Cover Page – Protocol

Study Official Title: A Phase 2 Pharmacodynamic Trial of Ezogabine on Neuronal Excitability in Amyotrophic Lateral Sclerosis

NCT #: NCT02450552

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A Phase 2 Pharmacodynamic Trial of Ezogabine on Neuronal Excitability in Amyotrophic Lateral Sclerosis

Regulatory Sponsor:	Brian J. Wainger, MD, PhD
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Study Product:	Ezogabine (Potiga®)/Retigabine
Version Number:	5.0
Original Date:	09 March 2015
ALS Consortium:	Northeast ALS Consortium (NEALS)

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, "A Phase 2 Pharmacodynamic Study of Ezogabine on Neuronal Excitability in Amyotrophic Lateral Sclerosis" dated **09 March 2015** (Version Number 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 guidelines identified in 21 CFR Parts 11, 50, 54, and 312, local Institutional Review Board (IRB) guidelines and policies, and the Health Insurance Portability and Accountability Act (HIPAA).

Site Investigator: _____

Signed: _____ Date: _____

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LIST OF ABBREVIATIONS

ADR	Adverse Drug Reaction
AE	Adverse Event
ANOVA	Analysis of Variance
ALS	Amyotrophic Lateral Sclerosis
ALSFRS-R	Amyotrophic Lateral Sclerosis Functional Rating Scale - Revised
APB	Abductor Pollicis Brevis
AUA	American Urological Association
BID	Twice a Day
CBC	Complete blood count
CFR	Code of Federal Regulations
CMAP	Compound motor action potential
CSF	Cerebrospinal Fluid
DSMB	Data and Safety Monitoring Board
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HHD	Hand Held Dynamometry
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
iPSC	Induced Pluripotent Stem Cell
IRB	Institutional Review Board
ITT	Intent to Treat
LMN	Lower Motor Neuron
LP	Lumbar Puncture
MEP	Motor Evoked Potential
NEALS	Northeast ALS Consortium
MOP	Manual of Procedures
NCRI CC	Neurological Clinical Research Institute Coordination Center
NCS	Nerve Conduction Studies
PHI	Protected Health Information
PI	Principal Investigator
PK	Pharmacokinetic
QA	Quality Assurance
QC	Quality Control
QD	Once a Day

SAE	Serious Adverse Event
SI	Site Investigator
SICI	Short-Interval Intracortical Inhibition
SMaRT	Safety Management Resource Team
SOP	Standard Operating Procedure
SVC	Slow Vital Capacity
TID	Three Times a Day
TMS	Transcranial Magnetic Stimulation
UMN	Upper Motor Neuron
US	United States
WOCBP	Women of Child Bearing Potential

PROTOCOL SUMMARY

Study Title

A Phase 2 Pharmacodynamic Study of Ezogabine on Neuronal Excitability in Amyotrophic Lateral Sclerosis

Version Number

5.0

Study Indication

Amyotrophic Lateral Sclerosis (ALS)

Phase of Development

2

Rationale for the Study

A significant body of literature from human neurophysiology studies of upper and lower motor neurons supports the hypothesis that neuronal hyperexcitability may contribute to neurodegeneration in both sporadic and familial ALS, and lower motor neuron excitability indices have been found prospectively to predict survival duration in recently diagnosed sporadic ALS patients. Data from the SOD1 mouse model also support this hypothesis, and now we have found that induced pluripotent stem cell-derived (iPSC) motor neurons from ALS patients are hyperexcitable compared to control-derived motor neurons. Based on the identified mechanism of reduced delayed-rectifier potassium channel currents (discussed below), we determined that the Kv7 agonist ezogabine (retigabine/Potiga) could reduce the *in vitro* motor neuron hyperexcitability. Consistent with the importance of hyperexcitability in the ALS disease process, we also found that ezogabine improved the *in vitro* survival of ALS-derived motor neurons, returning the survival to that of control-derived motor neurons. We now propose to determine how ezogabine affects neurophysiological measures of upper and lower motor neuron excitability in ALS patients as assessed by transcranial magnetic stimulation and threshold tracking nerve conduction studies, respectively, as such studies could be used as pharmacodynamic markers of a drug's effect on blocking motor neuron hyperexcitability. We will also draw subject blood samples to obtain and characterize iPSC-derived motor neurons from select trial subjects. Thus, a phase two trial investigating ezogabine in ALS patients could both evaluate a promising drug using neurophysiological excitability indices as pharmacodynamic monitors and serve as a proof of concept study for determining how iPSC-derived motor neurons might provide clinically relevant information with minimal time delay. Despite failed trials of multiple other anti-epileptics, ezogabine's unique mechanism combined with the strong preliminary data makes it an attractive candidate for the proposed translational study. Documentation of an effect on reducing hyperexcitability in ALS subjects, as well as

validating safety and determining dose, could pave the way for a larger trial to investigate effect on ALS using a combined measure of function/survival.

Study Design

This is a multicenter, randomized, double-blind, placebo-controlled 14-week study evaluating the effect of ezogabine treatment on neuronal excitability in ALS subjects.

Study Objectives and Endpoints

The primary efficacy outcome is change in paired pulse short-interval intracortical inhibition (SICI) assessed by transcranial magnetic stimulation (TMS) after treatment with 600 mg/day or 900 mg/day of ezogabine vs. matched oral placebo. Change in motor evoked potential (MEP) threshold is the key secondary efficacy outcome. Additional upper motor neuron secondary outcomes include MEP amplitude, MEP latency, cortical silent period, and paired pulse facilitation, assessed by TMS. Lower motor neuron excitability outcomes include change in electrotonus, recovery cycle and strength duration time constant as determined by threshold tracking axonal nerve conduction studies (NCS) and CMAP amplitude. The effect of retigabine on strength will be assessed using hand held dynamometry, and muscle symptoms will also be assessed utilizing a subject muscle symptom diary, in which the subjects will record the frequency, location and severity of their muscle cramps and fasciculations over the course of study treatment. Additional secondary outcomes include dosing, tolerability and safety of ezogabine in ALS patients. Serum and optional cerebrospinal fluid (CSF) sampling will be collected (at Week 6) for limited pharmacokinetic (PK) analysis and banking.

Exploratory outcomes include *in vitro* analysis of subject iPSC-derived motor neurons and *in vitro* response of motor neuron firing to ezogabine treatment. We will also recruit approximately 72 healthy control subjects to perform quality control analysis for the neurophysiology testing (approximately 60 healthy controls) and to correlate differences between ALS subjects and healthy controls as assessed using *in vitro* measurements of hyperexcitability compared to using the *in vivo* neurophysiological ones (approximately 12 healthy controls). Healthy control subjects will undergo neurophysiological testing but not be treated with drug or placebo. Approximately sixty (60) healthy control subjects will be evaluated prior to ALS subjects, to assess test-retest and inter-investigator variability for the neurophysiology measurements. Approximately twelve (12) healthy control subjects will be recruited in parallel with the ALS subjects, to allow an age and site-matched comparison between ALS and healthy control subjects. These 12 healthy control subjects will also undergo blood sampling for iPSC generation.

Study Locations

Approximately 12 Northeast ALS Consortium (NEALS) centers in the US will participate in the study.

Number of Planned Subjects

Approximately 192 subjects will be enrolled in this study. Approximately sixty (60) healthy controls will be enrolled for assessment of short-term reliability of TMS and NCS measurements (approximately five (5) from each site) prior to recruiting ALS subjects. Approximately twelve (12) additional healthy controls will be age and site-matched and enrolled in parallel with the ALS subjects. An initial sample of 45 ALS subjects will be recruited and randomized prior to an interim futility analysis. If the trial of one of the two active treatment groups is not stopped early for futility or safety, then approximately 120 ALS subjects will be recruited in total.

Study Population

This study will be conducted in ALS subjects 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 a slow vital capacity (SVC) $\geq$ 50% of expected, and must provide written informed consent prior to screening. Subjects on a stable dose of riluzole and those not taking riluzole, and women of child-bearing age at screening are eligible for inclusion as long as they meet specific protocol requirements. This study will also be conducted in healthy controls that do not have a history or family history of possible motor neuron disease. Detailed criteria are described in the body of the protocol.

Treatment Groups

ALS subjects will be randomly assigned in a 1:1:1 ratio to oral ezogabine (Potiga® or Retigabine) 600 mg/day, 900 mg/day or matching placebo.

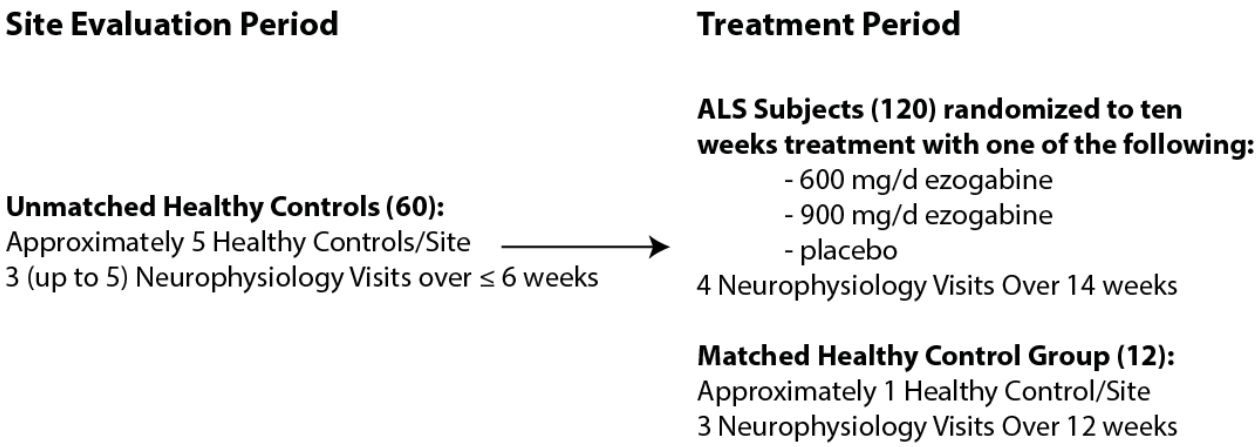
Healthy controls will not be randomized nor receive study drug.

Duration of Treatment and Follow-up

ALS subjects will remain on randomized, placebo-controlled, double-blind treatment for 10 weeks as detailed in the Schedule of Activities. Each randomized subject will also have a Week 14 Telephone Call to assess for adverse events (AEs), changes in concomitant medications and to administer the ALSFRS-R.

Healthy controls will have neurophysiological testing but will otherwise only undergo limited components (see separate Schedule of Activities). The number of healthy controls recruited and the number of their study visits may vary based on the ongoing recommendation of the study Steering Committee, which will review the incoming data dynamically. Approximately five (5) unmatched healthy control subjects at each site will undergo three (3) study visits each for neurophysiological testing and test-retest reliability estimates over up to a six week period. Based on review of the incoming data by the Steering Committee, changes in the number of unmatched healthy controls and number of visits required may occur, with a maximum potential number of five (5) visits per subject. The Steering Committee will determine when individual sites will be activated to begin recruitment of ALS subjects based on test-retest and inter-investigator reliability.

Study Workflow



SCHEDULE OF ACTIVITIES (ALS SUBJECTS)[1]

Activity	Screening Visit	Baseline Visit[2]	Week 2 Call	Week 4 Visit[3]	Week 6 Visit[3]	Week 8 Visit[3]	Week 10 Call	Week 12 Visit	Week 14 Call
	CLINIC	CLINIC	CALL	CLINIC	CLINIC	CLINIC	CALL	CLINIC	CALL
	-21 days	Day 0	Day 14 ± 3	Day 28 ± 3	Day 42 ± 3	Day 56 ± 3	Day 70 ± 3	Day 84 ± 3	Day 98 +5
Written Informed Consent	X								
Inclusion/Exclusion Review	X								
Medical History	X								
Demographics	X								
ALS Diagnosis History	X								
Concomitant Medication Review	X	X	X	X	X	X	X	X	X
Adverse Event Review[4]	X	X	X	X	X	X	X	X	X
Physical Examination	X							X	
Neurological Exam	X							X	
Manual Muscle Testing (MMT)	X							X	
Vital Signs[5]	X	X		X	X	X		X	
12-Lead Electrocardiogram (ECG)	X	X[15]			X			X	
Height & Weight[6]	X	X		X	X	X		X	
Safety Labs[7]	X			X	X	X		X	
Urological Symptom Score	X			X		X		X	
ALSFRS-R	X	X		X	X	X		X	
Slow Vital Capacity (SVC)	X	X		X	X	X		X	
Hand Held Dynamometry (APB only)	X	X		X	X	X		X	
Assign Global Unique Identifier (GUID)	X								
Blindedness Questionnaire and Exit Survey								X	
Suicide Assessment (C-SSRS)		X		X	X	X		X	
Edinburgh Handedness Inventory – short form	X								
Blood collection for iPSC Lines				X[8]	(X[8])	(X[8])			
Neurophysiological Testing (TMS/NCS)	X	X			X	X			
Ophthalmologic Examination[9]		X						X	
Lumbar Puncture (Optional)					X[16]				
Pharmacokinetics (PK)/Biobanking					X				
Telephone Call[10]			X				X		X
Randomization[11]		X							
Administer/Dispense Study Drug		X[12]		X					
Drug Accountability/ Compliance		X		X[13]	X[13]	X[13]		X[13]	
Review of Muscle Symptoms Diary[14]		X		X	X	X		X	
Fasciculation Assessment Form		X		X	X	X		X	

[1] All clinic visits after the Baseline Visit will have a +/- 3 day window.
[2] Screening procedures must be completed prior to the Baseline Visit, which will be scheduled up to 21 days after the Screening Visit.
[3] Study staff should document the time the last dose of ezogabine was administered.
[4] Adverse events that occur AFTER signing the informed consent form will be recorded.
[5] Vital signs include systolic and diastolic pressure in mm Hg, respiratory rate/minute, heart rate/minute and temperature.
[6] Height measured at Screening Visit only.
[7] Labs include Hematology (CBC with differential), Complete Chemistry Panel, Liver Function Tests, Urinalysis and serum pregnancy test (WOCBP, screening visit only).
[8] Blood for iPSC lines can be collected at either week 4, 6 or 8.
[9] Ophthalmologic Examinations may be scheduled separately, but within 14 days prior to Baseline Visit and within 14 days of Week 12 Visit.
[10] Subjects will be called at the designated time points, as a reminder to escalate up or taper off study drug, when appropriate.
[11] Randomization should occur at or prior to the Baseline Visit.
[12] Administer first dose of study drug AFTER all Baseline Visit procedures are completed (with the exception of ECG testing). Subjects to take study drug until the Week 10 call.
[13] Review dosing diary
[14] Muscle symptoms will be completed every day, starting at the Baseline visit, with a Muscle Symptom Diary and should be reviewed by study staff at every visit after Baseline.
[15] ECG testing should be performed 3 hours post initial study drug administration.
[16] Lumbar puncture is optional

SCHEDULE OF ACTIVITIES (UNMATCHED CONTROLS)[1]

Activity	Visit 1 (Screening)	Visit 2[5]	Visit 3[4,5]
	CLINIC	**CLINIC**	**CLINIC**
Written Informed Consent	X		
Inclusion/Exclusion Review	X		
Medical History	X		
Demographics	X		
Vital Signs[2]	X		
Height and Weight	X		
Edinburgh Handedness Inventory – short form	X		
Neurophysiological Testing (TMS/NCS)	X	X	X
Concomitant Medication Review	X	X	X
Adverse Event Review[3]	X	X	X

[1]Visits are defined by the required components, which do not need to take place on the same day. Specifically, TMS and NCS sessions can be on separate days. Please note that a screening TMS and NCS test must be complete and meet eligibility criteria prior to additional TMS and NCS testing performed. All three NCS sessions can occur in a single day if preferred. Up to two TMS sessions can occur in a single day, but the three TMS sessions must be spread out over at least two days.

