300 mg/day) from day 3 until their week 4 visit. In the 600 mg/day arm, bottles #1 and #2 will contain 150 mg capsules of mexiletine. Dosage of mexiletine will be titrated as follows: 150 mg at their baseline visit, 150 mg on the morning of day 2, 150 mg BID (300 mg/day) on days 3 and 4, 300 mg QAM and 150 mg QPM (450 mg/day) on days 5 and 6, and 300 mg BID (600 mg/day) from day 7 until their week 4 visit.

2.7 Allocation Concealment

The randomization schedule is known only by the unblinded study statistician who generated the schedule and by the research pharmacist at each site. Concealment of the true treatment allocation of specific participants is achieved by using anonymous subject identifiers to link participants with specific study drug kits, by over-encapsulating study drug in #00 Swedish Orange Opaque (4188) capsules, and by using matching titration schedules. Clinical members of the Steering Committee, site investigators and other site staff, clinical coordination and data

management staff, the medical monitor, and all participants are blinded to participant treatment allocations.

2.8 Schedule of Assessments

Activity	Screening Visit ¹	Baseline Visit ²		Treatment Period			Final Safety Visit ¹²
		Pre-Dose	Post-Dose	Week 1 Call	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) procedures.

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 the informed 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.

¹² The Final Safety Visit is only completed by participants who discontinue study drug prior to their Week 4 visit.

3. Statistical Methodology

3.1 General Considerations

3.1.1 Statistical Software

All statistical analyses will be performed using SAS (SAS Institute, NC, USA) or R (R Foundation for Statistical Computing, Vienna, Austria).

3.1.2 Summary Statistics

Data will be summarized with respect to disposition, demographics, pre-treatment characteristics, safety outcomes, tolerability, and TMS, TTNCS, pharmacologic, clinical, and quality of life outcomes. Summary statistics for continuous variables will include the number of subjects, the mean, median, standard deviation, and range. For categorical data, summaries will include counts and percentages.

3.1.3 Precision

Results will generally be reported to 3 significant figures. Percentages will generally be reported to 0.1 percentage points. P-values will be reported to two digits when greater than or equal to 0.095, to three digits when greater than or equal to 0.00095 and less than 0.095, and as <0.001 for all smaller values.

3.2 Study Endpoints

3.2.1 Pharmacodynamic Endpoints

The primary pharmacodynamic endpoint is 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.

Secondary pharmacodynamic endpoints include change in the following measures made before treatment, after 4 weeks of treatment, and then again after a 4-week washout.

TMS measures:

- Maximum MEP amplitude (as a percentage of maximum CMAP),
- Slope of the input/output curve describing the relationship between stimulus strength (in percent of maximum stimulator output) and motor evoked potential (MEP) amplitude (in mV),
- Cortical silent period (CSP).

TTNCS measures:

- Strength duration time-constant (SDTC),
- Depolarizing and hyperpolarizing electrotonus at 90 to 100 ms (TEd(90-100 ms) and TEh(90-100 ms)),
- Recovery cycle measures of relative refractory period (RRP), superexcitability, and subexcitability, and
- Compound motor action potential (CMAP).

Exploratory pharmacodynamic measures include: intracortical facilitation (ICF) by TMS; rheobase, TEd(40-60 ms), TEh(10-20 ms), TEd(peak), subexcitability after second pre-pulse, and stimulus for 50% maximum response assessed by TTNCS.

3.2.2 Clinical and Quality of Life Endpoints

Exploratory clinical and quality of life endpoints include change in the following measures made before treatment, after 4 weeks of treatment, and then again after a 4-week washout.

Clinical measures:

- ALS Functional Rating Scale-Revised (ALSFRS-R) total score,
- Slow vital capacity (SVC),
- Abductor pollicis brevis (APB) muscle strength by hand-held dynamometry (HHD),
- Muscle cramping frequency and pain, and
- Fasciculation occurrence and interference with daily life.

Quality of Life measures:

- Physical and mental summary scores from the RAND-36 Health Survey.

