University of Kansas Medical Center
RESEARCH PROTOCOL INVOLVING HUMAN SUBJECTS
TEMPLATE WITH GUIDANCE

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Study Title: Safety and efficacy of ranolazine for the treatment of amyotrophic lateral sclerosis

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I. Purpose, Background and Rationale

A. Aim and Hypotheses

1. Amyotrophic Lateral Sclerosis (ALS) is a progressive debilitating and fatal neurodegenerative disease involving the motor neurons in the primary motor cortex, corticospinal tracts, brainstem and spinal cord with 5,000 newly diagnosed patients per year in the USA[1]. There is a pressing need for additional therapies, as the only two FDA-approved drugs for ALS, riluzole and edaravone, showed prolongation of median survival of only two to three months and only a modest benefit in daily functioning, respectively[2, 3]. The ability to identify FDA approved drugs which can be repurposed to ALS, and which may slow disease progression, alleviate symptoms, or prolong survival will have an immediate positive impact of the lives of patients with ALS and their family members.
2. **Hypothesis:** Ranolazine, an FDA approved drug for angina which inhibits the late Na^+ current and intracellular $Ca2^+$ accumulation may be neuroprotective in ALS by reducing neuronal hyperexcitability, may slow disease progression and reduce cramp frequency.
   Aims:
   Aim 1: Determine safety and tolerability of two doses of ranolazine in patients with ALS.
   Aim 2: Evaluate for preliminary signs of drug-target engagement.

B. Background and Significance

1. Study Significance: The ability to identify FDA approved drugs which can be repurposed to ALS, and which may slow disease progression, alleviate symptoms, or prolong survival will have an immediate positive impact of the lives of patients with ALS and their family members.
2. ALS is a neurodegenerative disease involving the motor neurons in the primary motor cortex, corticospinal tracts, brainstem and spinal cord. It is a progressive debilitating and fatal disease affecting 3.9 per 100,000 persons in the USA with 5,000 newly diagnosed patients per year[1]. Riluzole, a glutamatergic neurotransmission blocker in the CNS, and edaravone, a free radical scavenger that reduces oxidative stress, are the only FDA-approved medications for ALS[2, 3]. There is a pressing need for additional therapies, as Riluzole only prolongs median survival by two to three months, has a small effect on bulbar and limb function, and no benefit on muscle strength [2]; and edaravone requires a complicated intermittent intravenous administration and only has modest effect on slowing functional decline[2, 3].
3. Literature Review: Many studies suggest neuronal hyperexcitability plays a key pathological role in ALS. Studies in ALS patients have shown evidence of neuron hyperexcitability in the

central nervous system (cortex and spinal cord) with reduction of corticomotor threshold using transcranial magnetic stimulation (TMS)[4-6]; corticomotor excitability precedes onset of familial ALS[5]; and motor nerve threshold changes using threshold electrotonus in the median nerve suggesting impairment of axonal K^+ channels of ALS patients[7]. Animal models also showed support for neuronal hyperexcitability playing a key role in ALS: 1) in pre-symptomatic G93A- SOD1 mouse models, cultured spinal neurons showed elevated electrical excitability induced by the SOD1 toxic gain-of-function through abnormal action potential generation[8]; 2) spinal cord cultures exposed to medium derived from mutant $SOD1^{G93A}$ astrocytes had increased Na^+ currents, repetitive firing and intracellular Ca^{2+} transients leading to motor neuron death[9]; 3) persistent Na^+ current was significant higher in the SOD1 mutated neurons while the fast transient Na^+ current was unaffected[10, 11]; and 4) mutated $SOD1^{G93A}$, $SOD1^{G86R}$ and $TDP43^{A315T}$ triggered motoneuron death through a common pathogenic pathway involving Na^+ channel activity[12].

4. Mexiletine is an FDA approved drug for ventricular arrhythmias which decreases axonal excitability by reducing nodal Na^+ currents[13]. Mexiletine has been shown to prolong motor neuron survival in ALS astrocyte cell cultures, and prolong survival in an ALS mouse model[9]. In a small clinical trial in ALS mexiletine decreased muscle cramps, but was not well tolerated at higher doses, leading to 32% of patients discontinuing therapy[14].
5. Ranolazine is an FDA approved drug for angina which inhibits the late Na^+ current and intracellular Ca^{2+} accumulation[15]. The rationale for ranolazine is similar to that of mexiletine, in that it inhibits neuronal sodium currents. Ranolazine is well tolerated, and does not have a black box warning for recent myocardial infarction like mexiletine.