[2] Vital signs include systolic and diastolic pressure in mmHg, respiratory rate/minute, heart rate/minute and temperature.

[3] Adverse events that occur AFTER signing the informed consent form will be recorded.

[4] As determined by Steering Committee review of neurophysiology data, unmatched controls could be asked to return for up to 5 study visits.

[5] All visits must be completed < 6 weeks from the screening visit.

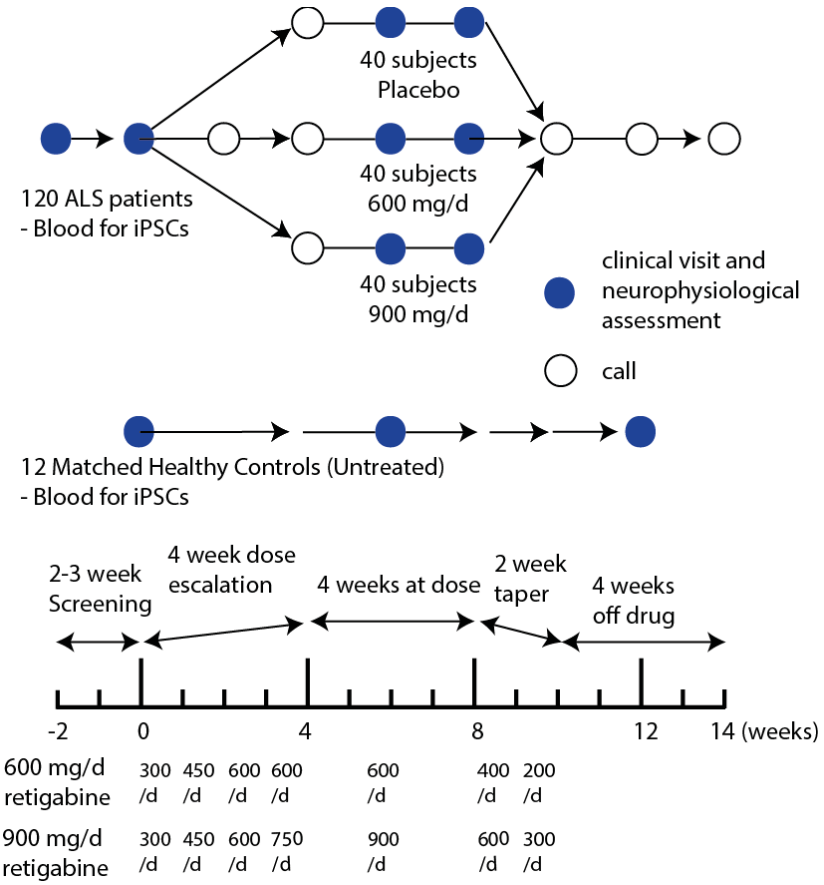
SCHEDULE OF ACTIVITIES (MATCHED CONTROLS)[1,4]

Activity	Screening Visit	Week 6 Visit	Week 12 Visit
	CLINIC	CLINIC	CLINIC
		42 days ±10	84 days ±10
Written Informed Consent	X		
Inclusion/Exclusion Review	X		
Demographics	X		
Medical History	X		
Neurological Examination	X		
Vital Signs[2]	X	X	X
Height and Weight	X		
Blood collection for iPSC Lines	X	(X[5])	(X[5])
Edinburgh Handedness Inventory – short form	X		
Neurophysiological Testing (TMS/ NCS)	X	X	X
Concomitant Medication Review	X	X	X
Adverse Event Review[3]	X	X	X

[1] All clinic visits after the Screening/Baseline Visit will have a +/- 10 day window.
[2] Vital signs include systolic and diastolic pressure in mmHg, respiratory rate/minute, heart rate/minute and temperature.
[3] Adverse events that occur AFTER signing the informed consent form will be recorded.
[4] Visits are defined by the required components, which do not need to take place on the same day. Specifically, TMS and NCS sessions can be on separate days.
[5] Blood for iPSC lines can be collected at Screening, Week 6 **or** Week 12

EZOGABINE WORKFLOW FOR ALS SUBJECTS AND MATCHED HEALTHY CONTROLS

Study Schematic: Randomized, double-blind, placebo-controlled parallel Phase 2 study of ezogabine at 2 active doses for 10 weeks treatment



600 mg/d retigabine	300 /d	450 /d	600 /d	600 /d		600 /d	400 /d	200 /d
900 mg/d retigabine	300 /d	450 /d	600 /d	750 /d		900 /d	600 /d	300 /d

1 ETHICS/PROTECTION OF HUMAN SUBJECTS

1.1 Institutional Review Board (IRB)

This study will be conducted in compliance with current Good Clinical Practices (GCP) and Title 21 Part 56 of the US 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 US 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. Subjects who wish to give their written consent to participate in the study will be provided with a copy of the fully executed consent form for their records.

2 INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

This is a phase 2, multi-center, randomized, double-blind, placebo-controlled 14 week study (10 weeks of drug treatment) evaluating the safety and tolerability, dosing, and effect on neurophysiological excitability measures of ezogabine in ALS subjects.

Multiple lines of evidence demonstrate the importance of motor neuron hyperexcitability as a disease mechanism in ALS. Human neurophysiological studies using nerve conduction threshold testing demonstrate axonal hyperexcitability of motor neurons in both sporadic and familial ALS patients (Bostock et al., 1995; Vucic & Kiernan, 2010) and parallel studies using TMS have shown cortical motor neuron hyperexcitability (reviewed in Vucic et al., 2012). Recently, an initial prospective stratification by axonal excitability was found to predict survival in sporadic ALS patients, in that subjects with higher excitability indices survived for shorter periods than subjects with lower initial excitability indices (Kanai et al., 2012).

Several independent groups have documented hyperexcitability in primary mouse $SOD1^{G93A}$ spinal motor neurons (Pieri et al., 2003; Kuo et al., 2004; van Zundert et al., 2008). Riluzole, the only FDA-approved ALS medication, blocks the persistent sodium current – a likely contributor to motor neuron hyperexcitability in ALS (Kuo et al., 2005). Furthermore, the toxic effects of $SOD1^{G93A}$ astrocyte-conditioned media may be mediated through increasing motor neuron excitability (Fritz et al., 2013). Thus, motor neuron hyperexcitability may represent a convergence of both cell-autonomous and non cell-autonomous disease mechanisms, making it an ideal therapeutic target.

In our study (Wainger et al., 2014), we identified primary motor neuron hyperexcitability markers in motor neurons derived using induced pluripotent stem cells (iPSCs) from ALS patients compared to healthy controls. We recorded decreased delayed-rectifier potassium currents in the ALS compared to control-derived motor neurons. As ezogabine's primary mechanism of action is to increase such currents, we hypothesized that the drug could compensate for the observed current reduction we found in ALS motor neurons. Indeed, we observed that ezogabine blocks hyperexcitability and improves motor neuron survival *in vitro*, thus showing that the increased excitability is likely linked to motor neuron death. We observed phenotypic motor neuron hyperexcitability and its reduction by ezogabine treatment *in vitro* in motor neurons derived from a range of familial ALS patients, including those due to SOD1 mutation, C9orf72 expansion, and fused-in-sarcoma mutation.

Despite the broad range of studies suggesting the importance of upper and lower motor neuron hyperexcitability in ALS, few studies have tried to employ the specific neurophysiological tests

used to identify clinical hyperexcitability in ALS subjects as pharmacodynamic markers. Caramia and colleagues showed that gabapentin and diazepam, but not riluzole, reduced cortical hyperexcitability in ALS subjects (Caramia et al., 2000). Using similar outcomes, Stefan and colleagues did find that riluzole reduced cortical hyperexcitability (Stefan et al., 2001). One may argue that most anti-epileptics reduce cortical excitability and that there is no reason to suspect otherwise in ALS subjects. However, the magnitude of such effects in ALS subjects will be important for determining dosing of a potential larger study.

A relatively small number of studies have shown the proposed neurophysiological outcome measures of neurophysiological excitability are stable enough in ALS subjects over the proposed study time course to reliably compare to baseline (Floyd et al., 2009; Cheah et al., 2012). For this reason, it is critical that we include a placebo-treated control group and a matched and untreated healthy control group as comparisons.

2.2 Rationale

The overarching rationale for the proposal is that motor neuron hyperexcitability contributes to neurodegeneration in ALS and that reduction of neuronal hyperactivity may slow the disease course. The clinical neurophysiological techniques used to demonstrate hyperexcitability may be useful as biomarkers to indicate reduction in excitability with treatment. Studies involving clinical neurophysiology, mouse motor neuron models and human iPSC-derived motor neurons from ALS patients all suggest that motor neuron hyperexcitability may contribute to motor neuron death in ALS. In particular, *in vitro* treatment of ALS patient-derived motor neurons with ezogabine can reverse the hyperexcitability and improve motor neuron survival. On account of these data, the primary goal of this study is to determine whether ezogabine treatment safely reduces neurophysiological indices of motor neuron hyperexcitability in ALS subjects.

The upper dose limit for this study was chosen based on tolerability in previous epilepsy studies (Porter et al, 2007; Brodie et al, 2010; French et al, 2011). Oral doses of 600 mg/day (all doses divided TID), 900 mg/day, and 1200 mg/day were used in two of the three studies. At 1200 mg/day, subject drop out due to AEs was nearly 30% (vs. 20% in the 900 mg/day dose) in Porter et al. (2007). On the opposite end, there was no statistically significant benefit in the 600 mg/day dose compared to placebo, while a clear reduction in seizure frequency was observed with 900 and 1200 mg/day treatments. There is no indication, given the stable linear PK properties over multiple doses and lack of drug-drug interactions in repeated studies, that pharmacokinetics or tolerability would differ markedly in ALS subjects (no CYP metabolism; predominantly renal clearance). We thus chose a fixed dosing strategy with 600 mg/day and 900 mg/day arms to include two doses that were both likely to be tolerated and likely to reduce excitability. Importantly, we will determine whether the CSF concentration approaches the known EC50 of ~ 2 μM from both our *in vitro* studies and heterologous expression of Kv7 channels (Wickenden et al., 2000). CSF levels were not measured in the epilepsy studies. With regard to study duration, the observed effects on hyperexcitability in the most relevant clinical studies (Caramia et al.,

2000; Stefan et al., 2001) were observed within the time frame (4 weeks of maintenance dose treatment) proposed in the study.

2.3 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 ezogabine treatment, lumbar puncture (LP), blood draws, neurophysiological studies, risk due to compromise of medical information and psychological risk.

2.3.1.1. Warnings and Precautions

In pivotal controlled trials, urinary retention, urinary hesitation, and dysuria were reported in 0.9%, 2.2%, and 2.3% of patients on ezogabine, respectively, and in 0.5%, 0.9%, and 0.7% of patients on placebo, respectively. The rate of urinary retention in combined Phase II/Phase III studies was 2%. Of the 29 patients of 1,365 treated who had urinary retention, 5 (17%) required catheterization. Four of 5 of these patients discontinued ezogabine and were able to void spontaneously following discontinuation, but 1 of these 4 continued intermittent catheterization. The fifth patient continued treatment with ezogabine and was able to void spontaneously after catheter removal. Hydronephrosis occurred in 2 patients, one of whom had associated renal impairment that resolved upon treatment discontinuation. The risk of urinary retention is larger in patients who take anticholinergic medications.

In 813 subjects treated with ezogabine in placebo-controlled epilepsy trials, major neuro-psychiatric symptoms included confusional state (75, 9% compared to 11, 3% in placebo), psychosis (9, 1% compared to 0 in placebo) and hallucinations (14, 2% compared to 2, <1% in placebo). Dizziness and somnolence were reported in 23% of patients treated with ezogabine compared to 9% of patients treated with placebo. Ezogabine produced a mean 7.7 ms QT prolongation in healthy volunteers titrated to 400 mg 3 times daily. Pooled analyses of anti-epileptic drugs showed treatment increased the risk of suicide by about two-fold. Ezogabine has been associated with elevations in liver function tests (1-2%) of treated subjects across multiple dose ranges. Withdrawal seizures are a risk of rapid medication discontinuation and for this reason a taper is recommended. A recent FDA safety alert has been posted for the risk of blue skin and retinal epithelium discoloration. Discoloration of the skin, nails, lips and/or mucosa has been described as blue grey color and has been observed generally at higher doses and after several years of treatment.

All known cases of retinal pigment abnormalities were reported after an exposure to ezogabine of at least three years. It is not known if the abnormalities can begin earlier, and it is also not known whether such changes affect visual acuity. Such changes were present in approximately one-third of patients who had eye examinations. Approximately one-third of the patients with retinal pigment abnormalities had no skin discoloration. Relevant information from the FDA is described in the following websites: *<http://www.fda.gov/Safety/MedWatch/SafetyInformation/SafetyAlertsforHumanMedicalProducts/ucm349847.htm> and <http://www.fda.gov/Drugs/DrugSafety/DrugSafetyPodcasts/ucm350120.htm>*.

