

CLINICAL STUDY PROTOCOL

STUDY TITLE: **A Multicenter, Double-Blind, Placebo-Controlled, Study to Investigate the Safety and Efficacy of Lithium in combination with Riluzole in Volunteers with Amyotrophic Lateral Sclerosis (ALS)**

PROTOCOL NUMBER: **LALS-001**

STUDY DRUG: **Lithium**

US IND No: **Exempt**

HEALTH CANADA No: **9427-S2019-42C**

PROTOCOL DATE: **August 13, 2008**

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1.0 INVESTIGATOR'S AGREEMENT

I have read the attached protocol entitled, "A Multicenter, Double-Blind, Placebo-Controlled, Study to Investigate the Safety and Efficacy of Lithium in combination with Riluzole in Volunteers with Amyotrophic Lateral Sclerosis," dated **August 13, 2008** (Version **4.0**) and agree to abide by all described protocol procedures. I agree to comply with the World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects, the International Conference on Harmonisation Tripartite Guidelines on Good Clinical Practice, applicable U.S. Food and Drug Administration (FDA) regulations and guidelines identified in 21 CFR Parts 11, 50, 56, and 312.7, the applicable provisions of sections 402(i) and 402(j) of the U.S. Public Health Service Acts (PHS Act) [42 U.S.C. §§ 282 (i) and (j)], amended by Title VII of the FDA Amendments Act of 2007 (Public Law No. 110-85, 121 Stat.904), all Health Canada applicable regulations and guidelines including the Therapeutic Products Directorate's Guideline for Good Clinical Practice, local Institutional Review Board (IRB) guidelines and policies, and the U.S. Health Insurance Portability and Accountability Act (HIPAA).

Site Principal Investigator Signature: _____ **Date:** _____

Print Site Principal Investigator Name: _____

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3.0 LIST OF ACRONYMS, ABBREVIATIONS, AND DEFINITIONS OF TERMS

ACE	Angiotensin-Converting Enzyme
ADR	Adverse Drug Reaction
AE	Adverse Event
AIDS	Acquired Immunodeficiency Syndrome
ALS	Amyotrophic Lateral Sclerosis
ALSFRS-R	Amyotrophic Lateral Sclerosis Functional Rating Scores – revised
ALSSQOL	ALS-Specific Quality of Life Measure
ALT	Alanine aminotransferase
AMP	Adenosine Monophosphate
AST	Aspartate aminotransferase
Bcl-2	B-cell lymphoma/leukemia-2 gene
BDNF	Brain-Derived Neurotrophic Factor
BID	Bis in diem/twice a day
BMI	Body Mass Index
BUN	Blood Urea Nitrogen
CALS	Canadian ALS Clinical Trials and Research Network
CBC	Complete Blood Count
CRF	Case report form
CFR	Code of Federal Regulations
CNTF	Ciliary neurotrophic factor
COX-2	Cyclo-Oxygenase 2
CTCAE	Common Terminology Criteria for Coding Adverse Events
DM	Data Management
3D-MRI	Three Dimensional Magnetic Resonance Imaging
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders- IV
DSMB	Data and Safety Monitoring Board
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EDC	Electronic data capture
FALS	Familial amyotrophic lateral sclerosis
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GI	Gastrointestinal
GSK-3	Glycogen Synthase Kinase- 3
HC	Canadian Therapeutics Products Division of Health Canada
HCG	Human Chorionic Gonadotropin
HIPAA	Health Insurance Portability and Accountability Act
HSP	Heat Shock Protein
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ID	Identity Document
IEC	Independent Ethics Committee
IND	Investigational New Drug
IP3	Inositol-1,4,5-Triphosphate

IPCW	Inverse Probability of Censoring Weighted
IRB	Institutional Review Board
IS	Information Systems
ITT	Intent-to-Treat
LFT	Liver function test
MDD	Major Depressive Disorder
MGH	Massachusetts General Hospital
MPTP	N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
mRNA	Mono ribonucleic acid
MS	Microsoft
NCI	National Cancer Institute
NCTU	Neurology Clinical Trials Unit
NEALS	Northeast Amyotrophic Lateral Sclerosis
NIH	National Institutes of Health
NINDS	National Institute of Neurological Disorders and Stroke
NMDA	N-methyl-D-aspartate
NSAIDs	Non-Steroidal Anti-inflammatory Drugs
PCs	Personal computers
PEG	Percutaneous Endoscopic Gastrostomy
QIDS	Quick Inventory of Depressive Symptomatology
QIDS-C	Quick Inventory of Depressive Symptomatology- Clinician rated
QIDS-SR	Quick Inventory of Depressive Symptomatology- Self report
QOL	Quality of Life
REB	Research Ethics Board
RBC	Red blood cells
SAE	Serious adverse event
SALS	Sporadic amyotrophic lateral sclerosis
SAS	Statistical Analysis Software
SHSC	Sunnybrook Health Sciences Centre
SOD1	Superoxide dismutase-1
SQL	Structured Query Language
SSKI	Potassium Iodide
SSL	Secure sockets layer
SUNY	State University of New York
Tmax	Time to Peak Serum Concentration
TSH	Thyroid Stimulating Hormone
US	United States
VC	Vital capacity
WBC	White blood cells
Wk	Week

4.0 SYNOPSIS

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Primary Objective

The primary study objective is to compare the efficacy of lithium in combination with riluzole to riluzole in combination with placebo in volunteers with ALS. Primary efficacy will be assessed by analyzing disease progression as measured by a 6-point drop in the ALS Functional Rating Scale - Revised (ALSFRS-R) from baseline value or death.

Secondary measures of efficacy will include a comparison of the rate of decline of ALSFRS-R and Slow VC in participants randomized to lithium versus those treated with placebo, and a comparison of tracheostomy-free survival compared to the historical controls from the NEALS trial database. In addition, the rate of ALSFRS-R decline in the placebo group will be compared before and after initiation of lithium. Other measures include mean changes over time in responses to selected questions from the ALS Specific Quality of Life Questionnaire (ALSSQOL) and mean changes in the summary values for the Quick Inventory of Depression Self Report Questionnaire (QIDS-SR₁₆) over time, in those treated with lithium versus those treated with placebo.

Secondary Objective

The secondary objective of this study is to determine the safety of long-term (12 months) administration of lithium in volunteers with ALS as measured by adverse events (AEs), lithium levels, ability to remain on assigned treatment (tolerability), physical examinations, laboratory test results, vital signs, weight/body mass index (BMI), and use of concomitant medications. The safety data will be summarized according to treatment group.

Study Rationale and Significance

Amyotrophic lateral sclerosis is a neurodegenerative disorder that affects predominantly motor neurons. Lithium carbonate has been FDA approved since the early 1970's for treatment of bipolar disorder. Lithium has been shown to be neuroprotective in models of acute brain injury and chronic neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, tauopathies, and Huntington's disease. In a recent study by Fornai et al. lithium was neuroprotective in the mutant SOD1 G93A transgenic mouse model of motor neuron disease [1]. Lithium treatment prolonged the mean survival time and increased disease duration compared with saline treated G93A transgenic mice. Lithium delayed cell death within lamina IX of the spinal cord and cranial motor neurons while it increased the number of lamina VII Renshaw-like neurons. Lithium also decreased reactive gliosis, rescued spinal cord mitochondria, and produced a marked regression of alpha-synuclein, ubiquitin, and SOD1 aggregates. The authors proposed that the likely molecular target for lithium in ALS involves blocking IP3 activity, which acts as an endogenous autophagy inhibitor. Other putative neuroprotective mechanisms of lithium include inactivation of NMDA receptors through inhibition of NR2B tyrosine phosphorylation, activation of cell survival factors such as the PI 3-kinase/Akt signaling pathway, and induction of neurotrophic/neuroprotective proteins including brain-derived

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neurotrophic factor, heat-shock protein, and Bcl-2.

Fornai and colleagues also compared riluzole (50 mg bid) and lithium carbonate at dosages of 300-450 mg daily (titrated to a plasma level of 0.4-0.8 mEq/liter) to riluzole alone in people with ALS. No deaths occurred in the 16 participants treated with lithium and riluzole after 15 months, whereas 29% of participants taking only riluzole died. The ALSFRS-R, a validated functional measure, declined 1/3 as rapidly in lithium and riluzole treated patients. This small pilot study was not blinded and requires confirmation in a controlled study. Based on this small study, people with ALS in the United States and Canada have started taking lithium off label. Therefore, any controlled study of lithium in ALS may face recruitment challenges, so that close collaboration with the patient community and education of all stakeholders on the need for a controlled study will be important. The proposed design is thought to be more appealing to participants because the time on placebo may be shorter than 12 months in those with relatively rapid disease progression. The study is designed to determine whether the effect seen in the human pilot study is real. Early stopping rules for futility, efficacy and safety are included.

Study Design and Methodology

This is a double blind, randomized, placebo-controlled clinical trial evaluating the safety and efficacy of lithium and riluzole compared to placebo and riluzole. All participants enrolled in this study will be taking a stable dose of riluzole 50 mg PO BID for at least 30 days and have an ALS disease duration (from symptom onset) of no more than 36 months at enrollment.

Approximately 250 volunteers will be recruited from multiple centers in the US and Canada that are part of the Northeast ALS Consortium (NEALS) and the Canadian ALS Clinical Trials and Research Network (CALC). Enrollment will occur in a staged manner. Initially 84 participants will be enrolled in the trial. First interim analysis will occur once the 84th participant has been accrued. At this point, based on the available data the Data Safety and Monitoring Board (DSMB) appointed by the National Institutes of Health (NIH) may decide to stop the trial for efficacy or futility or stop accrual and request that follow-up continue on the 84 participants enrolled in the trial or continue accrual. Participants will be randomized 1:1 to lithium/riluzole or placebo/riluzole. After screening and randomization, participants will be followed every 4 weeks for the first 12 weeks. Subsequent in-person visits will occur every 8 weeks with a final visit at Week 52. Between in-person visits, telephone interviews will take place every 4 weeks to administer the ALSFRS-R. A follow-up telephone interview will occur at week 56 (off study medication) to review adverse events.

The primary outcome measure is disease progression defined as the time to decline of 6 points in the ALSFRS-R scale or death. The ALSFRS-R will be administered at the Baseline Visit; this will be considered the baseline score for each participant. A drop of 6 points or more in the ALSFRS-R will be defined as disease progression and treatment

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failure. Participants will then be treated with lithium for the period of the study (up to 52 weeks total). Participants, Investigators and study site staff will remain blinded to initial group assignments. Participants and Clinical Evaluators administering ALSFRS-R will remain blinded to the total ALSFRS-R scores and will be unaware of the date when a participant is switched from placebo to lithium.

Unless participants withdraw consent prior to intervention, this intent to treat study will follow participants per protocol regardless of their compliance with assigned treatment. If they are unable to return for in-person visits, the in-person visits can be replaced by the ALSFRS-R and assessment of medical status over the phone.

Study Population and Main Criteria for Inclusion/Exclusion

Inclusion Criteria

Volunteers meeting all of the following criteria will be eligible:

1. Familial or sporadic ALS.
2. Participants diagnosed with laboratory supported probable, clinically possible, probable or definite ALS according to the World Federation of Neurology Revised El Escorial criteria [2] (Appendix 1).
3. Disease duration from symptom onset no greater than 36 months at the Screening Visit.
4. Aged 18 years or older.
5. Capable of providing informed consent and complying with trial procedures.
6. On a stable dose of riluzole 50mg bid for at least 30 days prior to screening.
7. Vital capacity (VC) equal to or more than 60% predicted value for gender, height and age at the Screening Visit.
8. Creatinine \leq 1.5 mg/dl (133 μ mol/L).
9. Participants maintained on thyroid medication must be euthyroid for at least 3 months before the Screening Visit.
10. Participants with psoriasis must have inactive disease for at least 30 days before the Screening Visit.
11. Women must not be able to become pregnant (e.g., post menopausal for at least one year, surgically sterile, or practicing adequate birth control methods) for the duration of the study. Adequate contraception includes: oral contraception, implanted contraception, intrauterine device in place for at least 3 months, or barrier method in conjunction with spermicide. Women of childbearing potential must have a negative serum pregnancy test at the Screening Visit and be non-lactating.
12. Geographic accessibility to the study site.
13. Fluency in English, Spanish or French (Canadian).