C. Rationale

1. Neuronal hyperexcitability in ALS is potentially amenable to therapy. Hyperexcitable motor neurons have increased persistent Na^+ channel conduction and reduction in K^+ currents, predisposing axons to generate fasciculation and cramps[16]. Our hypothesis is that ranolazine may be neuroprotective in ALS by reducing neuronal hyperexcitability, may slow disease progression, reduce cramp frequency, and will be better tolerated than mexiletine.

2. This study will provide information on safety and tolerability of ranolazine in patients with ALS to serve as a preliminary data for future clinical trials of ranolazine in ALS. In addition, this study will look for preliminary evidence for drug-target engagement using a variety of approaches: including patient-reported cramp frequency; fasciculation frequency on muscle ultrasound; and electrical excitability using a cramp fasciculation protocol on electromyography.

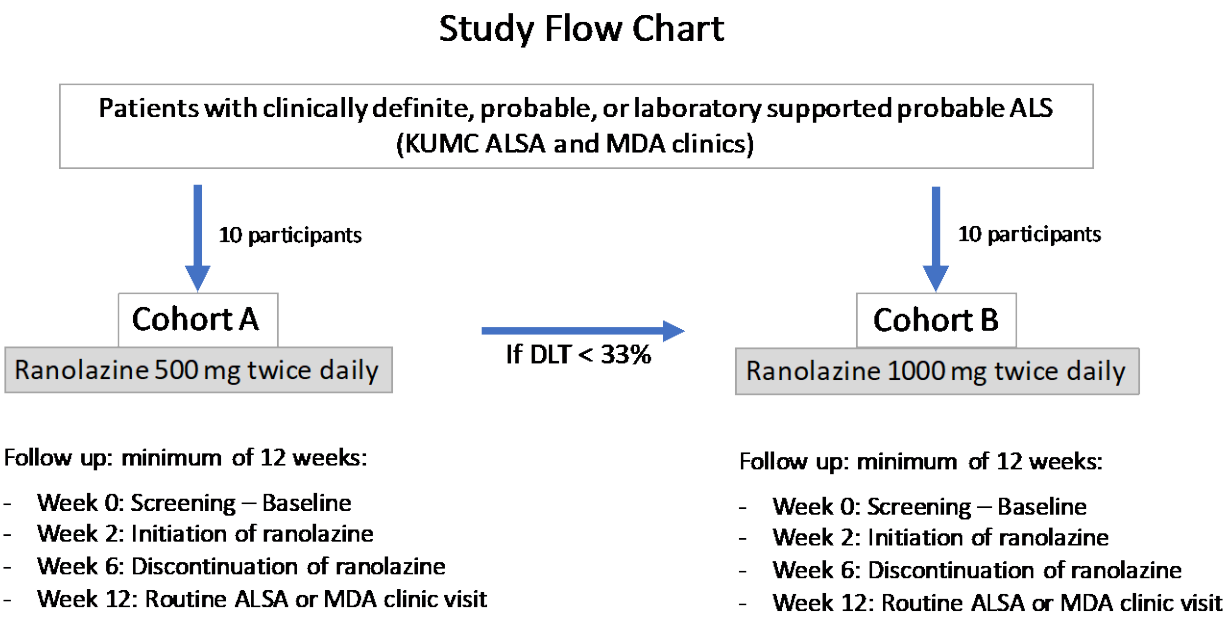
3. The ability to identify FDA approved drugs which can be repurposed to ALS, and which may slow disease progression, alleviate symptoms, or prolong survival will have an immediate positive impact of the lives of patients with ALS and their family members.

II. Research Plan and Design

A. Study Objectives: The goal of this study is to determine the safety and tolerability of 2 doses of ranolazine in patients with ALS, and to look for preliminary evidence for drug-target engagement.

B. Study Type and Design: We will conduct an open-label dose-ascending study of ranolazine in ALS. We will enroll a total of 20 participants in 2 sequential cohorts. Our primary outcome will

be dose limiting toxicities (DLT) defined as any drug-related serious adverse event, or drug-related adverse event necessitating study withdrawal. If a dose has < 33% DLTs it will be considered tolerable and the study will continue. Each cohort will run for a total of 12 weeks: there will be a 2-week run-in to determine eligibility; then participants will receive ranolazine for 4 weeks; then there will be a 6 week follow up for safety. Cohort 1 (n=10) will receive ranolazine 500 mg orally twice daily. If there are no DLTs after the first 6 participants reach week 6, then we will start recruiting for cohort 2, who will receive ranolazine 1000 mg oral twice daily (Figure 1. Study Flow Chart).