The most common adverse reactions leading to withdrawal in patients receiving ezogabine treatment were dizziness (6%), confusional state (4%), fatigue (3%), and somnolence (3%). Overall, the most frequently reported adverse reactions in patients receiving ezogabine (≥4% and occurring approximately twice the placebo rate) were dizziness (23%), somnolence (22%), fatigue (15%), confusional state (9%), vertigo (8%), tremor (8%), abnormal coordination (7%), diplopia (7%), disturbance in attention (6%), memory impairment (6%), asthenia (5%), blurred vision (5%), gait disturbance (4%), aphasia (4%), dysarthria (4%), and balance disorder (4%). In most cases the reactions were of mild or moderate intensity. Ezogabine was associated with dose-related weight gain, with mean weight increasing by 0.2 kg, 1.2 kg, 1.6 kg, and 2.7 kg in the placebo, 600 mg/day, 900 mg/day, and 1,200 mg/day groups, respectively. Following is a list of adverse reactions reported by patients treated with ezogabine during all clinical trials: rash, nystagmus, dyspnea, leukopenia, muscle spasms, alopecia, nephrolithiasis, syncope, neutropenia, thrombocytopenia, euphoric mood, renal colic, coma, and encephalopathy. Although abrupt withdrawal of antiepileptic drugs can be associated with a risk of withdrawal seizures, there is not a known risk of withdrawal seizures after abrupt cessation of ezogabine in non-epileptic patients.

Ezogabine has been associated with the following adverse reactions, labeled as very common (≥1/10), common (≥1/100 to 1/10), uncommon (≥1/1,000 to 1/100), and rare (≥1/10,000 to 1/1,000):

- Metabolism and nutrition:
    - Common: weight increased, increased appetite
- Psychiatric disorders:
    - Common: Confusional state, psychotic disorders, hallucinations, disorientation, anxiety
- Nervous system disorders
    - Very Common: Dizziness, somnolence
    - Common: Amnesia, aphasia, coordination abnormal, vertigo, paraesthesia, tremor, balance disorder, memory impairment, dysphasia, dysarthria, disturbance in attention, gait disturbance, myoclonus.
    - Uncommon: Hypokinesia.
- Eye disorders:
    - Common: Pigment changes (discoloration) of ocular tissues, including the retina (particularly after prolonged use).

    - Common: Diplopia, blurred vision
- Gastrointestinal Disorders
    - Common: Nausea, constipation, dyspepsia, dry mouth.
    - Uncommon: Dysphagia.
- Hepatobiliary disorders
    - Common: Increased liver function tests.
- Skin and subcutaneous tissue disorders
    - Common: Blue-grey discoloration of the nails, lips and/or skin have been observed, generally at higher doses and after several years of treatment.
    - Uncommon: Hyperhidrosis.
- Renal and urinary disorders
    - Common: Dysuria, urinary hesitation, hematuria, chromaturia.
    - Uncommon: Urinary retention.
- General disorders and administration site conditions.
    - Very common: Fatigue.
    - Common: Asthenia, malaise, peripheral edema.

Both ALS subjects and matched healthy control volunteers will have a blood draw to collect a blood sample (approximately 45mL) that will be used for isolating human somatic cells, which then can be cultured and reprogrammed into subject-derived iPSCs. Motor neurons and other relevant cell types can be differentiated *in vitro* from the iPSCs.

Blood and cerebrospinal fluid (CSF) will be collected for biobanking. Approximately 40 mL of blood will be collected from ALS subjects. Risks of blood collection include the possibility of fainting, pain, bruising, infection, and blood clot at the site of intravenous (IV) catheter insertion. Approximately 30 mL of CSF will be collected using standard clinical procedures. Risks include discomfort, bleeding, infection, headache, and pain at the site. Approximately 1 out of 3 people who have a LP develop a post-LP headache, which can range in severity from mild to severe. If the headache lasts more than 3 days, a blood patch may be performed, in which blood taken sterilely from the research subject is removed and inserted into the epidural space. The clotting of the blood should stop further CSF leaking and resolve the headache.

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 tests can be associated with mild to moderate discomfort and transient skin irritation due to either electrodes or stimulation.

Sample distribution (blood & CSF) will occur only of encoded samples. Non-study investigators who are provided access to study data for subsequent analyses of these samples will not seek or have access to subject identifying information. All subject identifying information will be kept on password-protected computers and will only be accessible to the study investigators. Despite study investigators taking all appropriate precautions, there is still a small chance of compromise

of identifying information. Standards for subject confidentiality, particularly with regard to genetic analysis, will be tightly followed according to the IRBs at all participating sites.

There is potential emotional and psychological discomfort when participating in this study, including during the blood draw and LP.

2.3.2 Known 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 ezogabine will be helpful or harmful for ALS subjects. It is possible that results from the study may yield benefits to future ALS patients.

3 OBJECTIVES

3.1 Study Objectives

The primary purpose of the proposed study is to determine whether ezogabine reduces neurophysiological excitability indices in ALS subjects as assessed by *axonal threshold NCS* and TMS. The study also will evaluate in ALS subjects dosing, safety and tolerability, and limited serum and CSF PK prior to a larger study designed to evaluate effect on a combined score of function and survival, should reduction in neurophysiological excitability indices be achieved.

Although motor neuron hyperexcitability has been repeatedly demonstrated in ALS subjects, two central questions relate to the use of such measures in clinical trials. First, it is not known if reducing excitability will influence the disease course. That is, the hyperexcitability may be a downstream property that results as a consequence of the neurodegenerative disease process, and blocking it might not affect clinical progression. While *in vitro* evidence from our work and that of other groups (Fritz et al., 2013) suggests that reducing activity may improve motor neuron survival *in vitro*, accurate mouse models exist only for SOD1 familial ALS (2% of total ALS cases) and thus the question can only be properly addressed in clinical studies of human subjects. Second, should a reduction in motor neuron excitability be capable of influencing the disease progression, it is not known what magnitude of reduction – particularly in the neurophysiological markers described in this proposal – would be clinically significant. Still, given the well-established differences between healthy control and ALS patients in both the TMS and *axonal hyperexcitability assessments*, a reasonable assumption is that reduction of excitability to the levels of controls may constitute a clinically relevant difference, and this study is sufficiently powered to detect that.

Because the dose of ezogabine is well-defined by prior studies in epilepsy and on account of the favorable metabolic profile of the drug and the lack of reason to believe that dosing and tolerability would be substantially different in ALS patients, we believe that the proposed approach and outcomes in this study are reasonable. Two oral doses of ezogabine (600 mg/day and 900 mg/day, both with generally TID dosing) will be compared to matched placebo. The PK analysis will provide data on the relationship between dose of ezogabine and CSF concentrations. We will attempt to estimate the dose-dependent maximum CSF concentration to determine if zero, one, or both doses reach physiologically relevant levels, approximately 2 μM as determined by our dose response studies in iPSC-derived motor neurons and those evaluating ezogabine on heterologously expressed Kv7 channels (Wickenden et al., 2000).

3.2 Study Outcome Measures

3.2.1 Primary Outcome Measure

The primary efficacy outcome is the change in paired pulse short-interval intracortical inhibition (SICI) assessed by transcranial magnetic stimulation (TMS).

3.2.2 Secondary Outcome Measures

Secondary outcomes include additional measures of efficacy and measures of safety, tolerability, pharmacokinetics, and *in vitro* response. Secondary efficacy outcomes include measures of upper and *lower motor neuron excitability and measures of clinical progression*. Motor evoked potential (MEP) threshold is the key secondary efficacy outcome, pre-specified for secondary efficacy analysis ahead of all other secondary efficacy outcomes. Additional upper motor neuron secondary outcomes include MEP amplitude, MEP latency, cortical silent period, and paired pulse facilitation, assessed by TMS. Lower motor neuron excitability outcomes include change in electrotonus, recovery cycle and strength duration time constant as determined by threshold tracking axonal nerve conduction studies (NCS) and compound motor action potential (CMAP) amplitude. The effect of retigabine on strength will be assessed using hand held dynamometry, and muscle symptoms (muscle cramps and fasciculations) will also be assessed utilizing a subject daily diary, in which the subjects will record the frequency, location and severity of their cramps over the course of study treatment.

Clinical progression outcomes include change in ALS Functional Rating Scale-Revised (ALSFRS-R) and slow vital capacity (SVC). Safety outcomes include adverse and serious adverse events, laboratory tests (including hematology, white cell differentials, blood chemistry, liver function tests, and urinalysis), weight loss, changes in electrocardiographic (ECG) parameters, changes in ophthalmologic parameters, and suicidality. Tolerability is defined as the proportion of participants able to reach their target dose and remain on study drug until planned discontinuation. Pharmacokinetic outcomes include peak concentration and time to peak concentration in serum and cerebrospinal fluid (CSF) after 2 weeks at target dose. *In vitro* exploratory outcomes include the following assessments of iPS-derived motor neurons: assessment of correlation between the primary clinical outcome (SICI using TMS) prior to treatment with ezogabine and the spontaneous firing rate of the *in vitro*-derived motor neurons; and correlation of the magnitude of the *in vivo* response to ezogabine detected by TMS and the *in vitro* EC50 for blocking motor neuron spontaneous action potential firing by ezogabine.

4 STUDY DESIGN

4.1 Overall Study Design and Plan

During the enrollment period, approximately 150 ALS patients will be screened from approximately 12 Northeast ALS Consortium (NEALS) centers in the US. Approximately 120 eligible ALS subjects will be randomly assigned in a 1:1:1 ratio to oral ezogabine 600 mg/day, 900 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 0). An interim futility analysis will be performed after the first 45 ALS subjects have completed Week 12 of the study. One or the other active treatment groups or the full trial may be terminated based on results of the futility analysis and/or periodic review of safety data by the DSMB.

4.2 Study Centers

The study will be conducted at approximately 12 NEALS Centers in the US. Sites will be selected based on recruitment records from prior trials, compliance with prior study protocols and regulations, clinical research expertise, availability of necessary resources, and test-retest reliability of TMS and NCS assessments of unmatched healthy control subjects.

4.3 Study Duration

Subjects will remain on randomized, placebo-controlled, double-blind treatment from Baseline until Week 10 (10 weeks). Each randomized subject will also have a Week 14 Follow-up Telephone Interview, in addition to the Week 12 Clinic Visit, to assess for AEs, changes in concomitant medications and to administer the ALSFRS-R.

4.4 Protocol Adherence

Each Site Investigator (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 (CC) or their representative 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.

5 STUDY ENROLLMENT AND WITHDRAWAL

5.1 Number of Study Subjects

Approximately 72 healthy control volunteers will be enrolled in the study. In addition, approximately 120 ALS subjects will be enrolled and randomized in a 1:1:1 ratio to oral ezogabine 600 mg/day, 900 mg/day or matching placebo. Additional ALS subjects may be randomized if any randomized subjects withdraw from the study prior to initiating study drug.

5.2 Inclusion and Exclusion Criteria for ALS Subjects

5.2.1 Inclusion Criteria

Study subjects meeting all of the following criteria will be allowed to enroll in the study:

1. Male or female, aged 18 to 80.
2. Sporadic or familial ALS diagnosed as possible, laboratory-supported probable, probable, or definite as defined by revised El Escorial criteria.
3. Slow vital capacity (SVC) measure ≥ 50% of predicted for gender, height and age at the Screening Visit, OR in the opinion of the SI, ability to perform and safely complete all study visit procedures.
4. 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).
5. Subjects must be able to swallow oral medication at the Screening Visit and expected to be able to swallow tablets throughout the course of the study.
6. Capable of providing informed consent and following trial procedures.
7. Geographically accessible to the site.
8. Women must not be able to become pregnant (e.g., post menopausal, surgically sterile, or using adequate birth control methods) for the duration of the study and three months after study completion. Adequate contraception includes: abstinence, hormonal contraception (oral contraception, implanted contraception, injected contraception or other hormonal contraception, for example patch or contraceptive ring), intrauterine device (IUD) in place for ≥ 3 months, barrier method in conjunction with spermicide, or another adequate method.
9. 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.
10. TMS shows sufficient MEP amplitude and/or NCS studies show sufficient CMAP amplitude (amplitudes defined in MOP).

5.2.2 Exclusion Criteria

Study subjects meeting any of the following criteria during screening evaluations will be excluded from entry into the study:

1. Medical condition, laboratory finding, or physical exam finding that precludes participation.
2. Serum AST or ALT value >2.0 times the upper normal limit
3. Clinically significant conduction abnormalities on electrocardiogram or a known history of cardiac arrhythmia, myocardial infarction within the past 24 months, or congestive heart failure.
4. Estimated glomerular filtration rate < 50 mL/min at Screening Visit.
5. Concomitant digoxin treatment.
6. Known allergic reactions to components of the study product(s).
7. Exposure to any other agent currently under investigation for the treatment of patients with ALS (off-label use or investigational) within 30 days of the Screening Visit including ezogabine, exposure to cell replacement therapy within six months of the Screening Visit or any prior intraparenchymal cell replacement injection within the spinal cord or brain at anytime in the past.
8. Presence of tracheostomy at the Screening Visit.
9. History of clinically significant urinary retention, or current use of medications to treat urinary retention.
10. History of drug and or alcohol abuse within 12 months of the Screening Visit.
11. The presence of unstable psychiatric disease, cognitive impairment, or dementia that would impair ability of the subject to provide informed consent, according to SI judgment.
12. Clinically significant history of unstable or severe cardiac, oncologic, hepatic, or renal disease, or other uncontrolled medical condition.
13. Presence of feeding tube.
14. Current use of antipsychotic, antiepileptic (except benzodiazepines, gabapentin, pregabalin) or class 1 (e.g. flecainide) or class 3 (e.g. amiodarone) antiarrhythmic medications. Quinidine or a quinidine-containing drug is allowed if the quinidine dose is not greater than 20 mg/day (for a full list of medications, please reference the study MOP).
15. Pregnant women or women currently breastfeeding.
16. Contraindication to TMS studies including ferromagnetic metal in the head or neck (potentially found in aneurysm clips, implanted medication pumps, implanted brain stimulators, pacemakers, cochlear implants), or history of epilepsy. Dental fillings are permitted.
17. Anything else that, in the opinion of the SI, would place the subject at increased risk or preclude the subject's full compliance with or completion of the study.