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Exclusion Criteria

Volunteers meeting any of the following will not be eligible:

1. History of known sensitivity or intolerability to lithium or to any other related compound.
2. Prior exposure to lithium within 90 days of the Screening Visit.
3. Exposure to any investigational agent within 30 days of the Screening Visit.
4. Participants who are malnourished, dehydrated or on a sodium-free diet will be excluded due to the potential side effects of lithium carbonate.
5. Use of digoxin or iodide salts [e.g. calcium iodide, hydrogen iodide (hydriodic acid), iodide, iodinated glycerol (Organidin), iodine, potassium iodide (SSKI), and sodium iodide supplementation beyond table salt].
6. Presence of any of the following clinical conditions:
 - a. Substance abuse within the past year.
 - b. Unstable cardiac, pulmonary, renal, hepatic, endocrine, hematologic, or active malignancy or infectious disease.
 - c. AIDS or AIDS-related complex.
 - d. Clinically active psoriasis within 30 days of the Screening Visit.
 - e. Unstable psychiatric illness defined as psychosis (hallucinations or delusions) or untreated major depression within 90 days of the Screening Visit.
 - f. Screening serum creatinine greater than or equal to 1.5 mg/dL (133 umol/L), TSH > 20% above the upper limit.
 - g. Presence of any clinically significant conduction abnormalities on ECG.
 - h. Female volunteers who are breast-feeding or who have a positive serum pregnancy test at the Screening Visit.

Number of Volunteers

A total of 250 study volunteers will be equally randomized to receive either riluzole/lithium or riluzole/placebo.

Test Product & Reference Therapy, Dose and Mode of Administration

Capsules will contain 150 mg lithium carbonate or matching placebo. Participants will start at a total daily dosage of 450 mg (one capsule in the morning and two in the evening) and the dosage will be titrated as necessary with monthly adjustments to maintain plasma levels between 0.4 to 0.8 mEq/L. Paired sham dosage modifications will be made for participants taking matching placebo capsules.

Duration of Treatment

Double-blind treatment period is 52 weeks (12 months).

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Criteria for Evaluation

Efficacy

The following efficacy variables will be evaluated:

Primary Outcome Variable:

- Disease Progression (defined as drop in the ALSFRS-R score by 6 points or more or death)

Secondary Outcome Variables

- Slow VC (Rate of decline)
- ALSFRS-R (Rate of decline)
- Quick Inventory of Depressive Symptomatology Self-Report (QIDS-SR₁₆)
- ALS-Specific Quality of Life Questionnaire
- Tracheostomy-free survival

Safety

The following safety variables will be assessed over 52 weeks:

- Adverse events
- Lithium levels
- Ability to remain on assigned treatment (tolerability)
- Clinical and laboratory data including physical examinations, vital signs, weight/BMI, ECG and laboratory test results (renal function, liver function, TSH, fasting blood glucose)
- Use of concomitant medications

Pharmacokinetics

Participants will be asked to provide trough blood samples (12 hours \pm 1 hour post last dose) for determination of lithium levels at Weeks 4, 8, 12 (\pm 3 days), 20, 28, 36, 44, and 52 (\pm 5 days).

Statistical Methods

There will be 125 participants on riluzole/lithium, and 125 participants on riluzole/placebo. All participants will be followed for 52 weeks. The primary endpoint of the trial (event) is time to failure defined as drop in the ALSFRS-R score by 6 points or more or death. Participants in the placebo/riluzole group whose ALSFRS-R drops by 6 points will be treated with lithium from that time.

We will use a group sequential design that establishes the time points for the interim analyses according to a mathematical function that is proportional to the number of events that have occurred. We expect a total of 167 events to occur in the trial. The first interim analysis (“first look”) will occur when 84 participants are accrued to the trial. At that time, one of the following four decisions will be made:

1. To stop the trial for futility.
2. To stop accrual and continue follow up on the 84 participants for a total

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treatment duration of 6 month and then repeat analysis.

3. To continue the trial accrual until the next interim analysis.
4. To stop the trial for efficacy.

If option (2) is selected, the second interim analysis will occur six months after the 84th participant has accrued. If option (3) is selected, the second interim analysis will occur after there are 55 events. At the second interim analysis (the “second look”), one of three decisions will be made:

1. To stop the trial for futility.
2. To continue accrual and look again after 100 events have occurred. This would be the last interim analysis. The final analysis will then occur after 250 participants have had 12 months of follow up.
3. To stop the trial for efficacy.

The stopping rules (used for determining how to proceed at each interim analysis) will be based on the monitoring method described in "Group Sequential Methods with Applications to Clinical Trials", Jennison C, Turnbull BW, Chapman & Hall/CRC, NY 2000. pp 145-169. The trial is designed to have over 80% power, that is, over an 80% chance of detecting a 40% decreased rate of decline in the treatment group using the stopping rules described separately in a detailed Interim Analysis Plan.

The secondary analysis will include a comparison of the rate of decline of ALSFRS-R and Slow VC of participants randomized to begin treatment on lithium as compared to participants treated on placebo and a comparison of tracheostomy-free survival compared to the historical controls from the NEALS trial database. In addition, the rate of ALSFRS-R decline in the placebo group will be compared before and after initiation of lithium. The mean changes over time (from Baseline to 52 weeks) in responses to selected questions from the ALS-Specific Quality of Life Questionnaire and mean changes in the summary values of the QIDS-SR 16 ratings over time (Baseline, Weeks 12, 36, 52) will be compared for the group of subjects treated with riluzole/lithium to those treated with riluzole/placebo.

Adverse events, withdrawal due to an AE (tolerability), serum lithium levels, abnormal findings on laboratory testing, physical exams, vital signs, weight/BMI, and use of concomitant medications will be assessed to characterize the safety profile of lithium in combination with riluzole in study volunteers.

Table 1: Schedule of Events

Activity	Screening Visit	Baseline Visit	Treatment Period														Final Safety Visit	
			Wk 4	Wk 8	Wk 12	Wk 16	Wk 20	Wk 24	Wk 28	Wk 32	Wk 36	Wk 40	Wk 44	Wk 48	Wk 52	Wk 56		
Visit	1	2	3	4	5	Phone 1	6	Phone 2	7	Phone 3	8	Phone 4	9	Phone 5	10	Phone 6		
Written Informed Consent	X																	
Inclusion/Exclusion Review	X	X																
Medical History/ Demographics	X																	
Vital Signs/ Weight/Height ¹	X	X	X	X	X		X		X		X		X		X		X	X
Safety Labs ²	X			X			X		X		X		X		X		X	X
Pre-Dose (Trough) Sample for Lithium	X		X	X	X		X		X		X		X		X		X	X
12-Lead ECG	X																	
Concomitant Medication Review	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical Examination	X														X		X	X
Neurological Examination	X														X		X	X
Vital Capacity	X	X	X	X	X		X		X		X		X		X		X	X
Randomization		X																
Dispense Study Medication		X	X	X	X		X		X		X		X					
Drug Accountability/ Compliance			X	X	X		X		X		X		X		X		X	X
ALSFRS-R ^{3,4}		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ALS-Specific QOL		X							X						X		X	X
QIDS-SR ₁₆		X			X						X				X		X	X
Adverse Event Review		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

¹ Height measured at Screening Visit only.

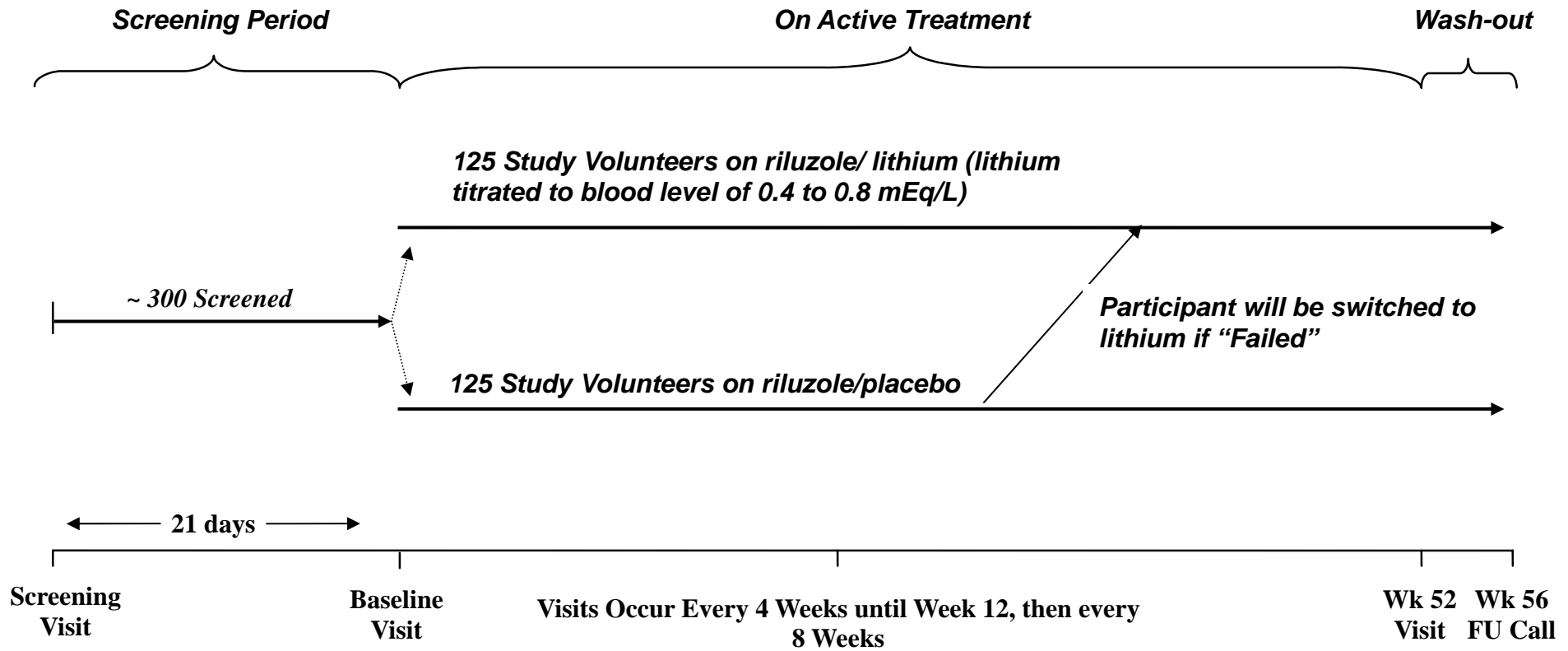
² BUN, creatinine, sodium, potassium, & liver function tests. CBC and serum pregnancy test (females only) at Screening Visit. TSH and blood glucose at Screening Visit and Weeks 28 & 52; glucose will be fasting at Weeks 28 & 52.

³ If a volunteer discontinues study drug early, Follow-Up Telephone Calls will be conducted instead of planned study visits for administration of ALSFRS-R and to assess medical status.

⁴ ALSFRS-R will be administered by telephone at Weeks 16, 24, 32, 40, 48

Visit windows: Baseline Visit must occur within 21 days of screening: ± 3 days for Weeks 4, 8, 12, and ± 5 days for all other visits.

Figure 4.1: Study Flow Chart



Volunteers who discontinue from the study early will be asked to return to the study site for Final Safety assessments, and will be asked to have a final Follow-Up Telephone Call at Week 56. Volunteers who discontinue study drug early but agree to be followed will remain in the study and will attend all assigned visits until the Week 56 final Follow-Up Telephone Call.

5.0 ETHICS

5.1 Independent Ethics Committee (IEC), Institutional Review Board (IRB) or Research Ethics Board (REB)

This study will be conducted in compliance with current Good Clinical Practices (GCP) and Title 21 Part 56 of the United States of America Code of Federal Regulations (CFR) relating to IRBs. This study will also be conducted in compliance with Health Canada's Food and Drugs Act and Regulations, Part A Administration, Part I, and Food and Drug Regulations, Part C, and the Health Canada Regulations Amendment (Schedule No. 1024) Clinical Trial Framework, Health Canada/ICH Guidance Documents E6: Guideline for GCP: Consolidated Guideline and E8: General Considerations for Clinical Trials, and all other applicable regulations pertaining to the conduct of clinical trials.

5.2 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.