3.2.3 Safety Endpoints

The following safety endpoints will be evaluated:

- Proportion of participants experiencing and number of unique events of each type of TEAE and serious TEAE classified by MedDRA system organ class and preferred term,
- Proportion of participants experiencing and number of unique events of TEAEs classified by seriousness, severity, relatedness to study drug, relatedness to TMS and TTNCS procedures, action taken with study drug, action taken with TMS/TTNCS, and outcome, summarized across all MedDRA terms,
- Instances of treatment-emergent and clinically significant laboratory abnormalities classified by assay, whether the abnormality is below or above the normal range,
- Mean change from baseline in weight
- Proportion of participants experiencing treatment-emergent and clinically significant ECG abnormalities by type of finding
- Proportion of participants experiencing treatment-emergent and clinically significant physical examination findings by body system
- Proportion of participants experiencing treatment-emergent neurological examination findings by type of finding

Reported proportions will use as their denominator all participants in the Safety and Tolerability sample (see Section 3.5 below).

3.2.4 Tolerability Endpoint

Participants will be judged tolerant of study drug if they reached their target dose and remain on study drug until planned discontinuation. Tolerability will be summarized separately for freedom from intolerance due to an AE and intolerance for any reason, in each case as the proportion of participants in a treatment group who are tolerant of study drug. The proportion of participants who report willingness to participate in a future trial that used TMS and TTNCS on the Exit Survey will be reported. Reported proportions will use as their denominator all participants in the Safety and Tolerability sample (see Section 3.5 below).

3.3 Measurement Definitions

3.3.1 TMS Measures

Transcranial magnetic stimulation (TMS) is a neurophysiologic test for assessing upper motor neuron function. A magnetic coil is positioned on the head above the motor cortex at a location that causes excitation of the abductor pollicis brevis (APB) muscle in a participant's dominant hand. High-frequency sampling of motor evoked potentials (MEP) in the APB is used to record neurophysiologic response to specific magnetic field strengths and sequences. The MEP traces are used to calculate a range of derived measures. MEPs that were below the limit of detect and recorded as zero mV were replaced by 0.005 mV.

- Resting motor threshold (RMT): RMT is the magnetic field strength, measured as a percentage of the maximum stimulator output, that produces at least a 0.05 mV response in at least 5 of 10 consecutive trials.

- Maximum MEP amplitude: Maximum MEP amplitude is defined as the maximum MEP elicited as a percentage of the maximum CMAP.
- Input/Output curve parameters: MEPs elicited by stimuli ranging in strength from 60% of RMT to 150% of RMT follow a sigmoidal curve with upper and lower thresholds and a smooth, symmetric transition between the thresholds. A four-parameter logistic function fit to the input/output curve after log-transformation of output potentials yields estimates of maximum amplitude, minimum amplitude, midpoint of activation, and slope at the midpoint of activation.
- Cortical silent period (CSP): CSP is the suppression of voluntary muscle contraction elicited by stimulation equal to 120% of RMT. CSP duration is measured from the time of the muscle activity suppression to return of muscle activity.
- Short-interval intracortical inhibition (SICI): Paired-pulse SICI is defined as the ratio of the response after a conditioning pulse equal to 80% of RMT is administered 3 ms prior to the signaling pulse divided by MEP amplitude. SICI is calculated as the geometric mean of replicate estimates.
- Intracortical facilitation (ICF): Paired-pulse ICF is defined as the ratio of the response after a conditioning pulse equal to 80% of RMT is administered 15 ms prior to the signaling pulse divided by MEP amplitude. ICF is calculated as the geometric mean of replicate estimates.

TMS tracings are centrally reviewed and processed. The central reader indicates the reliability of the overall TMS assessment and of individual measures using a set of quality flags:

- Overall quality: (0) poor quality, (1) acceptable quality, (2) raw data has no traces
- RMT estimation: (-1) RMT is missing, (0) poor quality estimate of RMT, (1) acceptable quality estimate of RMT, (2) RMT is greater than 100% of maximum stimulator output
- Input/Output curve estimation: (-1) no input/output traces obtained, (0) poor quality, (1) acceptable quality
- CSP estimation: (-1) no CSP traces obtained, (0) poor quality, (1) acceptable quality, (3) acceptable quality

TMS assessments that were evaluated as of acceptable quality overall and of acceptable quality for each category of measurement are used for primary analysis.