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

Lumbar Puncture. The lumbar puncture procedure is optional at the Week 6 visit. Subjects undergoing the lumbar procedure must not be currently taking anticoagulation medications such as warfarin that would be a contraindication to LP; aspirin and non-steroidal anti-inflammatories are allowed.

5.3 Inclusion and Exclusion Criteria for Healthy Controls (Unmatched and Matched)

5.3.1 Inclusion Criteria

Healthy control volunteers meeting all of the following criteria will be allowed to enroll in the study:

1. Male or female, aged 18 to 80.
2. Capable of providing informed consent and following trial procedures.
3. Geographically accessible to the site.
4. 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. Age (+/- 10 years and site-matched to a ALS participant within 6 months of their Baseline visit).**[Matched controls only]**
6. TMS shows sufficient MEP amplitude and/or NCS studies show sufficient CMAP amplitude (amplitudes defined in MOP).

5.3.2 Exclusion Criteria

Healthy control volunteers meeting any of the following criteria during screening evaluations will be excluded from entry into the study:

1. History of ALS or other neurodegenerative disease.
2. Presence of positive family history of ALS.
3. Presence of a neurological disorder.
4. Current use of an antipsychotic or antiarrhythmic medication
5. Definitely or possibly pregnant.
6. Contraindication to TMS studies including ferromagnetic metal in the head or neck (potentially found in aneurysm clips, implanted medication pumps, implanted brain stimulators, pacemakers, cochlear implants), or history of epilepsy. Dental fillings are permitted.
7. Anything that, in the opinion of the SI, would place the subject at increased risk or preclude the subject's full compliance with or completion of the study.

5.4. Treatment Assignment Procedures

Each ALS subject who meets all eligibility criteria, is accepted for the study, signs an informed consent form (ICF) and undergoes successful lead-in neurophysiological testing, will be randomized to receive either 600 mg/day or 900 mg/day oral ezogabine or matching placebo for 10 weeks of treatment.

5.5. Randomization Procedures

A permuted-block randomization schedule, stratified by site, will be developed by the study statistician with assignments in a 1:1:1 ratio among 600 mg/day oral ezogabine, 900 mg/day oral ezogabine, and placebo. If a randomized subject withdraws from the study prior to initiating study drug, their assignment will be released and re-used.

5.6. 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.

5.7. Handling of Withdrawals

The medical monitor should be notified in all instances if a subject chooses 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). These subjects will be encouraged to follow the study visits, off drug, up to the final visit. 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.

5.8. 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.

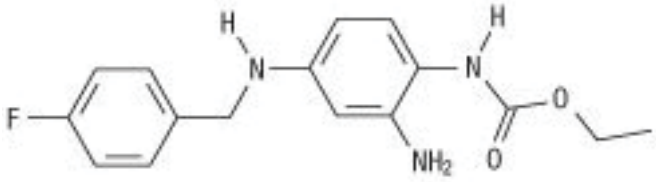
6 TREATMENTS ADMINISTERED

6.1 Treatments

Ezogabine (retigabine or trade name Potiga) is indicated as adjunctive treatment of partial-onset seizures in patients aged 18 years and older. An investigator's brochure (IB) will be available to all Study Investigators (SIs).

6.1.1 Study Product Description

The chemical name of ezogabine is N-[2-amino-4-(4-fluorobenzylamino)-phenyl] carbamic acid ethyl ester, and it has the following structure:



The empirical formula is $C_{16}H_{18}FN_3O_2$, with a molecular weight of 303.3 AMU. Ezogabine is a white to slightly colored, odorless, tasteless, crystalline powder. At room temperature, ezogabine is practically insoluble in aqueous media at pH values above 4, while the solubility is higher in polar organic solvents. At gastric pH, ezogabine is sparingly soluble in water (about 16 g/L). The pKa is approximately 3.7 (basic).

POTIGA® (ezogabine) is supplied for oral administration as 50-mg, 200-mg, 300-mg, and 400-mg film-coated immediate-release tablets. The 50, 200 and 300 mg tablets will be utilized for this study. Each tablet contains the labeled amount of ezogabine and the following inactive ingredients: cochineal (50 mg), croscarmellose sodium, FD&C Blue No. 2 (50 mg, 300 mg), hypromellose, iron oxide yellow (200 mg, 300 mg), lecithin, magnesium stearate, microcrystalline cellulose, polyvinyl alcohol, talc, titanium dioxide, and xanthan gum.

6.1.2 Placebo

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

Administration of matching placebo will be the same as for subjects in the treatment group.

6.2 Acquisition

Upon receipt of the study treatment supplies, an inventory must be performed and a drug receipt log filled out and signed by the person accepting the shipment. It is important that the designated

study staff counts and verifies that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable study drug in a given shipment will be documented in the study files. The SI must notify the study sponsor of any damaged or unusable study treatments that were supplied to the SI's site.

6.2.1 Formulation, Packaging, and Labeling

The study drug is prepackaged in bottles and ready for oral administration. The site pharmacy and SI have the responsibility to ensure that the integrity of packaged study drug is not jeopardized prior to dispensing. Each individual subject bottle must be dispensed as provided with no further repackaging or labeling done at the investigational site, unless required by the institution per institutional polices.

6.2.2 Product Storage and Stability

The SI must ensure that all investigational drug supplies are kept in a locked, safe area at controlled room temperature 59°-86°F (15°-30°C) with access limited to those directly involved in the study. Investigational drug supplies should not be repackaged in any way. It is recommended that each site utilize their Research Pharmacy for this study.

6.3 Dosage, Preparation and Administration of Study Intervention/Investigational Product

The sole investigational agent is ezogabine via oral administration. Generally, the study will include a combination of 50 mg ezogabine, 200 mg ezogabine and 300 mg ezogabine tablets or matching placebos for each of the groups as described in the table below. The study drug kits are comprised of two sub-kits (Weeks 1 through 4 and Weeks 5 through 10).

Subjects from all 3 ALS randomization groups will receive their first dose of study medication and drug sub-kit to take home at the Baseline Visit. At the Week 4 Visit, subjects will bring all study drug bottles for compliance, accountability and witnessed destruction from the first sub-kit and receive the second sub-kit (Weeks 5 through 10). In order to maintain the blind and escalate each group up to the appropriate dosage, the following bottles have been configured:

Week 1 – Week 4 (4 weeks)

- The placebo group will receive and take: 50 mg placebo; 50 mg placebo; 200 mg placebo.
- The 600 mg/day group will receive and take: 50 mg ezogabine; 50 mg placebo; 200 mg ezogabine.
- The 900 mg/day group will receive and take: 50 mg ezogabine; 50 mg ezogabine; 200 mg ezogabine.

Week 5 through 10 (6 weeks)

- The placebo group will receive and take: 200 mg placebo; 300 mg placebo.
- The 600 mg/day group will receive and take: 200 mg ezogabine; 300 mg placebo.
- The 900 mg/day group will receive and take: 200 mg placebo;300 mg ezogabine.

As described in the drug dosing schedule table below, equal number of tablets will be taken by all subjects during each week. Subjects will be instructed to take the specified number of tablets one, two, or three times a day at each week of the study according to the schedule found in Table 1.

Table 1. Study Drug Titration Schedule

Number of tablets per day

	Week 1	Week 2	Week 3	Week 4
50 mg	2 TID	3 TID	-	1 TID
200mg	-	-	1 TID	1 TID

Number of tablets per day

	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10
200 mg	1 TID	1 TID	1 TID	1 TID	1 BID	1 QD
300 mg	1 TID	1 TID	1 TID	1 TID	1 BID	1 QD

6.3.1 Missed Doses

Subjects who miss one or more doses should immediately take the most recently missed dose and then resume the current treatment, making sure to wait at least three hours in between doses. Missed doses should be documented at the subsequent clinic visit and in the subject's study drug diary.

6.3.2 Crushing Pills

Should a subject be unable to swallow the tablets over the course of the study, the tablets can be crushed and dispersed in water to form a suspension. The suspension should only be taken orally or delivered by a **silicone-based** gastrostomy tube.

6.3.3 Feeding Tube

Should a subject no longer be able to swallow and require a gastrostomy tube over the course of the study, the tablets can be crushed and dispersed in water to form a suspension to be delivered via the tube. The study drug cannot be given accurately via a non-silicone-based nasogastric or

non-silicone-based gastrostomy tube, thus the preference is for subjects to use only a silicone based tube. In the event that a subject becomes unable to swallow the study drug and receives a non-silicone-based nasogastric or non-silicone based gastrostomy tube over the course of the study, subjects should continue to take study drug regardless of the tube material. A notation on the eCRF will indicate the type and material of the feeding tube placed.

6.4 Modification of Study Intervention/Investigational Product for a Subject

Any dosage adjustment, including the reason for and dates of adjustment, will be documented in the CRF for each subject requiring this manipulation. The SI or licensed physician Sub-Investigator may reduce the dosage of study drug or discontinue study drug in its entirety for AEs thought to be related to study drug or for other reasons during the trial (the reason for and dates of suspension or dose reduction must be documented). If the AE is mild or moderate, the dosage may be reduced until the event improves. The SI may then choose to resume the higher dosage or maintain the subject at a reduced dosage. Reference the study Manual of Procedures for additional information.

If the event is serious or life threatening and deemed to be definitely drug related, study drug will be discontinued immediately. Study subjects must remain off study drug permanently. Subjects may not resume study drug. The medical monitor should be notified immediately, preferably prior to any dosage adjustments, if possible.

6.4.1. Dosage Discontinuation

Reasons for discontinuation of study drug may include an AE, Medical Monitor or SI recommendation, Sponsor termination, protocol deviation, loss to follow-up, patient request, or death.

Study subjects who discontinue study drug prematurely and decide not to continue study visits (wish to withdraw consent) will be encouraged to delay consent withdrawal in order to return for a Final Safety Visit within 14 days of expressing wish to withdraw consent and to permit a Follow-Up Telephone Call no sooner than 28 days after taking last known dose of study drug to monitor their safety and permit review of their medical records at the end of the study to document their vital status.

All subjects who discontinue study drug early and choose to continue study visits will be encouraged to follow the normal study visits, off drug, up to the time of the last visit (Follow-Up Telephone Call).

SAEs will be followed for resolution for 28 days (+5 days) after a subject's last known dose of study drug, regardless of whether they prematurely discontinued study drug or completed the treatment.

6.5 Accountability Procedures for Ezogabine

There will be a final reconciliation of drug shipped, drug consumed, and drug remaining. This reconciliation will be logged, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to destruction of unused study drug. Drug destroyed on site will be documented in the study files.

6.6 Assessment of Subject Compliance with Ezogabine

Subjects will be instructed to return empty and unused study medication containers at the Week 4 and Week 12 Visits 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 125% of study medication as determined by tablet counts. If a study subject is non-compliant with study medication, the SI and staff should re-educate and train the subject in administration of study drug. Data indicating compliance will be used in secondary analyses.

6.7 Prior and Concomitant Therapy

Throughout the study, SIs may prescribe concomitant medications or treatments deemed necessary to provide adequate supportive care provided that the medications are licensed in the United States. Study subjects should not receive other experimental agents during the study. This includes marketed agents at experimental dosages that are being tested for the treatment of ALS. All concomitant medications and/or treatments and significant non-drug therapies, including supplements and assistive devices, received by a subject should be recorded on the appropriate source document and eCRF.

6.7.1 Prohibited Medications and Contraindications

<u>Prohibited Medications</u>

Throughout the course of the trial, study subjects should not be treated with the following medications. If an investigator learns that a patient has begun therapy with any of these medications, this should be reported to the NCRI Coordination Center (CC) immediately and action should be taken to discontinue the prohibited medication. If, for safety or health reasons, or by subject choice, the prohibited medication cannot be stopped, then the study drug should be stopped. *For a complete and updated listing of prohibited medications and contraindications refer to the study Manual of Procedures.*

Prohibited medications include:

- Digoxin

The following medications are prohibited when given on an as needed (PRN) basis (full list is provided in Site Manual of Operations). 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
- Hypnotics (including anti-histamines)
- 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
- Antisense oligonucleotide treatment targeting SOD1 or C9orf72
- Mexiletine
- Ozanezumab (GSK 1223249)
- Rasagiline
- Fingolimod
- CK-2017357
- Tocilizumab

The medical monitor should be notified immediately, preferably prior to the start of any of these medications, if possible.

Pregnancy & Nursing Mothers

There are no adequate and well-controlled studies in pregnant women. Subjects or partners of male subjects should not become pregnant during the study or 1 month after stopping study drug. If a female subject becomes pregnant, study treatment must be discontinued immediately.

As it is not known whether ezogabine is excreted in human milk, caution should be exercised. No subject should nurse their infant while participating in this study.

7 STUDY SCHEDULE (UNMATCHED HEALTHY CONTROLS)

No study procedures should be performed prior to the signing of the ICF. All subjects will sign an ICF prior to undergoing any study tests or procedures. Components of each visit can be distributed on different days. TMS neurophysiology for all three visits can be scheduled on two or more days. NCS neurophysiology for all three visits can be scheduled on one or more days. One (1) TMS and one (1) NCS test must meet inclusion/exclusion criteria prior to performing additional neurophysiology tests. All visits must be completed < 6 weeks from the screening visit. Please reference the study Manual of Procedures (MOP) Outcome Measures for additional information.

7.1 Visit 1 (Screening)

The following procedures will be performed at an office visit to determine the subject's eligibility for the study:

- Obtain written informed consent
- Assess inclusion and exclusion criteria. If ineligible, record reason for screen failure.
- Collect demographic data
- Obtain medical history
- Review and document concomitant medications and therapies
- Assess and document AEs that occur after subject signs ICF
- Measure vital signs including height and weight
- Complete Edinburgh Handedness Inventory- Short Form
- Perform neurophysiological testing (TMS and NCS). At sites that have two TMS or two NCS physiologists, both should independently perform neurophysiological testing of unmatched healthy controls.
- Schedule next visit.