5.3 Subject Information and Consent

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

The informed consent must be presented to the study volunteer by the site Principal Investigator or licensed physician Sub-Investigator.

6.0 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

This will be a multi-center study with approximately 30 to 35 investigative sites. Approximately 6 to 10 study volunteers will be enrolled at each site.

Sponsor	NINDS/NIH ALS Association ALS Society of Canada
Study Principal Investigators	Swati Aggarwal, MD Lorne Zinman, MD, MSc Petra Kaufmann, MD, MSc
ALS Consortia	Northeast ALS Consortium (NEALS) www.alsconsortium.org Canadian ALS Clinical Trials and Research Network (CALN)
Project and Data Management Center	NEALS Coordination and Data Management Center Merit Cudkowicz, MD, MSc Neurology Clinical Trials Unit Massachusetts General Hospital Boston, MA
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Study Medical Monitor	Carl Leventhal, MD Retired Consultant
Study Drug Monitor	Francine Vriesendorp, MD NEALS Outcome Measures Center SUNY Upstate Medical University Syracuse, NY

Study Medical Reviewer	Veena Lanka, MBBS Data Manager Massachusetts General Hospital Boston, MA
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7.0 INTRODUCTION

7.1 Background and Significance

7.1.1 Clinical Features and Epidemiology of ALS

Amyotrophic lateral sclerosis is a rare degenerative disorder of large motor neurons of the cerebral cortex, brain stem and spinal cord that results in progressive wasting and paralysis of voluntary muscles [3]. The incidence of ALS is currently approximately 2/100,000/year [4, 5] and may be increasing [6]. The lifetime ALS risk is 1 in 600 to 1 in 1000. Even though the incidence of ALS is similar to that of multiple sclerosis [7], the prevalence is only 4-6/100,000 (about 25,000 patients in the United States), due to the higher mortality rate. Fifty percent of ALS cases die within three years of onset of symptoms and 90% die within five years [7]. The median age of onset is 55 years. The cause in most cases is unknown. Age and gender are the only risk factors repeatedly documented in epidemiological studies [8]. There is a slight male predominance (3:2 male to female ratio) in sporadic ALS. No treatment prevents, halts or reverses the disease, although riluzole use is associated with a slight prolongation of survival [9, 10].

The majority of ALS cases are sporadic (SALS); approximately 10% are familial (FALS). More than 100 point mutations in the gene encoding cytosolic copper-zinc superoxide dismutase (SOD1) have been demonstrated to cause typical FALS [11]. Essential features of ALS are progressive signs and symptoms of lower motor neuron dysfunction (atrophy, cramps, and fasciculations) associated with corticospinal tract signs (spasticity, enhanced and pathological reflexes) in the absence of sensory findings [5]. There is relative sparing of eye movement muscles and the urinary sphincters. The course is relentless with declines in strength, respiratory function and overall function during the active phase of the disease [12]. Natural history studies have determined that age at onset, site of onset, delay from first symptom to entering ALS clinic, and rate of change in respiratory function are significant covariates of survival [13-16].

7.1.2 Overview of ALS Pathogenesis

Many causes of ALS have been proposed including toxicity from excess excitation of the motor neuron by transmitters such as glutamate, free radical-mediated oxidative cytotoxicity, neuroinflammation, mitochondrial dysfunction, autoimmune processes, cytoskeletal abnormalities, and aberrant activation of cyclo-oxygenase [17]. It has also been suggested that atypical viral infections may trigger this disease (e.g. enteroviruses or atypical retroviruses) [18-20]. Whatever the cause, it is evident that there are multiple levels of cellular dysfunction as the disease progresses and that programmed cell death is activated in this disease [21-25]. Mutations in the gene encoding SOD1 account for about 25% of cases of FALS or 2-3% of all ALS cases [11]. Forced expression of high levels of a mutant SOD1 transgene causes progressive motor neuron disease in mice and rats [26]. Additional genes implicated in ALS-like syndromes include ALS2, which codes for a guanine-nucleotide exchange-like factor [27, 28] and the dynactin gene [29, 30]. Five genetic defects have now been reported to cause FALS [11, 27-32].

7.1.3 Lithium in Neurodegenerative Disorders

Chronic lithium treatment up-regulates cell survival molecules [e.g. Bcl-2, cyclic AMP-responsive element binding protein, brain-derived neurotrophic factor (BDNF), Grp 78, Hsp 70, and beta-catenin] [33-39], while down regulating pro-apoptotic activities (e.g. excitotoxicity, p53, Bax, caspase, cytochrome c release, beta-amyloid peptide production, and tau hyperphosphorylation)[36, 38, 39], thus preventing or even reversing neuronal cell death and neurogenesis retardation. Studies have demonstrated that lithium enhances neuronal survival and promotes regrowth of dendritic arbors [40-42]. Lithium pretreatment protects cultured brain neurons from glutamate-induced, N-methyl-D-aspartate (NMDA) receptor-mediated apoptosis.

7.1.3.1 The Neuroprotective or Neurogenic properties of lithium in animal models

A neuroprotective effect of lithium has been demonstrated in animal models of acute brain injury (e.g. ischemia) [43] and chronic neurodegenerative diseases (Alzheimer's disease, Parkinson's disease, tauopathies, and Huntington's disease).

Brain Ischemia. Lithium pretreatment of rats subjected to permanent ischemia due to occlusion of the left middle cerebral artery, reduced ischemic infarct size by 56%[44]. In rat model of 1-hour occlusion followed by reperfusion, infarct size reduced by 50% in a dose dependent manner, even when administered up to 3 hours after the onset of ischemia [45, 46]. Neuroprotective effects of lithium were associated with up-regulation of 70-kDa heat-shock protein (Hsp70) in neuronal cells [45].

Alzheimer's Disease. Lithium prevented beta-amyloid induced hippocampal neurodegeneration in rats injected with beta-amyloid fibrils into the dorsal hippocampus[47].

Tauopathies. Lithium treatment attenuated GSK-3-catalyzed phosphorylation of tau, reducing neurofibrillary tangle formation by 60% in transgenic mice expressing three missense mutations of tau [48, 49].

Parkinson's Disease. In a mouse model of Parkinson's disease, lithium pretreatment prior to N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) insult prevented MPTP-induced decreases of dopamine level and changes of Bcl-2 and Bax levels in striatum[50].

Huntington's Disease. Lithium prevented apoptosis by attenuating quinolinic acid-induced DNA damage and activation of caspase 3, concurrent with up-regulation of Bcl-2 in a rat model of Huntington's disease [51, 52]. In addition, lithium promoted neuronal and astroglial progenitor cell proliferation, protecting against apoptosis.

7.1.3.2 The Neuroprotective or Neurogenic properties of lithium in humans

In vivo neuronal protective and regenerative effects of lithium have been demonstrated in humans as well. In lithium-treated bipolar affective disorder subjects, sequential 3D-MRI scanning showed increase in grey matter volume [53-55], while quantitative proton magnetic resonance spectroscopy demonstrated a significant increase in N-acetyl-aspartate, a putative neuronal marker in all regions of brain [56].

7.1.4 Rationale for Choosing Lithium in ALS

Extensive evidence supports a causative role of programmed cell death (apoptosis) in motor neuron loss in ALS[57-59]. Dark and shrunken cytoplasm and nuclei, chromatin condensation, and apoptotic bodies characterize degenerating spinal cord and motor cortex neurons[22]. The ratio of apoptotic cell death genes Bax to Bcl-2 is increased at both mRNA and protein level in spinal cord motor neurons from patients with ALS and from SOD1-G93A mice[21]. Altered expression of Bcl-2 may contribute to the activation of mitochondrial apoptosis machinery such as caspase-9, caspase 3, and cytochrome c in spinal motor neurons of ALS transgenic mice and humans with ALS[60, 61]. Over expression of Bcl-2 prolongs survival and improves motor performance in mutant SOD1-G93A transgenic mice[60]. Similarly, prevention of apoptosis using caspase inhibitors prolongs survival and delays disease progression in transgenic ALS mice[62].

Recently, administration of lithium to G93A SOD1 mice prolonged survival compared with placebo [1]. Lithium delayed cell death within lamina IX of the spinal cord and cranial motor neurons while it increased the number of lamina VII Renshaw-like neurons. Lithium also decreased reactive gliosis, rescued spinal cord mitochondria, and produced a marked regression of alpha-synuclein, ubiquitin, and SOD1 aggregates. The authors proposed that the likely molecular target for lithium in ALS involves blocking of IP3 activity, which acts as an endogenous autophagy inhibitor. Lithium promoted autophagy in primary motor neuron cell cultures, increased the number of neurons in lamina VII, and suppressed glial cell activation suggesting multiple targets contributing to a neuroprotective effect of lithium.

A small pilot study was performed in Italy in participants with ALS. Sixteen participants were treated with 300 – 450mg/day of lithium (target serum level 0.4-0.8 mEq/L) and riluzole; 28 participants received only riluzole. Participants treated with lithium demonstrated a slower rate of functional decline as measured by the ALSFRS-R. In addition, there were no fatalities in the lithium group after 15 months while 29% of participants only on riluzole died.

The results of this pilot study, while encouraging, are limited by the trial design. The study was small and participants were not blinded to treatment. Lithium is an accessible medication with a well-known side-effect profile. The results need to be replicated in a randomized, double blind, placebo controlled study. The main question patients and investigators would like answered at this time is whether the large effect reported by Fornai and colleagues is a true finding. This can be accomplished efficiently with our proposed study design.

These results suggest a dramatic effect of lithium on rate of ALS progression; similar results have not been previously reported for any therapeutic agent in any ALS study, regardless of sample size or experimental design. However, there are compelling reasons to regard this result with caution. First, the number of subjects treated was small, and subject characteristics prior to treatment suggest that they may have had slowly progressive disease. Secondly, subjects were not blinded to treatment assignment. Third, it is not clear that control subjects were concurrently enrolled in the study. Finally, the preclinical evidence for activity in ALS related disease models is not as strong as some

other agents currently in therapeutic development. Thus, there is a compelling rationale based on human experience for performing a double blind, randomized, placebo controlled trial to test the hypothesis that lithium has a dramatic effect in slowing ALS disease progression. The study proposed here is powered to detect a large effect, and has built-in stopping rules to avoid needless volunteer exposure if it becomes clear that such an effect will not be forthcoming. This is a different design than what would be proposed for a drug whose rationale was based on a large body of preclinical evidence; in such cases, it is often critical to detect modest demonstrations of therapeutic activity.

7.1.5 Experimental Therapeutics in ALS

Riluzole, a drug that has multiple mechanisms of action including inhibition of glutamate release at pre-synaptic terminals, was reported in two controlled studies to extend survival by three months (about 11%) in participants with ALS and is the only approved agent for the treatment of ALS [9]. Trials of CNTF [63, 64], gabapentin [65, 66], BDNF [67], xaliproden [68, 69], topiramate [70], celebrex [71], creatine [72, 73] and minocycline [74, 75] failed to demonstrate efficacy in ALS clinical trials. Ceftriaxone and arimocloamol are currently being tested in clinical trials.

There are a number of strategies employed to select promising ALS therapeutics for development and investigation. To better understand the biology of ALS, various laboratory-based models have been established. *In vitro* models of disease consist of cultured isolated motor neurons and slices of the spinal cord [76]. These systems identified and/or validated the initial approaches for ALS therapy screening, including most of the trophic factors considered in ALS and riluzole. The development of transgenic mice and rat models expressing mutant forms of SOD1 [26, 77, 78] has provided a valuable tool for understanding pathways that can lead to motor neuron cell death. Nevertheless, the predictive value of the models with regard to the identification of therapeutics that are effective in humans with ALS is unknown [79].

7.1.6 Significance

Despite recent critical advances in understanding the pathogenesis of ALS, this remains an untreatable and uniformly lethal disease. The therapeutic potential of lithium in ALS is unknown. However, the pilot study in people with ALS has generated large interest in the ALS patient community. It is important to determine whether the reported large effect in people can be replicated in a randomized, placebo controlled trial. However, given the great optimism regarding lithium among many ALS patients, it is thought to be difficult to enroll ALS volunteers to a placebo arm for 12 to 18 months, the duration required for a study with survival as endpoint. The proposed time to progression design is thought to be more appealing to participants as it can minimize the time a volunteer is taking placebo in those with rapid disease progression. We also propose a group sequential design with early stopping boundaries for safety and efficacy. Any compound proven to slow the course of the illness will be of immediate clinical importance and will enhance our understanding of disease pathophysiology leading to development of additional effective treatments.