ALSA: Amyotrophic Lateral Sclerosis (ALS) Association, MDA: Muscular Dystrophy Association
ECG: Electrocardiogram, DLT: dose-limiting toxicity

Figure 1. Study Flow Chart

C. Sample size, statistical methods, and power calculation

1. Descriptive statistics (N, mean, SD, median, minimum, and maximum) will be provided for continuous demographic variables (age, weight, height) and frequency counts will be tabulated for categorical demographic variables (gender, race, ethnicity) by treatment and overall for each study part (Cohort 1 and 2). Frequency counts will be tabulated for disposition data and will consist of the number of patients completing treatment (Yes / No) along with frequency counts of primary reason for discontinuation (provided there is at least one patient who discontinued). Summaries will be provided by treatment and overall for each study part (cohorts 1 and 2). This is a pilot study. We plan to enroll a total of 20 patients.
2. For the primary outcome, the frequency of DLTs at each dose will be evaluated. If any cohort has > 33% DLTs the study will stop.
3. For secondary outcomes cramp frequency and severity, fasciculation frequency, and cramp potential duration will be compared from baseline to week 6, after treatment. As this is a pilot study we will evaluate trends in the data but we do not expect to see statistically significant effects in the pilot study. We will

evaluate the change from baseline to week 6 either using a paired t-test for continuous variables, or a Wilcoxon signed-rank test for ordinal data. The cramp questionnaire will also be completed at week 8 to look for persistence of effect. Adverse event frequency will be tabulated and compared between doses, and to known adverse event frequency for ranolozine per the FDA label.

D. Subject Criteria (See Vulnerable Populations appendix, if applicable):

1. Inclusion criteria: Patients with clinically definite, possible, probable, or laboratory supported probable ALS per revised El Escorial criteria[17]; > 18 years of age; cramp frequency $\geq$ 4 cramps per week during 2 week run in; ALS functional rating scale-revised (ALSFRS-R) $\geq$ 24;[18] able to lie on their back for study procedures; Women of childbearing age must be non-lactating and surgically sterile, or using an effective method of birth control and have a negative pregnancy test; and patients must be willing and able to give signed informed consent.
2. Exclusion criteria: tracheostomy invasive ventilation, or use of non-invasive ventilation > 12 hours per day; pregnant or lactating; participation in a prior experimental drug trial < 30 days prior to screening; patients taking ranolazine; for patients taking mexiletine, they would need to come off the drug for 1 week prior to screening; patients taking medications which are contraindicated for use with ranolazine such as strong CYP3 inhibitors (ketoconazole, clarithromycin, nelfinavir), and CYP3 inducers (rifampin, phenobarbital); patients with clinically significant medical comorbidities (hepatic, renal, cardiac, etc); patients with baseline QTc interval prolongation on ECG, patients pre-disposed to secondary QTc prolongation for other health conditions like family history of congenital long QT syndrome, heart failure, bradycardia, or cardiomyopathies. Although FDA label lists QTc interval prolongation under warning and precautions, there is no evidence that ranolazine prolongs the QTc interval [19]. We decided to exclude patients with baseline prolonged QTc interval to be safe.
3. Withdrawal/Termination criteria: Any serious drug related adverse event; any drug-related adverse event which per the investigator would put the participant at risk if they were to continue (e.g. prolongation of the QTc interval); Patient can be withdrawn at the investigator's discretion; any participant has the right to withdraw from the study at any time; failure to comply with study procedures. The QTc interval will considered prolonged if > 450 ms in men or > 470 ms in women. [20]
4. Participants can enroll in other non-drug research studies.