3.3.2 TTNCS Measures

Threshold-tracking nerve conduction studies (TTNCS) is a neurophysiologic test for assessing lower motor neuron function. The following measures were obtained using standard procedures (Kiernan et al. 2000): strength-duration measures of strength duration time-constant (SDTC) and rheobase; threshold electrotonus measures of depolarizing threshold electrotonus at 40 to 60 ms [TEd (40-60 ms)], 90 to 100 ms [TEd (90-100 ms)], and at peak [TEd (peak)] and hyperpolarizing threshold electrotonus at 10 to 20 ms [TEh (10-20 ms)] and 90 to 100 ms [TEh (90-100 ms)]; recovery cycle measures of RRP, superexcitability, subexcitability after first pre-pulse, and subexcitability after second pre-pulse; peak amplitude measures of compound motor evoked potential (CMAP) and stimulus for 50% maximum response.

TTNCS tracings are centrally reviewed and processed. A central reader indicates the reliability of the overall TTNCS assessment and of individual measures using a set of quality flags. For primary analysis, all measures were excluded if the overall quality flag indicated that the full assessment was unusable. Otherwise, acceptable quality for primary analysis was separately indicated for peak amplitude (CMAP, stimulus for 50% maximum response), strength-duration measures (SDTC, rheobase), threshold electrotonus (TEd (40-60 ms), TEd (90-100 ms), TEd (peak), TEh (10-20 ms), TEh (90-100 ms)), RRP, superexcitability, and subexcitability (after first and second pre-pulse).

3.3.3 ALSFRS-R

The ALSFRS-R (Cedarbaum et al. 1999) is a 12-item clinician-completed interview scale for assessing participants' function in four domains: bulbar, fine motor, gross motor, and respiratory. Each item is scored from 0 to 4 with higher scores indicating greater function. The ALSFRS-R total score is the sum of all items (range 0 to 48).

3.3.4 SVC

Slow vital capacity (SVC) is the maximum volume of air that can be slowly exhaled after slow, maximal inhalation. Trained technicians coach participants through 3 to 5 maneuvers using a spirometer. A minimum of 3 completed SVC maneuvers are required to calculate an estimate of maximum SVC for a given participant visit. The maximum volume expired is converted to percent of predicted normal. Normal values are calculated based on sex, age, and height using equations published by Knudsen et al. (1983).

3.3.5 APB Strength

Bilateral abductor pollicis brevis (APB) muscle strength will be measured quantitatively by hand-held dynamometry (HHD).

3.3.6 Cramping

Frequency of muscle cramping and maximum pain from muscle cramping is collected at baseline by retrospective report of the prior 7 and 30-day intervals. Prospective muscle cramping and pain from muscle cramping is recorded starting at baseline by participants in daily diaries and then summarized as weekly summaries of total number of cramps experienced and maximum and average daily maximum pain from muscle cramping.

3.3.7 Fasciculations

Data on muscle fasciculations are captured at baseline by retrospective report of the prior 14-day interval. Data are captured on the experience of any fasciculations, the average duration of fasciculations, the association of fasciculations with exertion or times of rest, the degree to which fasciculations interfere with daily activities, and interference of fasciculations with sleep. Prospective data on fasciculations are recorded starting at baseline by participants in daily diaries and then summarized as weekly summaries indicating the number of days with no fasciculations, with fasciculations after exertion that did not substantially interfere with daily activities, with fasciculations at rest or after exertion that did not substantially interfere with daily activities, and fasciculations that substantially interfered with daily activities.

3.3.8 RAND-36

The RAND-36 (Hays et al. 1993, 2001) is a 36-item, self-reported, generic profile, health-related quality of life instrument. Eight health domains are assessed: physical functioning (10 items), role limitations caused by physical health problems (4 items), role limitations caused by emotional problems (3 items), social functioning (2 items), emotional well-being (5 items), energy/fatigue (4 items), pain (2 items), and general health perceptions (5 items). An additional single item assesses change in perceived health during the last 12 months. Except for this single assessment of change during the last 12 months, the questions assess either current health or health over the previous 4 weeks. Each of the 35 items are transformed to a 0 to 100 range with 100 indicating maximal health, and items within a domain are averaged together. Two correlated physical and mental summary scores are derived from an oblique factor analysis. Higher scores indicate better health.