7.1.1 Screen Failures

Any subject who signs consent will be considered enrolled in the study. If a subject fails screening, *at a minimum*, the following information should be captured and entered in the Electronic Data Capture (EDC) System:

- Demographics
- Inclusion/Exclusion criteria
- Reason for screen failure

7.2 Visit 2

- Review and document concomitant medications and therapies
- Assess and document AEs

- Perform neurophysiological testing (TMS and NCS). At sites that have two TMS or two NCS physiologists, both should independently perform neurophysiological testing of unmatched healthy controls.
- Schedule next visit

7.3 Visit 3 (4 & 5 if needed)

- Review and document concomitant medications and therapies
- Assess and document AEs
- Perform neurophysiological testing (TMS and NCS). At sites that have two TMS or two NCS physiologists, both should independently perform neurophysiological testing of unmatched healthy controls.
- Schedule next visit, if applicable.

8 STUDY SCHEDULE (ALS SUBJECTS)

No study procedures should be performed prior to the signing of the ICF. All subjects will sign an ICF prior to undergoing any study tests or procedures. The slow vital capacity (SVC) should be performed first at the visits so as not to fatigue the subject with other testing; however, the order of testing will be at the discretion of each site investigator (SI).

Visit windows are consecutive calendar days and the target visit dates are calculated from the Baseline Visit, which should occur within 21 days of the Screening Visit. All clinic visits and calls after the Baseline visit will have a +/- 3 day window, except the Week 14 phone call which has a +5 day window only.

Subjects who withdraw consent or terminate from the study early will be asked to come in for a Final Safety Visit within 14 days of withdrawing consent, and will have a Final Telephone Call no sooner than 28 days after last known dose of study drug.

8.1 Screening Visit

The following procedures will be performed at an office visit to determine the subject's eligibility for the study:

- Obtain written informed consent
- Assess inclusion and exclusion criteria. If ineligible, record reason for screen failure.
- Collect demographic data
- Obtain medical history
- Obtain ALS diagnosis history
- Assign a Global Unique Identifier (GUID)
- Review and document concomitant medications and therapies
- Assess and document AEs that occur after subject signs ICF
- Perform physical examination
- Perform neurological examination, including MMT
- Measure vital signs including height and weight
- Administer ALSFRS-R questionnaire
- Administer the American Urological Association (AUA) Symptom Score survey
- Perform SVC
- Perform hand held dynamometry (HHD) APB bilaterally
- Complete Edinburgh Handedness Inventory- Short Form
- Perform neurophysiological testing (TMS and NCS)
- Collect blood samples and urinalysis for safety clinical laboratory assessments, pregnancy test (WOCBP)
- Perform 12-lead ECG
- Schedule Ophthalmologic visit (visit must be completed within 14 days prior to baseline visit)

- Schedule the Baseline Visit

8.1.1 Screen Failures

Any subject who signs consent will be considered enrolled in the study. If a subject fails screening but later becomes eligible, the subject may be re-screened once eligible. If the subject becomes eligible before the window between screening and baseline is up (<21 days), the subject will not need to be re-consented and study procedures will be repeated on a case by case basis only. If the subject is eligible outside the screening and baseline window (>21 days), then the subject must be re-consented and all study procedures will be repeated.

If a subject fails screening and is not eligible for rescreen, *at a minimum*, the following information should be captured and entered in the Electronic Data Capture (EDC) System:

- Demographics
- Inclusion/Exclusion criteria
- Reason for screen failure

Subjects who screen fail during the Screening Visit on account of TMS and NCS should be given the option of completing remaining components of the Screening Visit and the additional data should be captured and entered in the Electronic Data Capture (EDC) System:

- Concomitant medications and therapies
- Medical history including ALS diagnosis history
- ALSFRS-R
- Adverse Events (if applicable)
- Neurophysiology testing (if performed)
- Neurological Exam, MMT testing and HHD (APB)
- SVC (if performed)

8.2 Baseline Visit

The Baseline Visit (from which all other study time points will be determined) will occur not more than 21 days after the Screening Visit. The Baseline Visit for an individual subject can be spread over two consecutive days (both within +/- 3 day window) at the discretion of the SI. If the visit is spread over consecutive days it is recommended that the drug administration and associated activities be performed on the second day. The following procedures will be performed:

- Randomize subject (after the subject's successful Screening Visit and confirmation of inclusion criteria) 0 to 48 hours before the Baseline clinic visit as necessary to obtain study drug from the research pharmacy
- Review and document concomitant medications and therapies
- Assess and document AEs
- Measure vital signs including weight
- Administer ALSFRS-R questionnaire
- Perform SVC [Note: Baseline SVC is not exclusionary, even if below 50% of expected]
- Perform hand held dynamometry (HHD)

- Administer the suicide prevention (C-SSRS) questionnaire
- Perform neurophysiological testing (TMS and NCS)
- Perform ophthalmologic examination (may be scheduled separately, but within 14 days prior to the Baseline Visit and results signed by Ophthalmologist and SI prior to drug administration)
- Complete Baseline Muscle Cramp and Fasciculation Assessments
- Administer first dose of study drug. The subject will be observed at the site for 60 minutes by an appropriate healthcare staff member according to the site's institutional/state regulations to assess medical status and any immediate reaction to the study drug.
- Perform 12-Lead ECG three (3) hours after the first dose administration
- Provide subject with study drug for next four weeks and dispense Study Drug Diary to subject
- Distribute Muscle Symptom Diary
- Schedule next visit

8.3 Week 2 and Week 10 Telephone Calls

These phone calls will take place at Weeks 2 (14 ±3 days from Baseline) and Week 10 (70 ±3 days from Baseline). The following procedures will be performed:

- Review and document concomitant medications and therapies
- Assess and document AEs
- Confirm next visit

8.4 Week 4 Visit

This visit will take place 28 ± 3 days after the Baseline Visit (end of drug escalation phase). The following procedures will be performed:

- Review and document concomitant medications and therapies
- Assess and document AEs
- Measure vital signs including weight
- Collect blood samples and urinalysis for safety clinical laboratory assessments and iPSC generation
- Administer ALSFRS-R questionnaire
- Administer the American Urological Association (AUA) Symptom Score survey
- Perform SVC
- Perform hand held dynamometry (HHD - APB bilaterally)
- Administer the suicide prevention (C-SSRS) questionnaire
- Check study drug compliance and dosing diary
- Perform study drug accountability and collect all unused study drug and empty containers
- Provide study drug for the next six (6) weeks and dispense new dosing diary to subject
- Review returned Muscle Symptom Diary and dispense new Muscle Symptom Diary to subject
- Complete Fasciculation Assessment
- Schedule next visit

8.5 Week 6 Visit

This visit will take place 42 ± 3 days after the Baseline Visit (after two weeks of maintenance drug dose). The Week 6 Visit for an individual subject can be spread over two consecutive days (both within +/- 3 day window) at discretion of SI.

The following procedures will be performed:

- Review and document concomitant medications and therapies
- Assess and document AEs
- Perform 12-lead ECG
- Measure vital signs including weight
- Collect blood samples and urinalysis for safety clinical laboratory assessments
- Collect blood for biobanking at time of CSF collection
- Perform LP for CSF analysis (optional)
- Administer ALSFRS-R questionnaire
- Perform SVC
- Perform hand held dynamometry (HHD - APB bilaterally)
- Administer the suicide prevention (C-SSRS) questionnaire
- Perform neurophysiological testing (TMS and NCS) (may be performed on separate day if necessary)
- Check study drug compliance and dosing diary
- Review Muscle Symptom Diary with subject
- Complete Fasciculation Assessment
- Schedule next visit

8.6 Week 8 Visit

This visit will take place 56 ± 3 days after the Baseline Visit (after four weeks of maintenance drug dose). The following procedures will be performed:

- Review and document concomitant medications and therapies
- Assess and document AEs
- Measure vital signs including weight
- Collect blood samples and urinalysis for safety clinical laboratory assessments
- Administer ALSFRS-R questionnaire
- Administer the American Urological Association (AUA) Symptom Score survey
- Perform SVC
- Perform hand held dynamometry (HHD - APB bilaterally)
- Administer the suicide prevention (C-SSRS) questionnaire
- Perform neurophysiological testing (TMS and NCS)
- Check study drug compliance and dosing diary
- Review Muscle Symptom Diary with subject
- Complete Fasciculation Assessment
- Schedule next visit

8.7 Week 12 Visit

This visit will take place 84 ± 3 days after the Baseline Visit (two weeks after drug cessation). The following procedures will be performed:

- Review and document concomitant medications and therapies
- Assess and document AEs
- Perform physical examination
- Perform neurological examination, including MMT
- Perform ophthalmologic examination (may be scheduled separately, but within 14 days of Week 12 Visit)
- Measure vital signs including weight
- Perform 12-lead ECG
- Collect blood samples and urinalysis for safety clinical laboratory assessments
- Administer ALSFRS-R questionnaire
- Administer the American Urological Association (AUA) Symptom Score survey
- Perform hand held dynamometry (HHD - APB bilaterally)
- Perform SVC
- Administer the suicide prevention (C-SSRS) questionnaire
- Check study drug compliance and collect dosing diary
- Review returned Muscle Symptom Diary
- Complete Fasciculation Assessment
- Perform study drug accountability and collect all unused study drug and empty containers
- Schedule telephone call
- Complete Blindedness Questionnaire and Exit Survey

8.8 Week 14 Telephone Call

This phone call will take place 98 +5 days after the Baseline Visit. The following procedures will be performed:

- Review and document concomitant medications and therapies
- Assess and document AEs

8.9 Final Safety Visit & Telephone Call

ALS subjects who withdraw consent will be asked to come in for a Final Safety Visit and will be asked to have a final Follow-Up Telephone Call no sooner than 28 days after subject's last known dose of study drug.

The Final Safety Visit should be scheduled as soon as possible, but within 14 days of subject withdrawing consent. The following will be performed:

- Review and document concomitant medications and therapies
- Assess and document AEs
- Perform physical examination
- Perform neurological examination, including MMT

- Perform ophthalmologic examination (may be scheduled separately, but within 14 days of the Final Safety Visit)
- Measure vital signs and weight
- Perform 12-lead ECG
- Collect blood samples and urinalysis for safety clinical laboratory assessments
- Administer ALSFRS-R questionnaire
- Administer the American Urological Association (AUA) Symptom Score survey
- Perform SVC
- Perform hand held dynamometry (HHD - APB bilaterally)
- Administer the suicide prevention (C-SSRS) questionnaire
- Perform neurophysiological testing (TMS and NCS) unless the decision to withdraw consent was due to intolerance of neurophysiological testing.
- Check study drug compliance and collect dosing diary
- Review returned Muscle Symptom Diary
- Complete Fasciculation Assessment
- Perform study drug accountability and collect all unused study drug and empty containers
- Complete Blindedness Questionnaire and Exit Survey

The final Follow-Up Telephone Call phone call will take place no sooner than 28 days after the last known dose of study drug. The following procedures will be performed:

- Review and document concomitant medications and therapies
- Assess and document AEs

8.10 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.

8.11 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 study Manual of Procedures 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.

8.12 Survival Data

For all subjects, including those who have discontinued participation or are lost to follow-up, attempts may be made every 6 months to document vital status and collect future clinical data related to ALS progression (e.g. ALSFRS).

9 STUDY SCHEDULE (MATCHED HEALTHY CONTROLS)

No study procedures should be performed prior to the signing of the ICF. All subjects will sign an ICF prior to undergoing any study tests or procedures.

Visit windows are consecutive calendar days and the target visit dates are calculated from the Baseline Visit. All clinic visits after the Baseline visit will have a +/- 10 day window.

9.1 Screening Visit

The following procedures will be performed at an office visit to determine the subject's eligibility for the study and to collect baseline data if eligible:

- Obtain written informed consent
- Assess inclusion and exclusion criteria. If ineligible, record reason for screen failure.
- Review and document concomitant medications and therapies
- Assess and document AEs that occur after subject signs ICF
- Collect demographic data
- Obtain medical history
- Complete Edinburgh Handedness Inventory-Short Form
- Perform neurological examination
- Measure vital signs including height and weight
- Perform neurophysiological testing (TMS and NCS)
- Collect blood for iPS cell line generation
- Schedule the Week 6 Visit

9.1.1 Screen Failures

Any subject who signs consent will be considered enrolled in the study. If a subject fails screening, *at a minimum*, the following information should be captured and entered in the Electronic Data Capture (EDC) System:

- Inclusion/Exclusion criteria
- Demographics
- Reason for screen failure

9.2 Week 6 Visit

This visit will take place 42 ± 10 days after the Baseline Visit.

- Review and document concomitant medications and therapies
- Assess and document AEs
- Measure vital signs
- Perform neurophysiological testing (TMS and NCS)
- Collect blood for iPS cell line generation (if not done at Screening)
- Schedule next visit

9.3 Week 12 Visit

This visit will take place 84 ± 10 days after the Baseline Visit. The following procedures will be performed:

- Review and document concomitant medications and therapies
- Assess and document AEs
- Perform neurophysiological testing (TMS and NCS)
- Collect blood for iPS cell line generation (if not done previously)

10 CLINICAL ASSESSMENTS AND OUTCOME MEASURES

10.1 Clinical Assessments

Assessments will be performed at designated time-points throughout the study for clinical evaluation. In addition to the assessments evaluated below, subjects will provide information on their demographics, current medical conditions, medical history, as well as concomitant medication usage.

10.1.1 Vital Signs, Height & Weight

Vital signs will be obtained after the subject has been in a seated position for several minutes. Vital signs, including systolic and diastolic blood pressure, pulse rate (radial artery)/minute, respiratory rate/minute, temperature and weight will be assessed at specified visits. Height will be measured and recorded at the Screening Visit only.

10.1.2 Clinical Laboratory Assessments

The following laboratory tests will be performed to evaluate eligibility and for safety:

- Hematology with differential panel: complete blood count with differential (hematocrit, hemoglobin, platelet count, RBC indices, Total RBC, Total WBC, and WBC & differential)

- Blood chemistry panel/Liver function tests (LFTs): alanine aminotransferase (ALT (SGPT)), aspartate aminotransferase (AST (SGOT)), albumin, alkaline phosphatase, bicarbonate, blood urea nitrogen, calcium, chloride, creatinine, glucose, magnesium, phosphate, potassium, sodium, total bilirubin and total protein

- Urinalysis: blood, clarity, color, glucose, ketones, nitrate, pH, protein, specific gravity, and WBC screen

- Serum human chorionic gonadotrophin (hCG) for WOCBP (collected only at Screening Visit, and as necessary throughout course of study)

All subjects will have safety laboratory tests at the designated visits outlined in the protocol. These samples will be analyzed at a central laboratory. The SI may order additional testing, if needed, to further assess an AE, or if there is any suspicion that a subject may be pregnant, throughout the course of the study.