7.2 Lithium

Lithium was discovered in 1817 by Swedish chemist, J.A. Arfwedson working in the laboratory of J.J. Berzelius[80]. Lithium is the lightest known solid element and exists in its natural form as a salt. It is also manufactured for patient use as a salt. In 1949 the Australian psychiatrist John Cade discovered the use of lithium salts in the management of mania [81]. The FDA approved lithium for treatment of bipolar affective disorders in 1970.

7.2.1 Pharmacokinetics

7.2.1.1 Absorption

Lithium is an alkali metal and a monovalent cation. It is completely absorbed via the gastrointestinal tract and is unaffected by the presence of food [82]. Most absorption takes place in the small intestine with smaller amounts taken up in the large intestine and stomach. The time to peak serum lithium concentrations (t_{max}) is approximately 1 to 3 hours after single doses of lithium citrate and 2 to 4 hours after single doses of lithium carbonate. The t_{max} is extended to 5 hours with slow or controlled release preparations [83]. The bioavailability of lithium carbonate capsules/ tablets ranges from 80 to 104% [83].

7.2.1.2 Distribution

Lithium is water-soluble, minimally protein-bound and thus distributes throughout body water both intra-cellularly and extra-cellularly with a volume of distribution of 0.5 to 1.2 L/kg [84]. Lithium is able to cross the blood-brain barrier with a mean ratio of serum lithium to cerebral spinal fluid lithium concentrations of 3.6:1 [83]. Concentrations of ions in the lung, kidney, muscle, and heart are in equilibrium with plasma concentrations. In bone, thyroid, and white matter of the brain amounts are somewhat higher [80, 83].

The volume of distribution is slightly reduced in patients 65 years or older, leading to smaller daily dosage requirements than in younger patients [83]. Lithium crosses the placenta and fetal lithium concentrations equal maternal levels. Lithium also passes into human breast milk with breast milk levels at approximately half the serum maternal levels [85].

7.2.1.3 Metabolism and Elimination

Lithium does not undergo biotransformation, and is eliminated through the kidneys, where it competes with sodium for reabsorption in the proximal tubules [86]. It freely crosses the glomerular membrane similar to sodium. Approximately 80% is reabsorbed by passive diffusion in the proximal tubules. In humans, lithium has a reported clearance of 13 to 56 ml/min (usually 20% - 30% of the glomerular filtration rate) [84] and is 20% lower during the night than during the day. Therefore changes in renal function or in sodium and fluid balance significantly affect serum lithium concentrations. Lithium clearance decreases with dehydration, low sodium intake, treatment with thiazide diuretics, and some antihypertensive and non-steroidal anti-inflammatory drugs.

The elimination half-life of lithium is dependent on both the volume of distribution and clearance rate. The mean half-life of lithium ranges from 16 to 30 hours in subjects with normal renal function [83]. Steady-state lithium levels will often be reached within 3 to 4 days (e.g., 4-5 times the half-life) [87]. However, patients with reduced renal lithium carbonate clearance may have half-lives of 2 to 3 days and may not reach steady state for several weeks. In practice, lithium levels are typically monitored every 1-3 months during the first 6 – 12 months of maintenance therapy and less frequently thereafter[88].

7.2.2 Drug Interactions

The safety of combining lithium with other medications is a concern. Several pharmacologic classes of drugs can produce clinically significant inhibition of lithium excretion [89, 90]. These include ACE inhibitors, angiotensin receptor blockers, COX-2 inhibitors, NSAIDs, and thiazide diuretics. Lithium toxicity presenting with diarrhea, vomiting, drowsiness, sedation and muscle weakness has been reported with these agents when serum lithium concentrations exceed 1.5 mEq/L. The magnitude of the effect is proportional to serum lithium concentration. For patients requiring ACE inhibitors and/or angiotensin receptor blockers an alternative agent such as a calcium channel blocker should be considered. NSAIDs increase serum lithium concentrations. The interactions have been well documented for indomethacin in particular, although most NSAIDs such as ibuprofen, naproxen, mefenamic acid, piroxicam, diclofenac, ketorolac, and tiaprofenac have been implicated [83]. Salicylates have little effect on serum lithium concentrations and can be considered as alternatives to other NSAIDs. Acetaminophen does not alter lithium elimination. Thiazide diuretics trigger an increase in reabsorption of lithium in proximal tubule and can elevate lithium levels, whereas loop diuretics do not generally affect lithium levels [90]. Osmotic diuretics enhance lithium excretion and may result in reduced lithium levels, while potassium sparing diuretics cause minimal change in lithium levels [90]. In addition, xanthine diuretics, such as theophylline or caffeine, can increase lithium clearance [83, 90, 91]. The combination of lithium and iodine may result in alterations in thyroid function [92]. Lithium can prolong the neuromuscular blockade of succinylcholine and pancuronium and should be discontinued 48 to 72 hours before surgery and restarted on post-operative return of bowel sounds [93]. Lithium can potentiate digoxin toxicity by decreasing intracellular potassium. The combination can cause severe nodal bradycardia and slow atrial fibrillation [93]. The combination of lithium, digoxin, and thiazide diuretic is especially dangerous.

See section 9.5.5 ‘Excluded, Prior and Concomitant Medications’ for details regarding the use of medications that may affect lithium absorption and secretion.

7.2.3 Lithium Adverse Effects

Lithium primarily affects the renal (thirst, polyuria), nervous (tremor, memory complaints), metabolic (weight gain), and gastrointestinal (nausea, diarrhea) systems [94, 95]. The incidence of adverse drug reactions (ADR) involving these systems ranges from 20% to 40% depending on the patients’ lithium levels. Rare and idiosyncratic side effects occurring in less than 5% of patients involve the skin (acne, psoriasis), heart (rare sinus node dysfunction), and thyroid gland (hypothyroidism). Overdose may cause more serious complications.

Signs and symptoms of mild to moderate lithium toxicity (serum lithium levels of 1.5-2.0 mEq/L) may include abdominal pain, nausea, vomiting, dry mouth, tremor, ataxia, dizziness, slurred speech, nystagmus, muscle weakness, and lethargy or excitement. Signs and symptoms of moderate to severe lithium toxicity (serum lithium levels of 2.0-2.5 mEq/L) may include significant nausea and vomiting, anorexia, blurred vision, stupor, delirium, clonic limb movements, seizures, choreoathetotic movements, circulatory system failure, hyperactive deep tendon reflexes, and coma. The most severe cases of lithium toxicity (serum lithium levels above 2.5 mEq/L), may result in generalized seizures, renal failure, and possibly, death [93].

Approximately 60% to 90% of patients maintained on lithium at a target level of 1.0 mEq/L will experience at least one ADR. Maintaining lithium levels of 0.4 to 0.8 mEq/L (the target level in this study) will significantly reduce the number of patients experiencing ADR [88, 96].

Cardiac. T-wave flattening or inversion is the most common electrocardiographic abnormality, reported in 13%-100% of patients with therapeutic levels of lithium [97, 98]. T-wave changes may be related to displacement of intracellular potassium by lithium and are usually reversible within several days after lithium discontinuation. Sinus node dysfunction is an uncommon but potentially serious, lithium-related conduction defect [97]. Some patients are asymptomatic, others present with dizziness, fainting, paroxysmal tachycardia, and dyspnea. Participants should be monitored for these symptoms and their resting pulse should be assessed during scheduled visits. The abnormalities are usually reversible with discontinuation of lithium. Evidence linking lithium use at therapeutic doses to other conduction abnormalities is minimal. Lithium toxicity may be associated with sinoatrial block, AV block, AV dissociation, bradyarrhythmias, ventricular tachycardia, and ventricular fibrillation [97]. Patients with pre-existing conduction abnormalities should be monitored with an ECG after steady-state lithium levels are achieved.

Central Nervous System. Reversible and transient neurological symptoms and signs, including slurred speech, unbalanced gait, confusion, irritability, and restlessness, have been observed in patients with high serum concentration (1.3-2.0 mEq/L) of lithium [97, 99]. Concomitant electroconvulsive therapy or the use of other psychotropics increase the risk of neurotoxic adverse effects [97]. Dose-related impairment in cognition particularly memory loss, problems with recall or retrieval have been described. In a meta-analysis on the cognitive side effects of lithium, Honig et al. concluded that lithium in higher doses can have a deleterious effect on memory and can slow information processing [99].

Tremors affect up to 65% of patients treated with lithium [100]. Severe tremor may be a sign of toxicity.

Dermatologic. Therapeutic doses of lithium have been associated with new onset or exacerbations of acneiform eruptions [99]. Lithium-induced acneiform eruptions are often found in areas not usually affected by acne vulgaris, i.e., legs, upper arms, and forearms. The lesions tend to persist rather than occurring in cycles [97]. New onset or exacerbation of psoriasis has been described with use of lithium [99]. In addition, lithium-related psoriatic conditions may prove difficult to treat with standard agents and

may require lithium-dosage reduction or discontinuation. Lithium's inhibition of adenylate cyclase may play a role as psoriasis has been related to a possible defect in the adenylate cyclase-cyclic adenosine monophosphate (AMP) cascade [97]. Maculopapular eruptions likely related to inert ingredients, such as dyes and fillers, in lithium preparations have been described.

Endocrine. Lithium use has been associated with increased, decreased, and unchanged glucose tolerance [97]. In one study, the frequency of pathological glucose tolerance was significantly higher in patients between 35 and 65 years old when compared with age-matched subjects from epidemiologic reference studies [101]. Others have reported apparent lithium-related hypoglycemia or increased glucose tolerance. Lithium may have an insulin-like effect increasing glucose uptake and glycogen formation and inhibiting adenyl cyclase to decrease lipolysis [97]. This effect may be associated with lithium-induced weight gain ranging from 3 – 28 kg with an average of 8.5 kg over a range of 6 months to 17 years.

Hypofunction is the most common thyroid abnormality associated with lithium. Most studies have reported a 2-15% prevalence of hypothyroidism [97, 102]. Lithium related inhibition of T-cell suppressor function has been suggested as a possible mechanism for increased antibody titers causing autoimmune thyroiditis. Other studies have shown early and transient laboratory evidence of hypothyroidism [decreased levothyroxine (T4) and elevated thyroid-stimulating hormone (TSH)] without clinical manifestations that normalized with continued lithium treatment [97, 103]. Clinical manifestations of hypothyroidism appear to develop only in patients with markedly increased TSH (greater than 35mU/L) [97, 104]. Serum lithium levels do not correlate with the incidence and severity of hypothyroidism.

American Psychiatric Association guidelines recommend that lithium-treated patients be evaluated for thyroid dysfunction at baseline and every 6-12 months thereafter [105]. Patients with preexisting hypothyroidism should be adequately treated with hormone replacement and have routine evaluation of thyroid function while on lithium therapy with appropriate dosage adjustments of exogenous thyroid hormone.

Gastrointestinal. Mild and transient gastrointestinal (GI) complaints include epigastric bloating and slight abdominal pain. More severe symptoms such as nausea, vomiting, and anorexia occur most commonly with lithium concentrations greater than 1.5 mEq/L. These ADRs can produce water and electrolyte disturbances, most significantly loss of sodium, which may result in lithium retention and toxicity (see Renal). Lithium should be administered with food to minimize GI ADRs [106].

Renal. Renal effects reported with lithium treatment include tubular dysfunction, morphological changes, reduction in glomerular filtration rate, renal tubular acidosis, and nephrotic syndrome [94, 97]. Polyuria is a frequent renal side effect of lithium and can occur shortly after lithium treatment is started or after many years of treatment. In early studies, 2 – 35% of patients experienced urine volumes greater than 3 liters per 24 hours of urine output [107]. Milder cases of lithium-induced polydypsia may not require any specific treatment beyond maintenance of adequate hydration. For those with more severe polydypsia, treatment with thiazide diuretics or amiloride can be considered. Most

prevalence studies of lithium-induced polyuria involved patients receiving long-term lithium maintenance treatment with levels greater than 0.8 mEq/L. However, in the majority of patients with lithium levels less than 0.8 mEq/L, renal concentrating ability was usually not significantly impaired [94]. Maintaining a patient's lithium level between 0.4 and 0.8 mEq/L (the target range in this study) will minimize this ADR.