3.4 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 would 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, 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%.

3.5 Analysis Samples

The following analysis samples will be used for testing safety, tolerability, and efficacy endpoints:

- Safety and Tolerability (ST) Sample: ALS participants who are eligible, randomized, and take at least one dose of study drug, classified according to the actual treatment received. Observations made after premature permanent discontinuation of study drug are included in this sample, should such participants remain on study.
- Efficacy Modified Intent-to-treat (mITT) Sample: ALS participants who are eligible, randomized, and take at least one dose of study drug, classified according to their randomized treatment assignment. Observations made after premature permanent discontinuation of study drug are included in this sample, should such participants remain on study.

3.6 Baseline Characterization

Each analysis sample will be summarized overall and by treatment group for the following characteristics: randomization site; age, sex, race, ethnicity, El Escorial diagnosis, years since ALS symptom onset, delay between symptom onset and ALS diagnosis, site of ALS symptom

onset, use of riluzole at baseline, ALSFRS-R total score, SVC, APB muscle strength by HHD on the dominant hand, TMS parameters, TTNCS parameters, cramping frequency, cramping pain, fasciculation intensity, fasciculation interference with daily living, fasciculation interference with sleep, and RAND-36 physical and mental summary scores. The magnitude of differences between treatment groups will be summarized using Fisher's exact tests for nominal variables and two-sample t-tests for approximately continuous variables. Note that the p-values yielded by these tests do not estimate the probability that the observed differences are due to chance (which is 1 axiomatically).

3.7 Pharmacodynamic and Clinical Analyses

3.7.1 Primary Analysis of the Primary Pharmacodynamic Endpoint

The primary pharmacodynamic analysis will test the effect of mexiletine on RMT in the mITT sample using a shared-baseline mixed model with fixed effects for visit (4 levels: Screening, Baseline, Week 4, and Week 6), treatment group (3 levels: placebo and 300 and 600 mg/day mexiletine) x post-baseline visit (2 levels) and random participant-specific intercepts and slopes with unstructured covariance. The interaction between fixed effects for treatment group and visit will be restricted to post-baseline visits by including a numeric indicator variable (0 pre-treatment, 1 post-treatment) in the interaction. Use of a shared baseline reflects the true state of the population sampled prior to randomization and has the advantage of adjusting for any chance differences at baseline in a manner similar to analysis of covariance (Liang and Zeger, 2000) with potentially greater efficiency with more than one follow-up visit and drop-out. Random participant-specific slopes will be estimated per month from treatment initiation. Data from Final Safety Visits will be assigned to the closed scheduled post-baseline visit. The following SAS code specifies the model:

```
proc mixed data=xxx method=reml;
  class id trtrnd visit;
  model Value = visit post*trtrnd*visit;
  random intercept month / subject=id type=un;
```

The primary estimand will be the comparison of the average change in RMT from pre-treatment to Week 4 for the two active groups vs. placebo, tested using a two-tailed Wald-test at alpha = 0.05. The estimate and its 95% confidence bounds will be obtained by a linear contrast of adjusted means. The following SAS code specifies the linear contrast (with the sort order for treatment group being placebo, 300 mg/day mexiletine, and 600 mg/day mexiletine, and the sort order for visit being chronological):

```
estimate "3|ActvsPlb|dWk 04"
  post*trtrnd*visit 0 0 -2 0  0 0 1 0  0 0 1 0 / cl divisor=2;
```

A significant mean reduction in RMT from pre-treatment to the Week 4 among participants randomized to either 300 or 600 mg/day mexiletine relative to mean changes among participants randomized to placebo would be considered evidence that mexiletine is effective in reducing cortical hyperexcitability.

3.7.2 Secondary Analyses of the Primary Pharmacodynamic Endpoint

Several secondary analyses will be investigated to assess sensitivity of our estimates of treatment effect to alternative modeling assumptions and to evaluate additional hypotheses.