E. Specific methods and techniques used throughout the study

1. Schedule of events:

	Week 0-Baseline	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6 Stop Drug	Week 8	Week 12
Informed Consent	X								
Dispense Drug			X						
Drug Accountability							X		
Medical History/Meds	X								
Physical Exam	X		X				X		X
Safety Labs	X						X		
Electrocardiogram	X						X		
Adverse Events			X	X	X	X	X	X	X
ALSFRS	X		X				X		X
FVC	X		X				X		X
Cramp Questionnaire	X	X	X	X	X	X	X	X	X
Cramp-Fasciculation Protocol	X		X				X		
Muscle Ultrasound	X		X				X		
Phone Call/Email		X		X	X	X		X	

Study Procedures:

1. Laboratory tests/procedures: During the first visit, two weeks prior to initiation of the study drug, participants will be evaluated with a baseline electrocardiogram (or electrocardiogram performed in clinic within the last 6 weeks), baseline safety laboratory tests (or safety lab drawn in clinic within the last 6 weeks), (CBC, CMP, CK, and pregnancy test), answer a questionnaire for assessment of muscle cramps, undergo neurophysiologic study in the EMG laboratory (Cramp-Fasciculation Protocol) and undergo muscle ultrasound for evaluation of presence of muscle fasciculations. The same evaluation will occur four weeks after the initiation of the study drug.
2. Physical Exam will be performed at Baseline (physical from clinic may be used for baseline), weeks 2, 6 and 12.

3. Cramp and Muscle procedures:
   - Muscle cramp questionnaire: This questionnaire was modified from Katzberg [21]. It includes questions to estimate the following: cramp frequency per week, cramp severity on a scale from 1 to 10, cramp localization, cramp duration and association with quality of life. The questionnaire will be reviewed or collected via phone or email weeks 1, 2,

3,4,5, and 8. The estimated time to complete the questionnaire is 10 minutes. The FVC and the ALSFRS-R, both standard ALS disease measures will be completed at baseline, weeks 2, 6 and 12. The estimated time to complete the questionnaire is 10 minutes.

- Cramp-Fasciculation Protocol: Performed in the electrodiagnostic laboratory by a technician or a physician using an electroneurodiagnostic system. Two surface recording and reference electrodes are placed over the abductor hallucis brevis muscle and at the base of the first metatarsal, respectively. The posterior tibial nerve is stimulated at the ankle 8 cm proximal to the recording electrode. A total of five supramaximal stimuli are delivered at two frequencies, 2 and 5 Hz. Afterdischarges and/or cramp potentials will be recorded and evaluated qualitatively (presence or absence, change from cramp potential to afterdischarges) and quantitatively (time) [22]. Estimated time: 15 minutes.
- Muscle ultrasound: Performed in the electrodiagnostic laboratory or clinic during research or clinic visit using an ultrasonography machine with a real-time transducer [23]. Three bilaterally muscles (biceps, tibialis anterior, and gastrocnemius) will be studied for the presence or absence of fasciculations. Each muscle will be imaged for 30 seconds and graded in the following fashion: 0=no fasciculation; 1=1-3 fasciculations; 2=4-6 fasciculations; 3=>6 fasciculations. Performed by a physician. Estimated time: 15 minutes.

4. All procedures listed are for research.
5. Study visits will have a window of ±_ 2 days.

F. Risk/benefit assessment:

Physical risk: Both cohorts 1 and 2 will be taking ranolazine, which per the FDA label can be associated with the following side effects including: dizziness, constipation, nausea, asthenia, headache, peripheral edema, sweating, angina, palpitations, dyspepsia, malaise, dyspnea, and hypoglycemia. [24]

Safety lab risks: The risks of participating in the study of this proposal include blood drawing and a slight pain at the site of the needle stick, possibly developing a small bruise at the puncture site.

Electrocardiogram risk: some participants may have reaction to the skin electrodes.

Cramp-fasciculation protocol risk: Participants may experience pain during the stimulation as well as pain if muscle cramps occur.

Economic risk: There will be some cost for traveling to the study visits.

Potential benefit of participating in the study: There is no known benefit.

G. Location where study will be performed: The study will be conducted at either the Landon Center on Aging or at the Clinical Research Center (CRC).

H. Personnel who will conduct the study, including:

1. PI: Jeffrey Statland, MD
   Co-I: Anai Hamasaki, MD, Mazen Dimachkie, MD, Richard Barohn, MD, Omar Jawdat, MD, Melanie Glenn, MD.
   Study coordinators and EMG technicians
2. Primary responsibility for the following activities, for example:
   a. Determining eligibility: PI or their designee
   b. Obtaining informed consent: PI or their designee

c. Providing on-going information to the study sponsor and the IRB: PI or their designee
d. Maintaining participant's research records: PI or their designee
e. Completing physical examination: PI or their designee
f. Taking vital signs, height, weight: CRC staff or study coordinator
g. Drawing / collecting laboratory specimens: CRC staff
h. Performing / conducting tests, procedures, interventions, questionnaires: PI, Co-Is including research coordinators or their designee
i. Completing study data forms: PI or their designee
j. Managing study database: members of the study staff including the study coordinator.