Nephrotic syndrome is a rare adverse renal effect that can occur at therapeutic lithium levels. Biopsies have been consistent with minimum change disease and in all cases lithium discontinuation alone or combined with diuretics, hemodialysis, or prednisone resulted in improvement [97]. It is unknown if patients with preexisting kidney disease are at an increased risk for sustaining more damage secondary to lithium [97]. Lithium is contraindicated in acute renal failure; however, it may be used in chronic, stable failure. Lithium has been safely used in renal transplant patients [97].

7.2.4 Selection of Dosage in the Study

The typical daily dosage of lithium carbonate for acute mania is 1800 mg/day; with desired serum lithium levels ranging between 1.0 to 1.5 mEq/L, and maintenance therapy ranging between 900 to 1200 mg/day with desired serum lithium levels of 0.6 to 1.2 mEq/L. In the recent study by Fornai, et al. (2008), participants were treated with riluzole 50 mg b.i.d. and lithium carbonate at a dosage of 300-450 mg daily titrated to a plasma level of 0.4 to 0.8 mEq/L. There have been two studies of lithium in the mutant SOD1 transgenic mouse model, one at a dosage of 1mEq/kg daily [1] and another at 5mM lithium [108]. The study at the higher dosage suggested a slight neuroprotective effect of lithium (125.6 to 137.2 days) [108] as opposed to the one at lower dosage, which showed prolonged disease duration from a mean of 9 days to > 38 days [1]. It is possible that higher doses of lithium may have an alternate effect which may be detrimental to motor neurons. Based on these studies lithium may have a narrow therapeutic window for neuroprotection. Thus to account for significant inter-subject pharmacokinetic variability a concentration-controlled approach is selected for this study.

7.2.5 Safety and Clinical Use in Humans

Lithium carbonate is a mood stabilizer medication approved by the FDA and Health Canada for the treatment of bipolar disorder. Several safeguards will be in place to protect against the potential risks associated with lithium. After providing written informed consent, all participants will have screening laboratory tests and a medical history evaluation to confirm their appropriateness to be treated with lithium. Participants will be monitored closely, with scheduled visits every 4 weeks for the first 12 weeks, and then every 8 weeks thereafter with interim phone interviews. Unscheduled visits may occur at the discretion of the Investigator if deemed appropriate for the safety of the participant. Participants will be educated about the risks associated with lithium during the consent process and asked to cooperate with the research staff in monitoring for these risks. This education will include the signs and symptoms of lithium toxicity and instructions for emergency care. Participants will be provided with contact numbers for the research staff. All sites will have an appropriate staff member available 24 hours a day, 7 days a week in the event of an emergency. In addition, all participants will have periodic safety laboratory testing. Lithium levels will be closely monitored centrally.

Dosage adjustments will be recommended by the Drug Monitor if serum levels of lithium fall outside of the target window of 0.4-0.8 mEq/L. Investigators can suspend study drug if clinical signs and symptoms suggest lithium toxicity.

8.0 STUDY OBJECTIVES

8.1 Primary

The primary objective of this study is to determine if lithium carbonate in combination with riluzole is efficacious in volunteers with ALS. Volunteers diagnosed with ALS will be randomized in a 1:1 ratio to receive either lithium/riluzole or placebo/riluzole. Efficacy will be measured by comparing the time to disease progression (defined as a drop in overall ALS Functional Rating Scale-Revised (ALSFRS-R) score by 6 or more points) or death. When participants originally assigned to placebo reach this predefined level of decline (a 6 or more point drop in the overall ALSFRS-R score), they will be switched to active medication. This time to failure endpoint is novel for ALS trials, and was chosen in recognition of the intense interest in lithium by the ALS patient community. It is felt that patient acceptance of this trial would be enhanced by a design that switches participants to active drug after significant disease progression has occurred (defined as a 6 point decline in ALSFRS-R from baseline). This design accomplishes this while preserving a fully randomized double-blind comparison between lithium/riluzole and riluzole/placebo.

Secondary measures of drug efficacy will include change in the mean ALSFRS-R slope, pulmonary function (vital capacity - VC), effect on mood as measured by the Quick Inventory of Depressive Symptomatology Self-Report (QIDS-SR₁₆), quality of life as measured by the ALS-Specific Quality of Life Questionnaire (ALSSQOL) and tracheostomy-free survival at 12 months.

8.2 Secondary

The secondary objective of this study is to determine the safety of long-term (12 months) administration of lithium in combination with riluzole in participants with ALS as measured by adverse events, serum lithium levels, ability to remain on the assigned treatment (tolerability), physical examinations, vital signs, weight/body mass index (BMI), laboratory test results, and use of concomitant medications.

9.0 INVESTIGATIONAL PLAN

9.1 Overall Study Design and Plan

This is a double-blind, randomized, placebo-controlled study evaluating the efficacy and safety of lithium in combination with riluzole compared to placebo in combination with riluzole.

During the enrollment period 250 volunteers recruited from approximately 30 to 35 centers in the US and Canada will be randomized in a 1:1 ratio to one of two groups: lithium/riluzole or placebo/riluzole. The enrollment will occur in a staged manner. Initially 84 participants will be enrolled in the trial. First interim analysis will occur once

the 84th participant has been accrued. At this point, based on the available data the Data and Safety and Monitoring Board (DSMB) appointed by the National Institutes of Health (NIH) may decide to stop the trial for efficacy or futility or stop accrual and request that follow-up continue on the 84 participants enrolled in the trial or continue accrual. After screening and randomization, participants will be followed every 4 weeks (every 28 days \pm 3 days) for 12 weeks; subsequent visits will occur every 8 weeks (every 56 days \pm 5 days) up to 52 weeks with interim Follow-Up Telephone Interviews for administration of the ALSFRS-R at Weeks 16, 24, 32, 40, and 48 (\pm 5 days). There will be a 28-day (\pm 5 days) post treatment Follow-Up Telephone Interview to assess medical status and adverse events.

All participants will have the ALSFRS-R administered at the Baseline Visit. The score obtained at this time will be considered the baseline score for each participant. The ALSFRS-R will be administered to all participants monthly for evaluation of disease progression, either during in-person visits or through phone interviews. Previous studies have demonstrated that the ALSFRS-R score typically drops by approximately 1 point a month. If the overall ALSFRS-R score for a participant drops by 6 points or more from baseline they will be designated as a ‘treatment failure.’ All participants who have ‘failed’ will receive lithium starting at the next scheduled in-person study visit. A 6-point drop in ALSFRS-R will occur after approximately 6 months (midway through the study) and represents significant disease progression. However, a 6-point drop also provides the opportunity for participants to be switched to active compound in the phase of rapid disease progression. Therefore, participants originally randomized to placebo who “fail” (i.e. drop 6 or more points on the ALSFRS-R), will be reassigned to treatment with lithium for the duration of the study (up to 52 weeks). Participants originally randomized to lithium who fail would continue on lithium treatment for 52 weeks.

Drug assignment blinding (lithium or placebo) will be maintained for all participants, Clinical Evaluators, Study Coordinators, and the Site Investigators for the duration of the study. Both participants and Clinical Evaluators (and all study staff other than the unblinded Drug Distributor at each site) will be blinded to the ALSFRS-R scores and date of transition to active study medication if applicable. The ALSFRS-R scores will be completed by the blinded Clinical Evaluators, but the total score will not be tallied. The Clinical Evaluators and participants will not have access to total ALSFRS-R scores from previous assessments for comparison.

This study design limits the period of time participants are on placebo and provides the opportunity for all participants to receive lithium if they progress beyond the aforementioned endpoint. Participants who discontinue study medication early will be asked to return to the study site for a Final Safety Visit. All participants will be encouraged to follow per protocol regardless of their compliance with the assigned treatment. If they are unable to return for in-person visits, the in-person visits can be replaced by the ALSFRS-R questionnaire and assessment of medical status, adverse events and concomitant medications over the phone. An intention to treat analysis will be performed.

All visit windows are consecutive calendar days and are calculated from the day the participant starts study medication (the day of the Baseline Visit).

9.2 Study Centers

This study will be conducted at approximately 30 to 35 Northeast ALS Consortium (NEALS) and Canadian ALS Clinical Trials and Research Network (CALC) centers in the US and Canada selected because of their excellent record of recruitment, compliance with study protocols and regulations, clinical research expertise and resources.

9.3 Study Duration

There will be a start-up period of 16 – 20 weeks (4 – 5 months) to obtain IRB approval, develop electronic case report forms for data capture, and build a secure database. An enrollment period of approximately 21 weeks (5 months) is planned during which time first 84 volunteers will be screened and randomized. There will be a maximum allowed time of 21 days between the Screening and Baseline Visits (Randomization). Accrual will be temporarily held after the 84th participant has been enrolled and first interim analysis is completed. Study volunteers will receive study medication for a total of 52 weeks (12 months), followed by a 28-day (± 5 days) Follow-Up Telephone Interview. An additional 8- 12 weeks will be required for data analysis. Thus, the total study duration is estimated at approximately 108 - 130 weeks (27 - 30 months).

9.4 Selection of Study Population

9.4.1 Number of Study Volunteers

A total of 250 volunteers will be equally randomized to receive either placebo/riluzole or lithium/riluzole.

9.4.2 Inclusion Criteria

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

1. Familial or sporadic ALS.
2. Participants diagnosed with laboratory supported probable, clinically possible, probable or definite ALS according to the World Federation of Neurology Revised El Escorial Criteria [2] (Appendix 1).
3. Disease duration from symptom onset no greater than 36 months at the Screening Visit.
4. Aged 18 years or older.
5. Capable of providing informed consent and complying with trial procedures.
6. On a stable dose of riluzole 50mg bid for at least 30 days prior to screening.
7. Vital capacity (VC) equal to or more than 60% predicted value for gender, height and age at the Screening Visit.
8. Creatinine ≤ 1.5 mg/dl (133 μ mol/L).
9. Participants maintained on thyroid medication must be euthyroid for at least 3 months before the Screening Visit.
10. Participants with psoriasis must have inactive disease for at least 30 days before the Screening Visit.
11. Women must not be able to become pregnant (e.g., post menopausal for at least one year, surgically sterile, or practicing adequate birth control methods)

for the duration of the study. Adequate contraception includes: oral contraception, implanted contraception, intrauterine device in place for at least 3 months, or barrier method in conjunction with spermicide. Women of childbearing potential must have a negative serum pregnancy test at Screening Visit and be non-lactating.

12. Geographic accessibility to the study site.
13. Fluency in English, Spanish or French (Canadian).

9.4.3 Exclusion Criteria

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

1. History of known sensitivity or intolerability to lithium or to any other related compound.
2. Prior exposure to lithium within 90 days of the Screening Visit.
3. Exposure to any investigational agent within 30 days of the Screening Visit.
4. Participants who are malnourished, dehydrated or on a sodium-free diet will be excluded due to the potential side effects of lithium carbonate.
5. Use of digoxin or iodide salts [e.g. calcium iodide, hydrogen iodide (hydriodic acid), iodide, iodinated glycerol (Organidin), iodine, potassium iodide (SSKI), and sodium iodide supplementation beyond table salt].
6. Presence of any of the following clinical conditions:
 - a. Substance abuse within the past year.
 - b. Unstable cardiac, pulmonary, renal, hepatic, endocrine, hematologic, or active malignancy or infectious disease.
 - c. AIDS or AIDS-related complex.
 - d. Clinically active psoriasis within 30 days of the Screening Visit.
 - e. Unstable psychiatric illness defined as psychosis (hallucinations or delusions) or untreated major depression within 90 days of the Screening Visit.
 - f. Screening serum creatinine greater than or equal to 1.5 mg/dL (133 μ mol/L), TSH > 20% above the upper limit.
 - g. Presence of clinically significant conduction abnormalities on electrocardiogram (ECG).
7. Female volunteers who are breast-feeding or who have a positive serum pregnancy test at the Screening Visit.