- Each dose group vs. placebo: A secondary estimand of the efficacy of mexiletine will compare 300 mg/day and 600 mg/day treatment arms vs. placebo separately, each tested at two-tailed alpha = 0.027 using Dunnett's method to control for multiple comparisons. The following SAS code specifies the linear contrasts for these comparisons:

```
estimate "3|300vsPlb|dWk 04"
    post*trtrnd*visit 0 0 -1 0  0 0 1 0  0 0 0 0 / cl divisor=1 alpha=0.027;
estimate "3|600vsPlb|dWk 04"
    post*trtrnd*visit 0 0 -1 0  0 0 0 0  0 0 1 0 / cl divisor=1 alpha=0.027;
```

- 600 mg/day vs. 300 mg/day: The relative efficacy of 300 mg/day and 600 mg/day mexiletine will be compared to infer the dosage that more effectively reduces RMT. The following SAS code specifies the linear contrast for this comparison:

```
estimate "3|600vs300|dWk 04"
    post*trtrnd*visit 0 0 0 0  0 0 -1 0  0 0 1 0 / cl divisor=1;
```

- Effect of drug washout: If we observe an effect of mexiletine on change in RMT from pre-treatment to Week 4, then we will estimate the effect of washout from Week 4 to Week 8 relative to the 4-week change over that interval among placebo participants. The following SAS code specifies the linear contrasts for these comparisons:

```
estimate "3|ActvsPlb|iWk 08"
    post*trtrnd*visit 0 0 2 -2  0 0 -1 1  0 0 -1 1 / cl divisor=2;
estimate "3|300vsPlb|iWk 08"
    post*trtrnd*visit 0 0 1 -1  0 0 -1 1  0 0 0 0 / cl divisor=1;
estimate "3|600vsPlb|iWk 08"
    post*trtrnd*visit 0 0 1 -1  0 0 0 0  0 0 -1 1 / cl divisor=1;
```

3.7.3 Secondary Endpoints

Continuous secondary pharmacodynamic and exploratory clinical and quality of life endpoints will be analyzed using the same analysis samples and models as specified for the primary pharmacodynamic endpoint in Sections 3.7.1 and 3.7.2. Variables that are strongly right-skewed (e.g., maximum MEP amplitude) will be log-transformed prior to analysis, and estimates will be back-transformed for reporting.

Weekly cramping frequency data will be analyzed in similar generalized mixed models treating the number of cramps experienced in a week as a negative binomial random variable with log link and with the log of the number of days at risk for cramps as an offset. Similarly, weekly number of days with fasciculations will be analyzed in similar generalized mixed models treating the number of days with fasciculations in a week as a binomial random variable with logit link. Because diary data are only collected after baseline, fixed effects in the model will include treatment group, post-baseline week, and baseline retrospective report of number of cramps experienced in the prior 30-day interval or the experience of fasciculations in the prior 14-day interval.

3.7.4 Subgroup Analyses

Differences in treatment efficacy will be explored in several pre-defined subgroups: baseline RMT (median split) and baseline riluzole use (yes vs. no). Subgroup specific estimates will be obtained by including subgroup, subgroup \times visit, and subgroup \times treatment \times post-baseline visit terms to models for primary and secondary pharmacodynamic outcomes using the mITT sample.

3.7.5 Associations between Muscle Symptoms and TTNCS Parameters

Associations between frequency and intensity of muscle cramps and fasciculations and TTNCS parameters will be assessed at baseline and longitudinally and with respect to response to mexiletine. At baseline, Spearman correlations will be calculated between measures of muscle cramp frequency and pain and fasciculation frequency and interference and TTNCS parameters. Longitudinally, Spearman correlations will be calculated between rates of change in muscle cramp frequency and pain and fasciculation frequency and interference and rates of change in TTNCS parameters. To test whether any reduction in frequency and intensity of muscle cramps and fasciculations resulting from treatment with mexiletine is associated with changes in TTNCS parameters, we will estimate whether 4-week changes in TTNCS parameters mediate any effect of mexiletine on 4-week changes in muscle cramp frequency and pain and fasciculation frequency and interference using a causal mediation framework for analysis (Valeri et al. 2013).

3.7.6 Multiplicity Adjustments

A single primary comparison is planned, avoiding any need to adjust p-values for multiple comparisons. The secondary analysis of each dose of mexiletine on the primary pharmacodynamic outcome will be tested at two-tailed $p < 0.027$ following Dunnett's correction for comparing two active treatments to placebo.