I. Assessment of Subject Safety and Development of a Data and Safety Monitoring Plan

a. Elements of the plan include: Mamatha Pasnoor, MD will serve as the independent safety monitor. She is currently not involved in the ALSA and MDA clinics. She will be notified of all adverse events and follow the lab results. Some of the data she will look at will be the number of patients with drug-related adverse events, including serious adverse events that will lead to possible participant withdrawal, including dose-limiting toxicity greater than 33%. She will also be available for questions as needed. This is a short duration study. We expect her to look at the data in real time.
b. Adverse events will be monitored during scheduled research visits and at any time during the study by providing patients with contact number to the research staff. These events will be recorded and reported to the IRB. Serious adverse events are defined as any drug-related adverse event necessitating study withdrawal, including adverse events resulting in death, disability or life-threatening event; requiring hospitalization; or an important medical event based upon appropriate medical judgment. Any serious adverse event must be reported within 24 hours after the investigator becomes aware of the event.
c. In case of QTc prolongation, the study drug will be discontinued. If asymptomatic from cardiac standpoint, we will repeat the EKG in one week. If symptomatic, the subject will be referred to the emergency department for cardiac evaluation.
d. After a serious adverse event occurs and the investigator is notified, arrangements for appropriate management will be made and may include emergency room visit, hospitalization, office visit with research team or other specialty, discontinuation of the study drug and discontinuation of study participation. The participant will be followed until the event has resolved or stable.

III. Subject Participation

A. Recruitment:

Participants will be recruited from the ALSA, MDA, or neuromuscular clinics. In addition, patients in the neuromuscular research data base who have previously agreed to be contacted may be contacted for this study. Recruitment will be conducted by the PI or Co-Is.

B. Informed consent process and timing of obtaining of consent

1 The PI and their designee will discuss with the participant about the study. If the participant expresses interest, a copy of the consent form will be provided and the participant will be given time to read the consent form. After all questions have been answered, the participant will sign the consent form.
2 Study details will be discussed with patient and family during the subject’s routine office visit and questions will be answered. Written consent will be provided in lay language to patients willing to participate in the study. Decline or acceptance in participation in the study will not affect current treatment or follow up in ALSA or MDA clinic. To guarantee

patient confidentiality, study records, reports and communications we will identify the participant by the assigned participant identification number. Participant identifiers will not be publicly available. The information obtained from the study will be used towards the development of the investigational product and may be disclosed to regulatory authorities, other investigators or consultants as required.

3 We anticipate there would be no cognitive issues in the patient population.

C. Alternatives to Participation: This study is voluntary. Participants will be allowed to continue their standard of care medications.

D. Costs to Subjects: There won't be costs to the participants. The only anticipated cost may be travel to research visits.

E. How new information will be conveyed to the study subject and how it will be documented: This is an open label study. Participants will know the dose that they will be taking. Each participant will be notified at the end of the study regarding the results. The results of this study will be published in an ALS related journal. Also, we plan to present this information at different meeting throughout the coming year.

F. Payment, including a prorated plan for payment: There won't be reimbursement for participation in this study.

G. Payment for a research-related injury: There will be no payment for research-related injury.

IV. Data Collection and Protection

A. Data Management and Security:

Only members of the study team will have access to the study data. To guarantee patient confidentiality, study records, reports and communications, we will identify the participants by the assigned participant identification number. Participant identifiers will not be publicly available. The information obtained from the study will be storage in password protected computers at KUMC and will be used towards the development of the investigational product and may be disclosed to regulatory authorities, other investigators or consultants as required. Study data will be stored on password protected university computers. No mobile devices will be used for data collection or storage. This study is being conducted only at KUMC. No identifiable data will be sent outside of KUMC.

B. Sample / Specimen Collection: Safety labs will be collected but not stored.

C. Tissue Banking Considerations: There will be no tissue banking.

D. Quality Assurance / Monitoring

1. The investigators will permit trial-related monitoring, audits, ethics committee review, and regulatory inspections as requested by FDA or other health authorities and the designee. There is an internal monitor (Maureen Walsh) in the neurology department who will monitor as needed.
2. There are no plans to have third party monitoring.

V. Bibliography / References / Literature Cited

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