10.1.3 Blood Sample for iPSCs

ALS subjects will provide a one-time research blood sample following standard clinical protocols at the Week 4 visit. If subjects are unable to provide a sample at Week 4, the sample could also be collected at the Week 6 or 8 visit. Matched healthy control subjects will provide a one-time research blood sample at the Screening, Week 6 or Week 12 Visit following standard clinical protocols. These samples will be used to derive iPSCs for the exploratory aims of this study and for other future research both within and outside of motor neuron diseases. All samples will be labeled with a code. The code will not include any identifiable information. Coded blood samples will be stored at a central laboratory prior to iPSC generation and other research use. Third party safety protocols for iPSC generation require blood testing for infectious agents including HIV, Hepatitis B and Hepatitis C. Results will not be conveyed to study staff or to subjects.

10.1.4 12-Lead Electrocardiogram (ECG)

A standard 12-lead ECG will be performed. Tracings will be reviewed by a local on-site cardiologist, and a copy of the tracings will be kept on site as part of the source documents. QTc interval > 450ms in males or > 470ms in females should be brought to the attention of the medical monitor.

10.1.5 Physical Examination

A physical examination will be performed and recorded. The following systems will be examined: head/neck, eyes, ears, nose/throat, cardiovascular, lungs, abdomen, musculoskeletal, central nervous system, extremities, and skin. The skin assessment will include evaluation for discoloration of the nails, lips, skin and oral mucosa.

10.1.6 Neurological Examination

A neurological examination will be performed and recorded. Examination will include assessment of mental status, cranial nerves, motor and sensory function, reflexes, coordination, and stance/gait.

10.1.7 Columbia Suicide Severity Rating Scale (C-SSRS)

The US FDA recommends the use of a suicidality assessment instrument that maps to the Columbia Classification Algorithm for Suicide Assessment (C-CASA) (Posner et al., 2007). The C-CASA was developed to assist the FDA in coding suicidality data accumulated during the conduct of clinical trials of antidepressant drugs. One such assessment instrument is the Columbia Suicide Severity Rating Scale (C-SSRS) (US FDA Draft Guidance, 2010). The C-SSRS involves a series of probing questions to inquire about possible suicidal thinking and behavior.

At the Baseline Visit, the C-SSRS Baseline version will be administered. This version is used to assess suicidality over the subject's lifetime and specifically for the previous 6 month time period. Suicidality will also be assessed at follow-up visits over the course of the study.

10.1.8 Ophthalmological Examination

Ophthalmological examinations will be performed and recorded at the Baseline and Week 12 Visits (within 14 days prior of each). Exams will include visual acuity testing, dilated fundus photography, and a slit-lamp examination. If deemed warranted by the examining ophthalmologist, fluorescein angiogram, ocular coherence tomography, perimetry, and electro-retinogram may also be obtained.

10.1.9 Lumbar Puncture

The lumber puncture is optional.

For subjects consenting to a LP, it will be performed to collect CSF at the Week 6 Visit. Study staff should document the time of last ezogabine dose administered. The SI will discuss all potential LP risks to the subjects including:

- Local pain at injection site
- Reaction to anesthetic agents
- Bleeding at needle entrance site
- Infection at needle entrance site
- Post-LP low-pressure headache

Extensive experience with research LP in Alzheimer's disease reveals a very low incidence of complication, including the incidence of post-LP headache (Zetterberg et al., 2010). Fewer than 2.6% of patients in a memory disorder clinic developed post-LP headache, and only a single patient in a cohort of over 1,000 had a headache lasting more than 5 days (Zetterberg et al., 2010). No other local or generalized complications occurred.

The procedure must be performed by the SI or another licensed practitioner with experience and training in performing LPs, and must be listed on the site delegation log. When possible, LPs will be performed with an atraumatic Sprotte needle to reduce the risk of post-LP headache.

Coded CSF samples (with no subject identifying information) will be stored in the NEALS Biorepository located at Massachusetts General Hospital. They will be used for broad research purposes including retigabine drug levels.

10.1.10 Adverse Events

AEs will be documented at all study visits, including the Screening Visit once the ICF has been signed by the subject, and at all scheduled telephone calls, including the final telephone call. Any unresolved AEs at the time of study completion will be followed for up to 28 days after the final telephone call. Information on AEs will be determined at each visit by direct questioning of the subjects, review of concomitant medications, and vital sign results.

10.1.11 Blood Biobanking

Up to 40mL will be collected at the same time as the lumbar puncture (LP). The time of the most recent ezogabine dose will be recorded for this blood draw. Encoded blood samples (with no subject identifying information) will be stored in the NEALS Biorepository located at Massachusetts General Hospital. They will be used for broad research purposes.

10.1.12 Neurophysiological Testing

TMS measurements will be performed by a board-certified physician or experienced technician. NCS measurements will be performed by a neuromuscular physician. Each site will have up to two TMS physiologists and up to two NCS physiologists (overlap allowed). Experienced neuromuscular physicians will obtain TMS and threshold NCS training at the investigator meeting, as it is critical to minimize test-retest and inter-site variation.

For ALS subjects, neurophysiological testing will be performed on the side (right or left) as determined during the neurological examination. For unmatched and matched healthy control subjects, neurophysiological testing will be performed on the right side. Sites will have the option of including one additional recording on the left side for unmatched healthy control subjects at the third visit.

Cortical motor neuron excitability will be measured by TMS. Measurements will include SICI, paired pulse facilitation, MEP amplitude and threshold, and cortical silent period. These measurements typically take 60 minutes but can take up to 120 minutes to complete at each session.

Lower motor neuron excitability will be measured using NCS. For studies of lower motor neurons, median compound nerve action potentials will be recorded using surface electrodes in a standard belly/tendon montage using disposable self-adhesive ground and disk electrodes with constant temperature monitoring. For axonal threshold testing, data acquisition and stimulation for threshold tracking measurements will be performed using a computerized program, QTRACS with multiple excitability protocols (Institute of Neurology). We will obtain the following measurements: stimulus-response curve, strength-duration time constant, threshold electrotonus, and recovery cycle analysis (as described in detail in Kiernan et al., 2000; Nakata et al., 2006; Kanai et al., 2006; Vucic & Kiernan, 2006). When necessary, a clinical NCS machine may be

used for optimization of electrode placement and validation of inclusion criteria. Lower motor neuron excitability measurements typically take approximately 20 minutes to complete at each session but can take up to 40 minutes.

10.1.13 American Urological Association (AUA) Symptom Score

The AUA Symptom Score will be administered to subjects at baseline, and the Week 4, 8, and 12 Visits. This instrument is widely used to track symptoms of urinary dysfunction. Because ezogabine has a risk of urinary dysfunction including urinary retention, we will include this survey to help SIs and medical monitor assess symptoms.

10.1.14 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, threshold tracking NCS and lumbar puncture.

10.2 Clinical Outcome Measures

10.2.1 ALSFRS-R

The ALSFRS-R is a quickly administered (5 minutes) 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.

10.2.2 Slow Vital Capacity (SVC)

The SVC (percent of predicted normal) will be determined, using the upright SVC method. 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 difference between the highest and second highest relative to the highest SVC is 10% or greater for the first 3 trials. All values will be recorded. The highest SVC recorded is utilized for eligibility.

10.2.3 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.

10.2.4 Hand-held Dynamometry (HHD)

Hand held dynamometry (HHD) will be used as a quantitative measure of muscle strength of the abductor pollicis brevis (APB) muscle.

10.2.5 Manual Muscle Testing (MMT) is a procedure for the evaluation of the function and strength of individual muscles and muscle groups based on the effective performance of a movement in relation to the forces of gravity and manual resistance. MMT testing will be performed on the bilateral APB muscles only.

10.2.6 Training and Validation

All evaluators must be NEALS certified to perform the ALSFRS-R, SVC and HHD; specific certification requirements are outlined in the study operations manual. Repeat NEALS certification will follow NEALS standard practices for all outcome measures. It is strongly preferred that a single evaluator performs all measures throughout the study, if possible. NEALS certification is required for all evaluators prior to performing any study tests.

10.2.7 Assessment of Muscle Symptoms

Subjects will be provided with a Muscle Symptom Diary to record muscle cramp frequency and intensity and severity of fasciculations at home daily. At each subsequent visit, the site staff will review the diary and record the daily frequency and maximal intensity of muscle cramps and fasciculations before providing the subject with a new diary.

10.2.7.1 Muscle Cramps

The effects of retigabine on muscle cramps will also be assessed as a secondary endpoint. Cramps in ALS 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.

The visual analog scale (VAS) is a scale that measures pain associated with muscle cramping. It will be used to measure muscle cramp intensity in this study. The scale rating is from 0-10; 0 equals no symptoms, 10 equals most severe symptoms.

For the purposes of this study, a muscle cramp is defined as a sustained painful muscle contraction lasting seconds to minutes. At the Baseline Visit, subjects will be asked to recount the number of muscle cramps experienced in the previous 24 hours and the total number of cramps experienced in the previous 30 days. Subjects will also be asked to recount the maximum pain experienced with a muscle cramp in the previous 24 hours and the maximum pain experienced with a muscle cramp in the previous 30 days. The coordinator will record this on the source document at this visit. The VAS will also be explained at the Baseline Visit to all subjects. The coordinator will provide the subject with a muscle cramp diary to record muscle cramp frequency and intensity at home, daily. At each future visit (beginning at the Week 4 Visit), the coordinator will review the Muscle Cramp Diary.

10.2.7.2 Fasciculations

Fasciculations will also be recorded in the daily diary. At Baseline, Weeks 4, 6, 8 and 12, subjects will be asked several questions related to their fasciculations experienced in the previous 14 days. Reference the study Manual of Procedures for additional information.

For the purpose of this study, a fasciculation is a brief, spontaneous contraction affecting a small number of muscle fibers, often causing a flicker of movement under the skin. Defining interference with daily activities may be different for each subject and defining daily activities will be different for each subject.

11 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.

11.1 Definitions of AEs, Suspected Adverse Drug Reactions & SAEs

11.1.1 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.

11.1.1.1 Adverse Events of Special Interest

All subjects participating in this trial will be informed that they could be at risk for:

- new or worsened urinary retention (in the clinical judgment of the site investigator)
- retinal hyperpigmentation abnormalities and/or vision loss,
- pigmentation of non-retinal ocular tissues (e.g., conjunctiva, sclera, iris)
- discoloration of nails, lips, skin (including skin around the eyes) or mucosa.

Subjects must inform the SI of any of these symptoms immediately. The SI must contact the Medical Monitor to discuss the subject's continued use of study drug. Per the discretion of the SI, subjects may be referred to a dermatologist for skin discoloration at any point in the study.

Special attention must also be made for AEs of possible drug-induced liver injury with hyperbilirubinemia defined as ALT 3x ULN and bilirubin 2x ULN (with >35% direct).

11.1.2 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.

11.2 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.

11.2.1 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

11.2.2 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).

11.2.3 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, retinal pigmentation and/or vision loss, skin pigmentation changes or urinary retention, 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.

11.3 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)
- Events of special interest as described in Section 11.1.1.1.
- 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.

12 DATA SAFETY MONITORING

12.1 Data and Safety Monitoring Board

An independent DSMB will be assembled for the trial. The DSMB receives the blinded and unblinded summary reports of the frequency of all clinical AEs and safety laboratory tests for planned periodic meetings approximately every 3-6 months throughout the study. Meetings will be held via teleconference. In addition, the DSMB Chair may call ad hoc meetings.

Summaries of SAEs and enrollment will be provided approximately monthly to the DSMB by the Study Biostatistician. AEs occurring within 24 hours of dosing, AE(s) related to visual acuity, skin pigmentation, hyperbilirubinemia, and urinary retention, and any severe unexpected SAEs are considered events of interest and will be reported in the monthly summaries to the DSMB by the NCRI CC. The DSMB can ask to receive the SAE reports more frequently. As necessary, the DSMB can review the frequencies of clinical and laboratory abnormalities. Recommendations for modification or termination of the trial based on safety data will be made by the DSMB to the PI and Steering Committee. The DSMB will review safety data throughout the trial and may stop the trial for safety if it determines that there is a significant difference in the rate of a particular AE that would indicate a risk that is greater than the possible benefit of the study drug. A notable increase in the frequency of any AE should be examined by the DSMB although it may not lead to a recommendation by the DSMB. There will not be early stopping for efficacy in this trial.

Prior to each DSMB meeting, the NCRI CC will provide an update to the DSMB on enrollment, data quality (missing data) and protocol adherence. The NCRI CC will be responsible for communication with the DSMB.

Complete information can be found in the Data and Safety Monitoring Plan.

12.2 Safety Monitoring

12.2.1 Safety Management Resource Team

The NCRI CC Safety Management Resource Team (SMaRT) is comprised of physicians, project managers, data managers, and systems personnel. On a periodic basis, the SMaRT will review AEs, SAEs, abnormal laboratory measurements, ECGs and protocol deviations as detailed in the study's Safety Management Plan.

12.2.2 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 STATISTICAL CONSIDERATIONS

13.1 Analysis Populations

The unmatched healthy controls, matched healthy controls, and ALS subjects represent three distinct populations of individuals for the purposes of analysis. Some unmatched or matched healthy controls may be excluded from analysis if there is good reason to question their status as neurologically normal. All ALS subjects who are randomized and receive at least one dose of study drug will compose the modified intention to treat (mITT) population and will be included in primary analyses of safety, tolerability, and efficacy. For mITT analyses, subjects will be grouped according to their randomized treatment assignment regardless of treatment actually received or compliance with assigned treatment.

13.2 Analysis of Unmatched Healthy Control Subjects

Neurophysiological testing (TMS and NCS) will be performed on unmatched healthy control subjects at each site to evaluate test-retest variation of the primary outcome prior to activating a site for enrollment of ALS subjects. Approximately five unmatched healthy control subjects per site will each undergo three repeat neurophysiological testing sessions. The Steering Committee will review estimates of test-retest reliability and other measures of site performance dynamically and will determine when individual sites may begin enrolling ALS subjects. The Steering Committee may adjust the number of healthy controls and the number of testing sessions per subject at individual sites based on the existing data at the time of review. The decision to activate a site to enroll ALS subjects will take into consideration the test-retest variation relative to performance at other participating sites. The goal will be to activate eight (8) to twelve (12) sites to recruit ALS subjects. The Steering Committee may recommend additional healthy control testing and delayed activation of sites to recruit ALS subjects.