3.7.7 Missing Data

The planned mixed model yields estimates that are unbiased conditional on the observed scores under a missing at random assumption. Sensitivity analyses may be pursued to impute missing values or otherwise construct models for unobserved outcomes if more than 20% of participants are missing follow-up data for any reason.

3.8 Safety Analysis

3.8.1 Treatment-emergent Adverse Events

The incidence of TEAEs will be summarized by the number of events of a given classification experienced by participants in each treatment group and by the number and proportion of participants experiencing such an event in each treatment group in the ST sample. TEAEs will be summarized in aggregate across all MedDRA terms and separately by MedDRA system organ class and preferred term.

Aggregate summaries of TEAE grade will include characteristics of: (a) seriousness, (b) severity, (c) relatedness to study drug, (d) relatedness to TMS, (e) relatedness to TTNCS, (f) action taken with study drug, (g) action taken with study procedure, and (h) outcome. For each level of a given TEAE characteristic, summaries will include the number of events of a given classification and by the number and proportion of participants for which that level of a characteristic was the worst they experienced (treating any unknown characteristic as not worst).

3.8.2 Safety Labs

The absolute level and the absolute change from baseline for each safety laboratory assay will be summarized as means, standard deviations, medians, and ranges at each visit by treatment group. The proportion of participants with abnormal and clinically significant safety lab levels will be summarized by treatment group by visit and at any post-baseline visit.

3.8.3 Vital Signs and Weight

The absolute level and the absolute change from baseline for vital signs and weight will be summarized as means, standard deviations, medians, and ranges at each visit by treatment group.

3.8.4 Physical and Neurological Examinations

The proportion of participants with any treatment-emergent and clinically significant physical or neurological findings will be summarized by treatment group.

3.9 Other Analyses

3.9.1 Participant Disposition

The number of patients who were screened, randomized, completed scheduled follow up, and prematurely withdrew study participation will be summarized overall and by treatment group. Reasons for screen failure and for withdrawal from study will be presented.

3.9.2 Study Drug Compliance and Tolerance

The number of days of exposure to study drug will be summarized by treatment group. Compliance with study drug will be calculated as the number of doses taken divided by the scheduled number of doses taken prior to permanent discontinuation, expressed as a percentage. The proportion of participants judged tolerant of study drug, both those free of intolerance due to an AE and those free of intolerance for any reason, will be summarized by treatment group.

3.9.3 Prior and Concomitant Medication Use

Concomitant medications taken during the study period will be listed for each patient, coded using the World Health Organization Drug Dictionary Enhanced. The percentage of patients taking each class of medications will be summarized overall and by treatment group.

4. References

Cedarbaum JM, Stambler N, Malta E, Fuller C, Hilt D, Thurmond B, Nakanishi A. The ALSFRS-R: a revised ALS functional rating scale that incorporates assessments of respiratory function. BDNF ALS Study Group (Phase III). *J Neurol Sci.* 1999 Oct 31;169(1-2):13-21.

Hays RD, Sherbourne CD, Mazel RM. The RAND 36-Item Health Survey 1.0. *Health Econ.* 1993 Oct;2(3):217-27.

Hays RD, Morales LS. The RAND-36 Measure of Health-Related Quality of Life. *Ann Med.* 2001 Jul;33(5):350-357.

Kiernan MC1, Burke D, Andersen KV, Bostock H. Multiple measures of axonal excitability: a new approach in clinical testing. *Muscle Nerve.* 2000 Mar;23(3):399-409.

Knudson RJ, Lebowitz MD, Holberg CJ, Burrows B. Changes in the normal maximal expiratory flow-volume curve with growth and aging. *Am Rev Respir Dis.* 1983 Jun;127(6):725-734.

Liang KY, Zeger S. Longitudinal data analysis of continuous and discrete responses for pre-post designs. *Sankhyā Ser B.* 2000 Apr;62(1):134-148.

Valeri L, Vanderweele TJ. Mediation analysis allowing for exposure-mediator interactions and causal interpretation: theoretical assumptions and implementation with SAS and SPSS macros. *Psychol Methods*. 2013 Jun;18(2):137-50.