Riluzole. All volunteers participating in the study will be taking a stable dose of riluzole 50mg bid for 30 days prior to the Screening Visit. Approximately 60-70% of patients with ALS are currently taking riluzole in the United States. The vast majority of patients diagnosed with ALS in Canada are followed in academic subspecialty ALS/neuromuscular clinics and have access to riluzole at minimal to no cost. In the recent study by Fornai et al. [1], all volunteers participating in the study were on a stable dose of riluzole 50mg bid and lithium 300 to 450mg titrated to a blood level of 0.4 to 0.8 mEq/L. It is possible that the survival benefit and decrease in rate of decline of ALSFRS-R and VC reported in this study are due to the combined effect of riluzole and lithium

instead of the lithium alone. To verify the large effect reported in the previous study we will require all participants to be on riluzole during the period of the study (52 weeks).

9.4.4 Removal of Study Volunteers from Therapy or Assessments

A volunteer may choose to discontinue study medication at any time. However, the Site Investigator or designee will encourage participants to continue with follow-up, regardless of their compliance with the study medication. If the Site Investigator, Drug Monitor, or Coordination Centers are concerned about the use of a prohibited medication or other safety issues, then the study drug prescription may have to be modified or study drug may have to be held. However, the Site Investigator or designee will still encourage participants to follow-up per study protocol under the intent to treat principle. Loss to follow-up should be prevented whenever possible.

The Site Investigator will notify the appropriate Site Management Center via telephone as soon as possible when a volunteer is at risk of loss to follow-up. Early termination occurs when a volunteer withdraws consent or the sponsor terminates the study. Volunteers may choose to discontinue study participation (withdraw consent) at any time. Volunteers who withdraw consent, or who discontinue participation due to study termination by the Sponsor, should then return unused study medication and will be asked to return to the study site for a Final Safety Visit

9.5 Treatments

9.5.1 Treatments Administered

The two treatment arms include placebo/riluzole and lithium/riluzole. All participants will be taking a stable dose of riluzole 50 mg twice a day (b.i.d.) for at least 30 days prior to the Screening Visit. Lithium will be started at a dosage of 450 mg per day. Capsules will contain 150 mg of either placebo or lithium carbonate and will be administered two times a day (one capsule in the morning and two in the evening). Each bottle contains 105 capsules and study volunteers will receive 1-2 bottles at each study visit. Lithium and placebo will be supplied in bulk from the manufacturer to the Central Research Pharmacy. The Research Pharmacy located at the Clinical Trials Coordination Center at the University of Rochester in Rochester, NY, will serve as the Central Research Pharmacy for this trial. They will package the lithium and placebo into individual bottles and supply to an unblinded Drug Distributor at each site. An unblinded research pharmacist, physician, or research nurse who is not part of the study can act as the Drug Distributor for an individual site. Additional drug will be shipped to each site, as needed based on enrollment.

Lithium carbonate or the matching placebo will be taken orally as a capsule. If a volunteer develops swallowing difficulties, the capsule may be opened and the contents added to a small quantity of applesauce or other soft food and administered orally or through a g-tube, as appropriate. All volunteers will start study medication the day of their Baseline Visit (Visit 2) and continue treatment for 52 weeks (Visit 10). The day that a study volunteer starts treatment with study drug will be designated as Day 0. All subsequent visits must be scheduled from Day 0 (Baseline Visit), not the date of their last assessment. All volunteers will be instructed to take 3 capsules a day (1 capsule in the

morning and two in the evening). Additional capsules may be added to achieve desired blood levels of lithium between 0.4 and 0.8 mEq/L, as per the instructions of the Drug Monitor. Paired sham dosage modifications will be performed in the placebo group to maintain blinding. No dosage increases will be performed without approval of the Drug Monitor.

If serum lithium levels exceed 1.5 mmol/L the Drug Monitor will contact the Site Investigator to hold study drug for at least 2 days after which the daily dosage will be reduced by 300 mg or more, or permanently discontinued if needed, per the instructions of the Drug Monitor.

9.5.2 Method of Assigning Study Volunteers to Treatment Groups (Randomization)

Each volunteer who meets all eligibility criteria, is accepted for the study and signs an informed consent form will be randomized to receive lithium or placebo on a 1:1 basis. The randomization scheme will be independently developed by the Biostatistics Center at MGH and individual randomization sheets indicating the treatment assignment and the subject numbers to be used by each site will be provided to the unblinded Drug Distributor at each site. A programmer in the MGH Biostatistics Center will develop the plan under the guidance of the chief biostatistician.

9.5.3 Study Drug Adjustments and Dosage Reduction

Any dosage adjustment including the reason for and dates of adjustment will be documented in the source documents and the eCRF for each volunteer requiring this manipulation.

9.5.3.1 Dosage Initiation/ Escalation

Given the considerable variability between lithium dosage (mg/d) and lithium steady state levels, a concentration-controlled approach was chosen for this study. For those participants randomized to lithium/riluzole, lithium will be initiated at a dosage of 450mg/day. The lithium will be supplied as 150 mg capsules and each participant will start with 3 capsules a day taken as 1 capsule in the a.m. and 2 in the p.m. The dosage of lithium will be escalated as necessary to achieve a desired serum concentration of 0.4 to 0.8 mEq/L. Lithium follows first-order kinetics; therefore, as the dosage increases or decreases, the steady-state serum lithium concentration increases or decreases proportionately provided there is no change in the renal clearance of lithium. Thus, a patient with steady state concentration of 0.2 mEq/L at 300 mg/d is expected to have a steady-state concentration of 0.4 mEq/L if the dosage is increased to 600 mg/d. A generic form of lithium carbonate will be used for this study, purchased from the same source for all sites.

9.5.3.2 Dosage Reduction

Lithium levels will be closely monitored centrally by a Drug Monitor at the NEALS Outcome Measures Center, SUNY Upstate Medical University. Any adjustments to the dosage will not be made without approval from the Drug Monitor. The Drug Monitor will determine appropriate dosage adjustments if serum lithium levels fall outside of the target window of 0.4-0.8 mEq/L. For every dose adjustment of a participant on lithium, an

equivalent adjustment will be made to a placebo treated patient. The Site Investigator or licensed physician Sub-Investigator has the option of temporarily stopping the study medication for AEs thought to be related to the study drug. If the event is serious or life threatening, and deemed to be drug related, the study medication should be discontinued immediately.

9.5.3.3 Dosage Suspension

The Site Investigator or licensed physician Sub-Investigator may temporarily stop the study medication for AEs or any other reason during the trial (the reason for, and dates of suspension must be documented). If serum lithium levels exceed 1.5 mmol/L the Drug Monitor will contact the Site Investigator, who will contact the participant and instruct him/her to hold study drug for at least 2 days after which the daily dosage will be reduced by 300 mg or more, or permanently discontinued if needed, per the instructions of the Drug Monitor. Study volunteers may remain off the study medication until the condition improves or they may resume on a reduced dosage as advised by the Drug Monitor. Since an intention to treat analysis will be performed, no upper limit will be set for the length of allowable drug suspension. However, any resumption of drug after a suspension requires approval from the respective Site Management Center.

9.5.3.4 Dosage Re-Challenge

The Site Investigator or licensed physician Sub-Investigator may choose to re-challenge a volunteer at the previous dose or at a reduced dose as advised by the Drug Monitor if the AE resolves or it is felt that the volunteer can continue safely. Sites must receive approval from the respective Site Management Center prior to re-challenging volunteers after any suspension.

9.5.3.5 Dosage Discontinuation

Reasons for discontinuation of study medication may include an AE, Drug Monitor or Site Investigator recommendation, Sponsor termination, protocol violation, lost to follow-up, withdrawal of consent, or death. All serious adverse events (SAEs) that occur in a volunteer who has discontinued study medication must be recorded and reported up to the final Follow-Up Telephone interview (Week 56) within 24 hours of awareness. Study volunteers who discontinue study medication prematurely will be encouraged to return for a Final Safety Visit. All volunteers who discontinue early will be encouraged to follow per protocol regardless of their compliance with the assigned treatment. If they are unable to return for in-person visits, the scheduled in-person visits can be replaced by the ALSFRS-R questionnaire and assessment of medical status, adverse events and concomitant medications over the phone.

9.5.4 Blinding

The Randomization ID will be used to identify the volunteer's electronic case report forms (eCRFs), laboratory tests, study medication and all communications. Study Volunteers, Investigators, Coordinators, Clinical Evaluators (and all other study site staff), Study Monitors, Project Management, Clinical Laboratory and Data Management personnel, and the Sponsor will be blinded to treatment group assignment throughout the

study. Only the Drug Monitor, Drug Distributor, and Biostatistician developing the randomization schedule will be unblinded to the individual drug assignments in this study. Communications between these individuals and the blinded Investigators will be limited.

9.5.4.1 Emergency Unblinding

An emergency unblinding procedure will allow the Site Investigator the option of learning the treatment assignment for an individual volunteer if clinical circumstances require it. For unblinding, the Site Investigator may contact the respective Site Management Center, if time allows. If time does not allow (i.e. the subject's safety is dependent on immediate unblinding), the Site Investigator may contact the Drug Distributor at his/her site for the drug assignment. In this case, the site must inform the respective Site Management Center as soon as possible regarding the emergency unblinding and the circumstances surrounding it. Rarely should such an extreme action be taken. Experimental medications can usually be withdrawn without the need for unblinding. In the event that emergency disclosure of treatment assignment is necessary, the volunteer will be withdrawn from further participation in the trial. All AEs resulting in emergency unblinding will be followed up to the final Follow-Up Telephone interview at Week 56.

9.5.5 Excluded, Prior and Concomitant Medications

Throughout the study, Site Investigators or licensed physician Sub-Investigators may prescribe concomitant medications or treatments deemed necessary to provide adequate supportive care providing that the medications are licensed in the US or Canada. Study volunteers should not receive other experimental agents during the study. An up to date list of experimental agents will be provided to sites on the study portal. This includes marketed agents at experimental dosages that are being tested for the treatment of ALS. All concomitant medications/treatments and significant non-drug therapies (including supplements and assistive devices) received by a volunteer should be recorded on the appropriate source document and eCRF. Volunteers and/or primary caregivers should consult with the Site Principal Investigator or licensed physician Sub-Investigator prior to initiating any new medication, including non-prescription compounds or any other non-drug therapy.

As mentioned in Section 7.2.2, several pharmacological classes of drugs can produce clinically significant inhibition of lithium excretion and result in higher serum concentrations. These include ACE inhibitors, angiotensin receptor blockers, COX-2 inhibitors, non-steroidal anti-inflammatory drugs (NSAIDs), and thiazide diuretics. Xanthine derivatives such as theophylline can increase lithium clearance, which could decrease lithium levels. Participants already taking these medications would be encouraged to maintain a stable dose during the study. Participants starting on these medications during the study will be allowed to take these medications; however, the use of these drugs will be tracked in the electronic data capturing system. The participants starting any of these drugs during the study period should be observed for any change in the clinical status. They may need adjustment of lithium dosage to maintain desired serum concentration and avoid toxicity. Iodine use in combination with lithium can

produce alterations in thyroid function and this combination will thus be avoided. Occasional NSAID use not exceeding 200mg of naproxen every 8 hours is acceptable.

9.5.6 Treatment Compliance

Study volunteers will be instructed to return unused study medication capsules at each visit. Site personnel will review returned unused study medication to determine compliance. As lithium levels are checked at each visit, non-compliance in the placebo group (surreptitious use of lithium) will be ascertained. Data indicating non-compliance will be used in the end of study analysis, but will not be addressed with individual subjects during the course of the study.

Non-compliance will be otherwise defined as taking less than 80% or more than 120% of study medication as determined by pill counts. If a volunteer is non-compliant with study medication, the Site Investigator and staff should re-educate and train the participant in administration of study medication.

9.5.7 Study Drug Storage

The Site Investigator must ensure that all investigational drug supplies are kept in a locked, safe area with access limited to those directly involved in the study. The drug will be stored at room temperature (range 59-86 °F) away from excess heat and moisture as recommended. Investigational drug supplies should not be repackaged in any way.

9.5.8 Warnings/Precautions

Participants will be advised to maintain a normal diet with moderate salt intake and daily fluid intake of 2500-3500 mL. It is important to maintain adequate hydration as dehydration may result in increased lithium levels and toxicity. Vigorous exercise, prolonged exposure to the heat or sun, excessive sweating, fever, diarrhea, or vomiting may cause dehydration. Participants will be advised to call the doctor if they are losing a significant amount of body fluid as a result of sweating, diarrhea, or vomiting.