Estimates of test-retest variability will be obtained by analysis of the unmatched healthy control subjects using random effects models with random intercepts for site and subject and site-specific within-person heteroscedasticity,

$$Y = \mu + a_i + b_{ij} + \epsilon_{ijk}$$

where i indexes site, j indexes subject, and k indexes repeated testing sessions, $a_i \sim N(0,\mathrm{Var}[a])$, $b_{ij} \sim N(0,\mathrm{Var}[b])$, and $\varepsilon_{ijk} \sim N(0,\mathrm{Var}[\varepsilon]_i)$. Test-retest reliability will be calculated as the person-level intraclass correlation, the ratio of among-person variance over the sum of among-person and within-person variance for a given site. By assuming that among-person variance is not site-specific, a site can achieve high intraclass correlation only by ensuring low within-person variance, not by enrolling highly variable subjects. TMS and NCS parameters will be analyzed on their original scale or after log transformation depending on which scale yields residuals that

are more nearly normally distributed by the Anderson-Darling goodness of fit statistic. The Steering Committee may also consider the absolute magnitudes of TMS and NCS parameter estimates for each site in evaluating site performance.

13.3 Primary Analysis

We will test the effect of ezogabine on SICI using the mITT sample of ALS subjects in a shared-baseline mixed model with fixed effects for visit (6 levels: Screening, Baseline, and Weeks 4, 6, 8, and 12), treatment group (3 levels: placebo and 600 and 900 mg/day ezogabine) x post-baseline visit (4 levels) and random site-specific intercepts and random participant-specific intercepts and slopes with unstructured covariance. The use of a shared-baseline reflects the homogeneity of the trial cohort prior to randomization and the combination with participant-specific random effects induces an adjustment for any chance baseline differences similar to ANCOVA (Liu et al, 2009). We will use linear contrasts to test for dose-dependent differences in change from baseline to the average SICI over the stable dose period from weeks 6 through 8. A significant reduction of SICI (i.e., higher MEP amplitude) of either dose relative to placebo would be accepted as evidence of benefit from ezogabine treatment.

13.4 Secondary Analyses

We will use the same shared-baseline mixed model analysis to test other continuous secondary efficacy outcomes (additional TMS parameters, NCS parameters, ALSFRS-R, and SVC). MEP threshold, the key secondary outcome, will be tested prior to and separate from all other secondary efficacy outcomes. We will compare trajectories in TMS and NCS parameters between ALS and matched healthy control subjects in an augmented model with the addition of fixed effects for patient group (2 levels: ALS and healthy controls) and patient group x visit interactions (9 levels: 6 visits for ALS patients and 3 for matched healthy controls) and separate patient-level variance-covariance and residual variance estimates by patient group. Comparisons between matched healthy controls and ALS patients will be estimated using linear contrasts.

Safety and tolerability will be compared across treatments by log-rank test of time to first SAE, time to death, and time to dose discontinuation and by negative binomial regression of overall SAE and AE rates. The proportion of participants experiencing each MedDRA-classified AE will be compared across treatment groups by Fisher's exact test. Treatment-dependent differences in suicidality will be estimated from C-SSRS total scores analyzed using the same shared-baseline mixed model used for the primary analysis.

Serum PK estimates will be obtained for C_{max} and time to peak concentration.

Associations between firing rate of iPSC-derived motor neurons and baseline *in vivo* neurophysiological excitability indices will be compared within and between ALS and healthy control subjects by linear regression with *in vivo* paired pulse SICI as the dependent variable and

in vitro-firing rate, disease status and their interaction as predictors. A change-point model will be explored with a break at each participant's *in vitro*-estimated EC50 for blocking motor neuron spontaneous action potential firing by ezogabine. Spontaneous firing rate of the *in vitro*-derived motor neurons and *in vitro*-estimated EC50 for blocking motor neuron spontaneous action potential firing by ezogabine will be considered as moderators of treatment efficacy in tests of neurophysiological and clinical response by augmenting the shared-baseline mixed models with fixed effects for iPSC parameters, the two-way interaction with visit, and the three-way interaction with treatment group and post-baseline visit.

13.5 Missing Data

Following the ITT principle, all subjects in the mITT sample will be included in the primary efficacy analysis. Missing data will be handled by using models that are unbiased when data are missing at random. Alternative models that might achieve the missing at random assumption and alternative handling of missing values, e.g., by multiple imputation or explicit modeling of the missingness process, may be pursued as a sensitivity analysis, particularly if the active treatment arms have higher rates of missingness.

13.6 Multiplicity Adjustment for Primary and Key Secondary Endpoints

The effect of each dose of ezogabine on the primary efficacy outcome in the mITT sample will be tested at two-tailed $p < 0.027$ following Dunnett's correction for comparing two active treatments to placebo. If this primary analysis identifies a significant benefit from ezogabine treatment, then testing the key secondary efficacy outcome at $p < 0.027$ maintains an overall type I error rate at 5% under a closed testing sequential analysis. If significance of the key secondary outcome alone is accepted as evidence of a therapeutic benefit from ezogabine when the primary analysis is not significant, then the overall type I error rate will be limited to 10% or less (depending on the correlation between the primary and key secondary outcomes).

13.7 Interim Analysis

An interim futility analysis will take place after data are available from the forty-fifth (45th) ALS subject. Futility analysis will be performed independently for each study arm based on probability of "success", which we define as being at least 95% certain that ezogabine treatment is beneficial relative to placebo with regard to the primary outcome or the key secondary outcome given a sample size of 30 subjects in each group. Futility for a specific study arm will be met if the probability of "success" conditioned on the data from the initial forty-five subjects is less than 20%. If a single arm meets the futility requirement, that arm will be discontinued. If both arms meet the futility requirement, the study will stop pending completion of already enrolled subjects. Recruiting will continue during the interim analysis period.

In addition to early stopping for futility, the DSMB, SMaRT, and the medical monitor will review safety data throughout the trial. While no formal early stopping rules for safety have been

predefined given the diversity of possible safety signals that might arise, the DSMB may stop the trial for safety, for example, if they determine that there is a significant difference in the rate of a particular adverse event that would indicate a greater risk than the possible benefit of the study drug. The DSMB can also decide whether a specific subject must discontinue study drug due to concerns about recurrent or persistent serious side effects such as cardiac events. A significant increase in the frequency of any adverse event should be examined by the DSMB although it may not lead to a recommendation by the DSMB.

13.8 Power Calculations

Caramia et al. (2000) report mean (SD) paired pulse SICI at 3 ms among 16 ALS patients of 125.7 (25.7) percent of MEP control size. Stefan et al. (2001) report paired pulse SICI at 3 ms among 13 ALS patients of 96.9 (35.9) percent of MEP control size. Assuming a mean (SD) equal to 100 (30) percent of MEP and using a two-sided alpha of 0.027 based on Dunnett's correction for comparing two treatments to placebo, 40 patients in each group and up to 25% loss to follow-up would give 80% power to detect a 25% difference between placebo and each active treatment based on a simple two-sample t-test. This accommodates the loss of power due to early stopping for futility and is conservative relative to our proposed shared-baseline mixed model analysis. For reference, Caramia et al. (2000) report an effect of three-week exposure to gabapentin equal to an 80% reduction of the paired pulse amplitude in untreated patients, and Stefan et al. (2001) report an effect of 5 or more day exposure to riluzole equal to a 35% reduction of the conditioned stimulus amplitude.

14 DATA COLLECTION, MANAGEMENT AND MONITORING

14.1 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.

14.1.1 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.

14.1.2 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.

14.1.3 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 CC 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.

14.2 Clinical 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. More information regarding monitoring guidelines, timelines, and processes can be found in the study Manual of Procedures.

14.3 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.

14.3.1 Confidentiality

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.

14.3.2 Global Unique Identifier (GUID)

A patient Global Unique Identifier (GUID) will be used as an identifier for individuals participating in this study. The GUID is an 11-character string that is generated using encryption technology and algorithms licensed by the NCRI from the National Institutes of Health (NIH).

The GUID is generated on a secure website that utilizes 128-bit Secure Socket Layer (SSL). This website is not linked to the study database. 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.

The GUID is entered into the study database when the patient is being created in the system. As the same patient may participate in multiple studies, the study database will also allow capturing a study-specific ID for the patient.

14.3.3 Study Discontinuation

The study can be terminated at any time. Reasons for terminating the study may include the following:

- The incidence or severity of AEs in this or other studies indicates a potential health hazard to study subjects.
- Study subject enrollment is unsatisfactory.
- Data recording is inaccurate or incomplete.
- Sponsor withdraws funding.

14.3.4 Retention of Records

The SIs should retain all study documents and records in accordance with their site IRB policies.

14.3.5 Publications

The PI, Brian Wainger, MD, PhD, along with the Steering Committee 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.

14.3.6 Use of Samples

Blood and CSF samples as well as iPSCs generated from subject blood samples will be used for broad research purposes both related to and potentially unrelated to motor neuron disease. Encoded samples that do not contain subject identifying information will be stored in research

repositories from which researchers will be able to obtain them for research purposes only. Research using the samples may take place within both academic and non-academic settings. Although genetic information may be analyzed, no genetic information will be given to the subject nor will the information that may be obtained be placed in a subject's medical records.

There is no scheduled date on which the samples will be destroyed. Samples may be stored for research until they are used, damaged, decay or otherwise become unfit for analysis. Subjects have the option of declining participation in this portion of the study at any time by withdrawing their consent to have their sample used. However, it will not be possible to destroy samples that have already been submitted to a repository.

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16 APPENDICES

16.1 Appendix I: El Escorial World Federation of Neurology Criteria for the Diagnosis of ALS

Information obtained from the web site: ***www.wfnals.org***.

The diagnosis of Amyotrophic Lateral Sclerosis [ALS] requires:
A - The presence of:

(A:1) evidence of lower motor neuron (LMN) degeneration by clinical, electrophysiology cal 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 Probable - 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 gag reflex exaggerated snout reflex pseudobulbar features forced yawning pathologic DTRs spastic tone	clonic DTRs Hoffman reflex pathologic DTRs spastic tone preserved reflex in weak wasted limb	loss of superficial abdominal reflexes pathologic DTRs spastic tone	clonic DTRs - extensor plantar response pathologic DTRs spastic tone preserved reflex in weak wasted limb

16.2 Appendix II: ALS Functional Rating Scale – Revised (ALSFRS-R)

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

Evaluator's Initials: _____ _____ _____

16.3 Appendix III: Columbia-Suicide Severity Rating Scale (C-SSRS) Baseline Version

Information obtained from: http://www.cssrs.columbia.edu/

SUICIDAL IDEATION	
Ask questions 1 and 2. If both are negative, proceed to "Suicidal Behavior" section. If the answer to question 2 is "yes", ask questions 3, 4 and 5. If the answer to question 1 and/or 2 is "yes", complete "Intensity of Ideation" section below.	Lifetime: Time He/She Felt Most Suicidal
1. Wish to be Dead Subject endorses thoughts about a wish to be dead or not alive anymore, or wish to fall asleep and not wake up. Have you wished you were dead or wished you could go to sleep and not wake up? If yes, describe:	Yes No ☐ ☐
2. Non-Specific Active Suicidal Thoughts General, non-specific thoughts of wanting to end one's life/commit suicide (e.g., "I've thought about killing myself") without thoughts of ways to kill oneself/associated methods, intent, or plan. Have you actually had any thoughts of killing yourself? If yes, describe:	Yes No ☐ ☐
3. Active Suicidal Ideation with Any Methods (Not Plan) without Intent to Act Subject endorses thoughts of suicide and has thought of at least one method during the assessment period. This is different than a specific plan with time, place or method details worked out (e.g., thought of method to kill self but not a specific plan). Includes person who would say, "I thought about taking an overdose but I never made a specific plan as to when, where or how I would actually do it…and I would never go through with it." Have you been thinking about how you might do this? If yes, describe:	Yes No ☐ ☐
4. Active Suicidal Ideation with Some Intent to Act, without Specific Plan Active suicidal thoughts of killing oneself and subject reports having some intent to act on such thoughts, as opposed to "I have the thoughts but I definitely will not do anything about them." Have you had these thoughts and had some intention of acting on them? If yes, describe:	Yes No ☐ ☐
5. Active Suicidal Ideation with Specific Plan and Intent Thoughts of killing oneself with details of plan fully or partially worked out and subject has some intent to carry it out. Have you started to work out or worked out the details of how to kill yourself? Do you intend to carry out this plan? If yes, describe:	Yes No ☐ ☐
INTENSITY OF IDEATION	
The following features should be rated with respect to the most severe type of ideation (i.e., 1-5 from above, with 1 being the least severe and 5 being the most severe). Ask about time he/she was feeling the most suicidal. _____ _____ Most Severe Ideation: Type # (1-5) Description of Ideation	Most Severe
Frequency How many times have you had these thoughts? (1) Less than once a week (2) Once a week (3) 2-5 times in week (4) Daily or almost daily (5) Many times each day	____

Duration When you have the thoughts, how long do they last? (1) Fleeting - few seconds or minutes (4) 4-8 hours/most of day (2) Less than 1 hour/some of the time (5) More than 8 hours/persistent or continuous (3) 1-4 hours/a lot of time	____
Controllability Could/can you stop thinking about killing yourself or wanting to die if you want to? (1) Easily able to control thoughts (4) Can control thoughts with a lot of difficulty (2) Can control thoughts with little difficulty (5) Unable to control thoughts (3) Can control thoughts with some difficulty (0) Does not attempt to control thoughts	____
Deterrents Are there things - anyone or anything (e.g., family, religion, pain of death) - that stopped you from wanting to die or acting on thoughts of committing suicide? (1) Deterrents definitely stopped you from attempting suicide (4) Deterrents most likely did not stop you (2) Deterrents probably stopped you (5) Deterrents definitely did not stop you (3) Uncertain that deterrents stopped you (0) Does not apply	____
Reasons for Ideation What sort of reasons did you have for thinking about wanting to die or killing yourself? Was it to end the pain or stop the way you were feeling (in other words you couldn't go on living with this pain or how you were feeling) or was it to get attention, revenge or a reaction from others? Or both? (1) Completely to get attention, revenge or a reaction from others (4) Mostly to end or stop the pain (you couldn't go on (2) Mostly to get attention, revenge or a reaction from others living with the pain or how you were feeling) (3) Equally to get attention, revenge or a reaction from others (5) Completely to end or stop the pain (you couldn't go on and to end/stop the pain. living with the pain or how you were feeling) (0) Does not apply	____

SUICIDAL BEHAVIOR *(Check all that apply, so long as these are separate events; must ask about all types)*	**Lifetime**
Actual Attempt: A potentially self-injurious act committed with at least some wish to die, *as a result of act.* Behavior was in part thought of as method to kill oneself. Intent does not have to be 100%. If there is ***any*** intent/desire to die associated with the act, then it can be considered an actual suicide attempt. ***There does not have to be any injury or harm***, just the potential for injury or harm. If person pulls trigger while gun is in mouth but gun is broken so no injury results, this is considered an attempt. Inferring Intent: Even if an individual denies intent/wish to die, it may be inferred clinically from the behavior or circumstances. For example, a highly lethal act that is clearly not an accident so no other intent but suicide can be inferred (e.g., gunshot to head, jumping from window of a high floor/story). Also, if someone denies intent to die, but they thought that what they did could be lethal, intent may be inferred. ***Have you made a suicide attempt?*** ***Have you done anything to harm yourself?*** ***Have you done anything dangerous where you could have died?*** ***What did you do?*** ***Did you_____ as a way to end your life?*** ***Did you want to die (even a little) when you_____?*** ***Were you trying to end your life when you _____?*** ***Or did you think it was possible you could have died from_____?*** ***Or did you do it purely for other reasons / without ANY intention of killing yourself (like to relieve stress, feel better, get sympathy, or get something else to happen)?*** *(*Self-Injurious Behavior without suicidal intent) If yes, describe: **Has subject engaged in Non-Suicidal Self-Injurious Behavior?**	**Yes No** ☐ ☐ Total # of Attempts _____ **Yes No** ☐ ☐

Interrupted Attempt: When the person is interrupted (by an outside circumstance) from starting the potentially self-injurious act *(if not for that, actual attempt would have occurred).* Overdose: Person has pills in hand but is stopped from ingesting. Once they ingest any pills, this becomes an attempt rather than an interrupted attempt. Shooting: Person has gun pointed toward self, gun is taken away by someone else, or is somehow prevented from pulling trigger. Once they pull the trigger, even if the gun fails to fire, it is an attempt. Jumping: Person is poised to jump, is grabbed and taken down from ledge. Hanging: Person has noose around neck but has not yet started to hang - is stopped from doing so. ***Has there been a time when you started to do something to end your life but someone or something stopped you before you actually did anything?*** If yes, describe:			**Yes No** ☐ ☐ Total # of interrupted _____
Aborted Attempt: When person begins to take steps toward making a suicide attempt, but stops themselves before they actually have engaged in any self-destructive behavior. Examples are similar to interrupted attempts, except that the individual stops him/herself, instead of being stopped by something else. ***Has there been a time when you started to do something to try to end your life but you stopped yourself before you actually did anything?*** If yes, describe:			**Yes No** ☐ ☐ Total # of aborted _____
Preparatory Acts or Behavior: Acts or preparation towards imminently making a suicide attempt. This can include anything beyond a verbalization or thought, such as assembling a specific method (e.g., buying pills, purchasing a gun) or preparing for one's death by suicide (e.g., giving things away, writing a suicide note). ***Have you taken any steps towards making a suicide attempt or preparing to kill yourself (such as collecting pills, getting a gun, giving valuables away or writing a suicide note)?*** If yes, describe:			**Yes No** ☐ ☐
Suicidal Behavior: Suicidal behavior was present during the assessment period?			**Yes No** ☐ ☐
Answer for Actual Attempts Only	Most Recent Attempt Date:	Most Lethal Attempt Date:	Initial/First Attempt Date:
Actual Lethality/Medical Damage: 0. No physical damage or very minor physical damage (e.g., surface scratches). 1. Minor physical damage (e.g., lethargic speech; first-degree burns; mild bleeding; sprains). 2. Moderate physical damage; medical attention needed (e.g., conscious but sleepy, somewhat responsive; second-degree burns; bleeding of major vessel). 3. Moderately severe physical damage; *medical* hospitalization and likely intensive care required (e.g., comatose with reflexes intact; third-degree burns less than 20% of body; extensive blood loss but can recover; major fractures). 4. Severe physical damage; *medical* hospitalization with intensive care required (e.g., comatose without reflexes; third-degree burns over 20% of body; extensive blood loss with unstable vital signs; major damage to a vital area). 5. Death	*Enter Code* _____	*Enter Code* _____	*Enter Code* _____
Potential Lethality: Only Answer if Actual Lethality=0 Likely lethality of actual attempt if no medical damage (the following examples, while having no actual medical damage, had potential for very serious lethality: put gun in mouth and pulled the trigger but gun fails to fire so no medical damage; laying on train tracks with oncoming train but pulled away before run over). 0 = Behavior not likely to result in injury 1 = Behavior likely to result in injury but not likely to cause death 2 = Behavior likely to result in death despite available medical care	*Enter Code* _____	*Enter Code* _____	*Enter Code* _____

16.4 Appendix IV: Columbia-Suicide Severity Rating Scale (C-SSRS) Since Last Visit

SUICIDAL IDEATION	
Ask questions 1 and 2. If both are negative, proceed to "Suicidal Behavior" section. If the answer to question 2 is "yes", ask questions 3, 4 and 5. If the answer to question 1 and/or 2 is "yes", complete "Intensity of Ideation" section below.	Since Last Visit
1. Wish to be Dead Subject endorses thoughts about a wish to be dead or not alive anymore, or wish to fall asleep and not wake up. Have you wished you were dead or wished you could go to sleep and not wake up? If yes, describe:	Yes No □ □
2. Non-Specific Active Suicidal Thoughts General, non-specific thoughts of wanting to end one's life/commit suicide (e.g., "I've thought about killing myself") without thoughts of ways to kill oneself/associated methods, intent, or plan during the assessment period. Have you actually had any thoughts of killing yourself? If yes, describe:	Yes No □ □
3. Active Suicidal Ideation with Any Methods (Not Plan) without Intent to Act Subject endorses thoughts of suicide and has thought of at least one method during the assessment period. This is different than a specific plan with time, place or method details worked out (e.g., thought of method to kill self but not a specific plan). Includes person who would say, "I thought about taking an overdose but I never made a specific plan as to when, where or how I would actually do it…and I would never go through with it." Have you been thinking about how you might do this? If yes, describe:	Yes No □ □
4. Active Suicidal Ideation with Some Intent to Act, without Specific Plan Active suicidal thoughts of killing oneself and subject reports having some intent to act on such thoughts, as opposed to "I have the thoughts but I definitely will not do anything about them." Have you had these thoughts and had some intention of acting on them? If yes, describe:	Yes No □ □
5. Active Suicidal Ideation with Specific Plan and Intent Thoughts of killing oneself with details of plan fully or partially worked out and subject has some intent to carry it out. Have you started to work out or worked out the details of how to kill yourself? Do you intend to carry out this plan? If yes, describe:	Yes No □ □

INTENSITY OF IDEATION	
The following features should be rated with respect to the most severe type of ideation (i.e., 1-5 from above, with 1 being the least severe and 5 being the most severe). Most Severe Ideation: _____ _____ Type # (1-5) Description of Ideation	Most Severe
Frequency How many times have you had these thoughts? (1) Less than once a week (2) Once a week (3) 2-5 times in week (4) Daily or almost daily (5) Many times each day	____
Duration When you have the thoughts, how long do they last? (1) Fleeting - few seconds or minutes (4) 4-8 hours/most of day (2) Less than 1 hour/some of the time (5) More than 8 hours/persistent or continuous (3) 1-4 hours/a lot of time	____
Controllability Could/can you stop thinking about killing yourself or wanting to die if you want to? (1) Easily able to control thoughts (4) Can control thoughts with a lot of difficulty (2) Can control thoughts with little difficulty (5) Unable to control thoughts (3) Can control thoughts with some difficulty (0) Does not attempt to control thoughts	____
Deterrents Are there things - anyone or anything (e.g., family, religion, pain of death) - that stopped you from wanting to die or acting on thoughts of committing suicide? (1) Deterrents definitely stopped you from attempting suicide (4) Deterrents most likely did not stop you (2) Deterrents probably stopped you (5) Deterrents definitely did not stop you (3) Uncertain that deterrents stopped you (0) Does not apply	____
Reasons for Ideation What sort of reasons did you have for thinking about wanting to die or killing yourself? Was it to end the pain or stop the way you were feeling (in other words you couldn't go on living with this pain or how you were feeling) or was it to get attention, revenge or a reaction from others? Or both? (1) Completely to get attention, revenge or a reaction from others (4) Mostly to end or stop the pain (you couldn't go on (2) Mostly to get attention, revenge or a reaction from others living with the pain or how you were feeling) (3) Equally to get attention, revenge or a reaction from others (5) Completely to end or stop the pain (you couldn't go on and to end/stop the pain living with the pain or how you were feeling) (0) Does not apply	____

Information obtained from: http://www.cssrs.columbia.edu/

SUICIDAL BEHAVIOR (Check all that apply, so long as these are separate events; must ask about all types)	Since Last Visit

Actual Attempt: A potentially self-injurious act committed with at least some wish to die, as a result of act. Behavior was in part thought of as method to kill oneself. Intent does not have to be 100%. If there is any intent/desire to die associated with the act, then it can be considered an actual suicide attempt. There does not have to be any injury or harm, just the potential for injury or harm. If person pulls trigger while gun is in mouth but gun is broken so no injury results, this is considered an attempt. Inferring Intent: Even if an individual denies intent/wish to die, it may be inferred clinically from the behavior or circumstances. For example, a highly lethal act that is clearly not an accident so no other intent but suicide can be inferred (e.g., gunshot to head, jumping from window of a high floor/story). Also, if someone denies intent to die, but they thought that what they did could be lethal, intent may be inferred. Have you made a suicide attempt? Have you done anything to harm yourself? Have you done anything dangerous where you could have died? What did you do? Did you_____ as a way to end your life? Did you want to die (even a little) when you_____? Were you trying to end your life when you _____? Or did you think it was possible you could have died from_____? Or did you do it purely for other reasons / without ANY intention of killing yourself (like to relieve stress, feel better, get sympathy, or get something else to happen)? (Self-Injurious Behavior without suicidal intent) If yes, describe: Has subject engaged in Non-Suicidal Self-Injurious Behavior?	Yes No ☐ ☐ Total # of Attempts _____ Yes No ☐ ☐
Interrupted Attempt: When the person is interrupted (by an outside circumstance) from starting the potentially self-injurious act (if not for that, actual attempt would have occurred). Overdose: Person has pills in hand but is stopped from ingesting. Once they ingest any pills, this becomes an attempt rather than an interrupted attempt. Shooting: Person has gun pointed toward self, gun is taken away by someone else, or is somehow prevented from pulling trigger. Once they pull the trigger, even if the gun fails to fire, it is an attempt. Jumping: Person is poised to jump, is grabbed and taken down from ledge. Hanging: Person has noose around neck but has not yet started to hang - is stopped from doing so. Has there been a time when you started to do something to end your life but someone or something stopped you before you actually did anything? If yes, describe:	Yes No ☐ ☐ Total # of interrupted _____
Aborted Attempt: When person begins to take steps toward making a suicide attempt, but stops themselves before they actually have engaged in any self-destructive behavior. Examples are similar to interrupted attempts, except that the individual stops him/herself, instead of being stopped by something else. Has there been a time when you started to do something to try to end your life but you stopped yourself before you actually did anything? If yes, describe:	Yes No ☐ ☐ Total # of aborted _____
Preparatory Acts or Behavior: Acts or preparation towards imminently making a suicide attempt. This can include anything beyond a verbalization or thought, such as assembling a specific method (e.g., buying pills, purchasing a gun) or preparing for one's death by suicide (e.g., giving things away, writing a suicide note). Have you taken any steps towards making a suicide attempt or preparing to kill yourself (such as collecting pills, getting a gun, giving valuables away or writing a suicide note)? If yes, describe:	Yes No ☐ ☐
Suicidal Behavior: Suicidal behavior was present during the assessment period?	Yes No ☐ ☐
Suicide:	Yes No ☐ ☐
Answer for Actual Attempts Only	Most Lethal Attempt Date:

Actual Lethality/Medical Damage: 0. No physical damage or very minor physical damage (e.g., surface scratches). 1. Minor physical damage (e.g., lethargic speech; first-degree burns; mild bleeding; sprains). 2. Moderate physical damage; medical attention needed (e.g., conscious but sleepy, somewhat responsive; second-degree burns; bleeding of major vessel). 3. Moderately severe physical damage; medical hospitalization and likely intensive care required (e.g., comatose with reflexes intact; third-degree burns less than 20% of body; extensive blood loss but can recover; major fractures). 4. Severe physical damage; medical hospitalization with intensive care required (e.g., comatose without reflexes; third-degree burns over 20% of body; extensive blood loss with unstable vital signs; major damage to a vital area). 5. Death	Enter Code _____
Potential Lethality: Only Answer if Actual Lethality=0 Likely lethality of actual attempt if no medical damage (the following examples, while having no actual medical damage, had potential for very serious lethality: put gun in mouth and pulled the trigger but gun fails to fire so no medical damage; laying on train tracks with oncoming train but pulled away before run over). 0 = Behavior not likely to result in injury 1 = Behavior likely to result in injury but not likely to cause death 2 = Behavior likely to result in death despite available medical care	Enter Code _____

16.5 Appendix V: Retigabine Muscle Cramp Diary – Visual Analog Scale (Pain Scale)

	Scale	
No pain	0	
	1	
Mild, annoying pain	2	
	3	
Nagging, uncomfortable, troublesome pain	4	
	5	
Distressing, miserable pain	6	
	7	
Intense, dreadful, horrible pain	8	
	9	
Worst possible, unbearable, excruciating pain	10	

16.6 Appendix VI: 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