Participants and their caregivers will be instructed to call the Site Investigator if any signs of lithium toxicity are noted including diarrhea, vomiting, tremor, ataxia, muscle weakness, or drowsiness.

Lithium may induce or, more commonly, exacerbate active psoriasis, which may be intractable to usual treatments and therefore require drug discontinuation. However, lithium carbonate does not necessarily exacerbate inactive psoriasis in patients with a preexisting condition.

See section 7.2.2 'Drug Interactions' and section 9.5.5 'Excluded, Prior, and Concomitant Medications' for warnings and precautions regarding concomitant drug use.

9.6 Efficacy and Safety Variables

9.6.1 Efficacy Assessments

9.6.1.1 ALSFRS-R

The ALSFRS-R is a quickly administered (5 min) ordinal rating scale (ratings 0-4) used to determine volunteers' 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 [109-111]. 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, it is quickly administered, and the rate of decline correlates strongly with survival. With appropriate training the ALSFRS-R can be administered with high inter-rater reliability and test-retest reliability. The ALSFRS-R can be administered by phone, again with good inter-rater and test-retest reliability [112]. The equivalency of phone versus in-person testing, and the equivalency of study volunteer versus caregiver responses have also recently been established [113]. Therefore, if necessary, the ALSFRS-R may be given to the study volunteer over the phone reducing the number of dropouts and missing data.

9.6.1.2 Vital Capacity (Pulmonary Function Testing/Spirometry)

The vital capacity (VC) (percent of predicted normal) will be determined, using the slow VC method. The VC can be measured using conventional spirometers that have had a calibration check prior to volunteer testing. A printout from the spirometer of all VC trials will be retained. All VC Evaluators must be NEALS certified. Three VC trials are required for each testing session, however up to 5 trials may be performed if the variability between the highest and second highest VC is 10% or greater for the first 3 trials. Only the 3 best trials are recorded on the CRF. The highest VC recorded is utilized for eligibility. Vital capacity results at baseline, even those less than 60%, will not preclude enrollment.

9.6.1.3 ALS-Specific Quality of Life Questionnaire

Previous studies using multiple instruments have shown that quality of life (QOL) does not correlate with disease severity, duration, or progression in those with ALS. Therefore, it is evident that QOL in patients with ALS is not easily determined by standard scales, such as the SF-36, that rely mainly on physical function as an indicator of QOL. The scale to be used in this study is the ALS-Specific Quality of Life Scale, which was developed, tested, and validated in patients with ALS, and is not a health-related QOL scale [114]. The Questionnaire consists of 59 questions, each rated on a 1 to 10 scale, that ask about severity of the symptoms of ALS, mood and affect, intimacy, and social issues.

9.6.1.4 Quick Inventory of Depressive Symptomatology Self-Report (QIDS-SR₁₆)

The QIDS ratings were constructed by selecting only items from the 30 item scales needed to assess the nine DSM-IV criterion diagnostic symptom domains. The scoring system of the QIDS converts responses to the 16 separate items into the nine DSM-IV symptom criterion domains. The nine domains comprise 1) sad mood; 2) concentration; 3) self criticism; 4) suicidal ideation; 5) interest; 6) energy/fatigue; 7) sleep disturbance (initial, middle, and late insomnia or hypersomnia); 8) decrease or increase in appetite or weight; and 9) psychomotor agitation or retardation. The total score ranges from 0 to 27. The items on the clinician rated and self report versions of the QIDS-C₁₆ and QIDS-SR₁₆ rate identical symptoms with equivalent anchors [115]. The self-report version QIDS-SR₁₆ was developed to be easy to use severity measure, providing a potentially more time efficient alternative to the QIDS-C₁₆ in both clinical or research settings.

QIDS-SR₁₆ is easy to administer, requires minimal training, and provides matched clinician and patient ratings. It is sensitive to changes in depressive severity, with medications, psychotherapy, or somatic treatments, making it useful for both research and clinical purposes. In a study of 596 adult outpatients with chronic, nonpsychotic MDD, Rush et al reported Cronbach's alpha of 0.86 for the QIDS-SR₁₆ [116]. Trivedi et al. reported the psychometric properties of the QIDS-SR₁₆. Cronbach's alpha was 0.86 (QIDS-SR₁₆) for the MDD patients [117].

9.6.1.5 Survival

A secondary measure of efficacy will be tracheostomy-free survival (free of tracheostomy and permanent assisted ventilation). Death from any cause, tracheostomy, or permanent assisted ventilation will be considered survival endpoints. Non-invasive ventilation techniques utilized for more than 22 hours daily for more than one week will be criterion for determining permanent assisted ventilation. The date and cause of death and/or tracheostomy will be recorded. Participants who elect to undergo permanent assisted ventilation or tracheostomy will continue on double-blinded treatment.

9.6.1.6 Training and Validation

Training for all outcome measures will be accomplished at a centralized Evaluator's meeting. All Evaluators must be NEALS certified for the ALSFRS-R and VC; specific certification requirements are outlined in the study manual of operations. Repeat NEALS certification will be required on an annual basis for all outcome measures. It is strongly preferred that a single Evaluator performs all measures throughout the study. NEALS certification is required for all Evaluators prior to performing any study tests.

9.6.2 Safety Assessments

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

9.6.2.1 Vital Signs, Height and Weight

Vital signs will be obtained after the volunteer has been in a seated position for at least 3 minutes. Systolic and diastolic blood pressure, and pulse rate (radial artery) will be obtained at specified visits. Height and weight will be measured and recorded at screening. Weight and vital signs will be assessed at each visit.

9.6.2.2 Clinical Laboratory Assessments

The clinical measurements are presented in the following table.

Serum Chemistry	CBC (Hematology)	Additional Tests
Glucose	Hemoglobin	HCG for women of childbearing potential
Sodium	Hematocrit	TSH
Bicarbonate	Total RBC	Lithium level
Blood urea nitrogen (BUN)	RBC Indices	
Potassium	Total WBC	
Calcium	WBC and Differential	
Creatinine	Platelet Count	
Chloride		
AST		
ALT		
Phosphate		
Magnesium		
Albumin		
Total Bilirubin		
Alkaline Phosphatase		

9.6.2.3 Serum Lithium Levels

All volunteers will have lithium levels tested at the Screening Visit. Pre-dose lithium trough levels will be measured at Weeks 4, 8, 12 (± 3 days), 20, 28, 36, 44, and 52 (± 5 days). These samples will be analyzed at a central laboratory. The Drug Monitor will receive all lithium levels from the central lab, make appropriate study drug dosage changes, and communicate with the sites. The Drug Monitor will also advise on paired, sham dosage modifications for the placebo arm to maintain blinding. The Drug Monitor should also be consulted for any dosage adjustments needed in relation to AEs thought to be related to study drug. The Drug Monitor will also advise the Drug Distributor regarding the switch from placebo to lithium for participants initially assigned to placebo if their ALSFRS-R score drops by 6 points or more.

Detailed instructions on sample handling, storage, and shipment are contained within the laboratory manual. This information will be reviewed with the Site Investigator and site personnel prior to enrollment of the first study volunteer.

9.6.2.4 12-Lead Electrocardiogram (ECG)

A standard 12-lead ECG will be performed at the Screening Visit. A copy of the tracing will be kept on site as part of the source documents.

9.6.2.5 Physical and Neurological Examinations

A physical examination will be performed and recorded at the Screening and Week 52 visits and will include the following systems: head/neck, eyes, ears, nose/throat, cardiovascular, lungs, abdomen, musculoskeletal, central nervous system, extremities, and skin. In addition, a complete neurological examination will be performed at screening and week 52. A physical examination and complete neurological examination will also be performed at any Final Safety visits that occur.

9.6.2.6 Adverse Events

Adverse events will be documented at each study visit and each Follow-Up Telephone Call. Information on adverse effects of study medication and on inter-current events will be determined at each visit by direct questioning of the volunteers, clinical examination, review of concomitant medications, vital signs and weight, and laboratory test results. Tolerability will be determined by the ability to complete the study on the assigned treatment. Of particular interest are side effects that would not be tolerable in a drug that might have to be administered life long. Given the severity of the disease fairly significant side effects might be tolerated.

9.7 Schedule of Study Procedures

No study procedures should be performed prior to the signing of the informed consent form. The VC should be performed first at each visit so as not to fatigue the volunteer with other testing. For visits requiring a pre-dose trough lithium level, subjects will hold the morning dose of study medication until after the blood draw. For visits requiring a fasting glucose level, subjects will fast for at least 8 hours (water and noncaloric liquids will be allowed) prior to the test. All subjects will sign an informed consent form prior to undergoing any study tests or procedures.

9.7.1 Visit 1 – Screening Visit

The following procedures will be performed at the Screening Visit:

- Obtain informed consent
- Assess inclusion and exclusion criteria
- Obtain medical history and demographics
- Review and document concomitant medications and therapies
- Measure vital signs including height and weight
- Perform VC (slow vital capacity)
- Perform 12-lead ECG
- Collect blood samples for clinical laboratory assessments including blood for serum pregnancy test for women of childbearing potential
- Collect blood samples for serum lithium level
- Perform physical and neurological examinations
- Schedule next Study Visit within 21 days

9.7.2 Visit 2 - Baseline Visit

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

Assess inclusion and exclusion criteria (Note: Baseline Visit VC is not exclusionary, even if below 60%)

Measure vital signs including weight

Review and document concomitant medications and therapies

Document AEs

Perform VC

Administer ALSFRS-R questionnaire

Administer QOL questionnaire (ALSSQOL)

Administer QIDS-SR₁₆ questionnaire

Randomize volunteer

Dispense study medication (1-month supply) and dosing diary and administer first dose of the study drug (Day 0).

Schedule next Study Visit in 28 ± 3 days

9.7.3 Visit 3 - Week 4

This visit will take place approximately 28 ± 3 days after Visit 2. The following procedures will be performed:

Measure vital signs including weight

Review and document concomitant medications and therapies

Document AEs

Check compliance and dosing diary

Perform VC

Administer ALSFRS-R questionnaire

Collect blood sample for pre-dose trough serum lithium level

Dispense study medication (1-month supply)

Schedule next Study Visit in 28 ± 3 days

9.7.4 Visit 4 – Week 8

This visit will take place approximately 28 ± 3 days after Visit 3. The following procedures will be performed:

Measure vital signs including weight

Review and document concomitant medications and therapies

Document AEs

Check compliance and dosing diary

Perform VC

Administer ALSFRS-R questionnaire

Collect blood sample for pre-dose trough serum lithium level

Collect blood samples for clinical laboratory assessments

Dispense study medication (1-month supply)

Schedule next study visit in 28 ± 5 days

9.7.5 Visit 5 – Week 12

This visit will take place approximately 28 ± 3 days after Visit 4. The following procedures will be performed:

Measure vital signs including weight
Review and document concomitant medications and therapies
Document AEs
Check compliance and dosing diary
Perform VC
Administer ALSFRS-R questionnaire
Administer QIDS-SR₁₆ questionnaire
Collect blood sample for pre-dose trough serum lithium level
Dispense study medication (2-month supply)
Schedule next study visit in 28 ± 5 days which will be by telephone

9.7.6 Phone Interview – Week 16

This phone call will take place approximately 28 ± 5 days after Visit 5/Week 12. The following will be performed:

Review and document concomitant medications and therapies
Document AEs
Administer ALSFRS-R questionnaire
Schedule next study visit in 28 ± 5 days

9.7.7 Visit 6 – Week 20

This visit will take place approximately 28 ± 5 days after the Week 16 Phone Call. The following procedures will be performed:

Measure vital signs including weight
Review and document concomitant medications and therapies
Document AEs
Check compliance and dosing diary
Perform VC
Administer ALSFRS-R questionnaire
Collect blood sample for pre-dose trough serum lithium level
Collect blood samples for clinical laboratory assessments
Dispense study medication (2-month supply)
Schedule next study visit in 28 ± 5 days which will be by telephone

9.7.8 Phone Interview – Week 24

This phone call will take place approximately 28 ± 5 days after Visit 6/Week 20. The following will be performed:

Review and document concomitant medications and therapies
Document AEs
Administer ALSFRS-R questionnaire
Schedule next study visit 28 ± 5 days

9.7.9 Visit 7 – Week 28

This visit will take place approximately 28 ± 5 days after the Week 24 Phone Call. The following procedures will be performed:

Measure vital signs including weight
Review and document concomitant medications and therapies
Document AEs
Check compliance and dosing diary
Perform VC
Administer ALSFRS-R questionnaire
Administer QOL questionnaire
Collect blood sample for pre-dose trough serum lithium level
Collect blood samples for clinical laboratory assessments including fasting blood glucose
Dispense study medication (2-month supply)
Schedule next study visit in 28 ± 5 days which will be by telephone

9.7.10 Phone Interview – Week 32

This phone call will take place approximately 28 ± 5 days after Visit 7/Week 28. The following will be performed:

Review and document concomitant medications and therapies
Document AEs
Administer ALSFRS-R questionnaire
Schedule next study visit in 28 ± 5 days

9.7.11 Visit 8 – Week 36

This visit will take place approximately 28 ± 5 days after the Week 32 Phone Call. The following procedures will be performed:

Measure vital signs including weight
Review and document concomitant medications and therapies
Document AEs
Check compliance and dosing diary
Perform VC
Administer ALSFRS-R questionnaire
Administer QIDS-SR₁₆ questionnaire
Collect blood samples for clinical laboratory assessments
Collect blood sample for pre-dose trough serum lithium level
Dispense study medication (2-month supply)
Schedule next study visit in 28 ± 5 days which will be by telephone

9.7.12 Phone Interview – Week 40

This phone call will take place approximately 28 ± 5 days after Visit 8/Week 36. The following will be performed:

Review and document concomitant medications and therapies
Document AEs
Administer ALSFRS-R questionnaire
Schedule next study visit in 28 ± 5 days

9.7.13 Visit 9 – Week 44

This visit will take place approximately 28 ± 5 days after the Week 40 Phone Call. The following procedures will be performed:

- Measure vital signs including weight
- Review and document concomitant medications and therapies
- Document AEs
- Check compliance and dosing diary
- Perform VC
- Administer ALSFRS-R questionnaire
- Collect blood samples for clinical laboratory assessments
- Collect blood sample for pre-dose trough serum lithium level
- Dispense study medication (2-month supply)
- Schedule next study visit in 28 ± 5 days which will be by telephone

9.7.14 Phone Interview – Week 48

This phone call will take place approximately 28 ± 5 days after Visit 9/Week 44. The following will be performed:

- Review and document concomitant medications and therapies
- Document AEs
- Administer ALSFRS-R questionnaire
- Schedule next study visit in 28 ± 5 days

9.7.15 Visit 10 – Week 52

This visit will take place approximately 28 ± 5 days after the Week 48 Phone Call. The following procedures will be performed:

- Measure vital signs including weight
- Review and document concomitant medications and therapies
- Document AEs
- Collect study medication and dosing diary and check compliance
- Perform VC
- Administer ALSFRS-R questionnaire
- Administer QOL questionnaire
- Administer QIDS-SR₁₆ questionnaire
- Collect blood samples for clinical laboratory assessments including fasting blood glucose
- Collect blood sample for pre-dose trough serum lithium level
- Record subject's best guess on whether initially randomized to lithium or placebo arm
- Schedule next study visit in 28 ± 5 days which will be by telephone

9.7.16 Week 56 – Follow-up Phone Interview

This call will take place approximately 28 ± 5 days after Visit 10/Week 52. The following will be performed:

- Review and document concomitant medications and therapies
- Document AEs

Administer ALSFRS-R questionnaire

9.7.17 Final Safety Visit

Measure vital signs including weight

Review and document concomitant medications and therapies

Document AEs

Collect study medication and dosing diary and check compliance

Perform VC

Administer ALSFRS-R questionnaire

Administer QOL questionnaire

Administer QIDS-SR₁₆ questionnaire

Collect blood samples for clinical laboratory assessments

Collect blood samples for pre-dose trough serum lithium level

Record subject's best guess on whether initially randomized to lithium or placebo arm

Schedule next visit in 28 ± 5 days per study protocol

All volunteers who discontinue study medication early will be encouraged to follow per study protocol regardless of their compliance with the assigned treatment. If they are unable to return for in-person visits, the scheduled in-person visits can be replaced by telephone interviews. Telephone Interviews will occur approximately every 4 weeks (every 28 ± 5 days) up to Week 56. The first Follow-up Telephone Call will occur at the next scheduled study visit ± 5 days after the Final Safety Visit. The following procedures will be performed via telephone up to Week 56:

Review and document concomitant medications and therapies

Document AEs

Administer ALSFRS-R questionnaire

Assess medical status

Visit windows for early discontinuation Follow-Up Telephone Calls are ± 3 days for Weeks 4 – 12, ± 5 days for Weeks 16 – 56. Visit windows are consecutive calendar days and are calculated from the day study medication is started; the day of the Baseline Visit for all volunteers.

9.7.18 Efficacy Variables

The primary efficacy variable is time to failure, defined as at least a 6-point drop in the overall ALSFRS-R score from the baseline value or death. Failure notification will come from the EDC core to the Drug Monitor after a participant's overall ALSFRS-R score demonstrates a 6-point drop. The Drug Monitor will then communicate with the Drug Distributor and participants initially assigned to placebo will then be switched to lithium starting with the next scheduled in-person study visit. Participants on lithium will continue taking active compound. The blind will be maintained as all medication bottles will be coded and distributed to participants at each visit by an unblinded Drug Distributor as directed by the Drug Monitor.

The secondary efficacy variables are:

Tracheostomy-free Survival
Pulmonary function (VC)
ALS Functional Rating Scale Revised Questionnaire (ALSFRRS-R)
Assessment of quality of life (QOL): ALS-Specific QOL Questionnaire
Quick Inventory of Depressive Symptomatology Self Report Questionnaire (QIDS-SR₁₆)

9.7.19 Safety Variables

The primary safety variables are:

Adverse events (AEs)
Treatment discontinuations due to AEs (tolerability)
Vital signs
Weight/BMI
Clinical laboratory test results (including lithium levels)
Physical examinations
Concomitant medication requirements

9.8 Data Monitoring Committee and Statistical and Analytical Plans

9.8.1 Data and Safety Monitoring Board

An independent Data and Safety Monitoring Board (DSMB) will be formed, and will be responsible for periodic evaluations of the clinical trial data to ensure continued study volunteer safety as well as the scientific validity of the study. The DSMB will receive listings and data summaries to review for planned periodic teleconference meetings approximately every six months throughout the study. In addition, the DSMB will receive SAE data monthly for safety review. In the event of a perceived need based on these data, the DSMB members will contact the Chair who will call an “ad hoc” meeting.

A separate Data and Safety Monitoring Plan will provide the details about the DSMB.

9.8.2 Analysis Populations

9.8.2.1 Intent-to-Treat Population

The intent-to-treat (ITT) population will include all study volunteers who are randomized and receive at least one dose of study medication. The ITT population will be considered for primary efficacy analyses. Analyses on this population will group volunteers according to randomized treatment, regardless of treatment actually received.

9.8.2.2 Safety-Analysis Population

The safety-analysis population will include all study volunteers who receive at least one dose of study medication.

9.8.3 Missing Data

The trial will be intent to treat. We will get follow up information for all participants whether or not they continue on treatment. If a participant is lost to follow up they will be censored in the primary analysis. A secondary sensitivity analysis will consider them to be failures when they stop follow up.

9.8.4 Baseline and Demographic Characteristics

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

Demographic and baseline characteristics will be compared between the treatment groups using Fisher's exact test for categorical variables, or a t-test for continuous variables. Any parameters showing statistically significant differences between treatment groups will be considered for inclusion in supplementary analyses on the primary endpoint.

9.8.5 Analysis of Primary Efficacy Variable

There will be 125 participants on riluzole/lithium, and 125 participants on riluzole/placebo. All participants will be treated for 52 weeks. The primary endpoint of the trial (event) is time to failure defined as drop in the ALSFRS-R score by 6 points or more or death. Participants in the placebo/riluzole group whose ALSFRS-R score drops by 6 points will be treated with lithium from that time.

9.8.6 Secondary Analyses and Analysis of Secondary Efficacy Variables

The secondary analysis will include a comparison of the rate of decline of ALSFRS-R and Slow VC of participants randomized to begin treatment on lithium as compared to participants treated on placebo and a comparison of tracheostomy-free survival compared to the historical controls from the NEALS trial database. In addition, the rate of ALSFRS-R decline in the placebo group will be compared before and after initiation of lithium. For each of these secondary endpoints, our primary analysis strategy will be to use a random effects analysis using PROC MIXED in SAS and LME in R. Here, the basic idea is that each patient has his or her own trajectory with a random slope, intercept and curvature, the average value of which may depend on treatment. Since the patient groups start out the same, the intercept will not depend on treatment but the slope and curvature may. This study contains many subjects who will eventually cross over from the placebo/riluzole group to the riluzole/lithium group, thus we will use a random effects model that has a change point and allows for the comparison of the crossover effect versus the treatment effect. Such a model makes use of available data on all the participants including those who have transferred from placebo to lithium.

Further analysis will compare the mean changes over time (from Baseline to 52 weeks) in responses to selected questions from the ALS-Specific Quality of Life Questionnaire for the group of subjects treated with riluzole/lithium to the group treated with riluzole/placebo, excluding those subjects originally in the placebo/riluzole group at any time point who have crossed over to receive lithium. The mean changes in the summary values of the QIDS-SR 16 ratings over time (Baseline, Weeks 12, 36, 52) will be compared for the group of participants treated with riluzole/Lithium to those treated with

riluzole/Placebo. Again, those subjects originally in the placebo/riluzole group at any time point who have crossed over to receive lithium will be excluded.

9.8.7 Analysis of Safety and Tolerability Variables

The safety data will be summarized according to treatment group. Adverse events will be coded using the National Cancer Institute's (NCI) Common Terminology Criteria for Coding Adverse Events (CTCAE) version 3.0. This system has been adapted for use in ALS trials by NEALS for the current clinical trial of ceftriaxone in ALS. The treatment groups will be compared with respect to occurrence of AEs. Total number of AEs, AEs that cause study medication withdrawal and abnormal laboratory tests will be compared between groups using Fisher's exact test. The p-values associated with these tests will be for explorative purposes only.

Adverse events, withdrawal due to an AE (tolerability), serum lithium levels, abnormal findings on laboratory testing, physical examinations, vital signs, weight/BMI, and use of concomitant medications will be assessed to characterize the safety profile of lithium in combination with riluzole in volunteers with ALS. Compliance data will be determined for each visit and by treatment group. The time to study volunteer refusal (voluntary withdrawal) will be compared between treatment groups to assess tolerability. This will be accomplished using inverse probability of censoring weighted (IPCW) log-rank tests, which account for informative censoring due to death [118]. Descriptive statistics denoting the changes from baseline to the final assessment visit with respect to key laboratory parameters and vital signs will also be provided.

9.8.8 Determination of Sample Size

In this trial, the null hypothesis is that there is no difference in the true rates of decline of the ALSFRS-R between the group of subjects taking placebo/riluzole and the group of subjects taking lithium/riluzole. The alternative hypothesis is that there is a difference in the true rate of decline of the ALSFRS-R between the group of subjects taking lithium/riluzole and the group of subjects taking placebo/riluzole. The trial is designed to have over 80% power, that is, over an 80% chance of detecting a 40% decreased rate of decline in the treatment group if in fact it exists, using the stopping rules for the interim analyses described in the detailed Interim Analysis Plan separately.

9.8.9 Interim Analysis

We will use a group sequential design that establishes the time points for the interim analyses according to a mathematical function that is proportional to the number of events that have occurred. We expect a total of 167 events to occur in the trial. The first interim analysis ("first look") will occur when 84 participants are accrued to the trial. At that time, one of the following four decisions will be made (see figure below):

1. To stop the trial for futility.
2. To stop accrual and continue follow up on the 84 patients for a total treatment duration of 6 month and then repeat analysis.
3. To continue accrual until the next interim analysis.
4. To stop the trial for efficacy.

If option (2) is selected, the second interim analysis will occur six months after the 84th patient has accrued. If option (3) is selected, the second interim analysis will occur after there are 55 events. At the second interim analysis (the “second look”), one of three decisions will be made:

1. To stop the trial for futility.
2. To continue accrual and look again after 100 events have occurred. This would be the last interim analysis. The final analysis will then occur after 250 participants have had 12 months of follow up.
3. To stop the trial for efficacy.

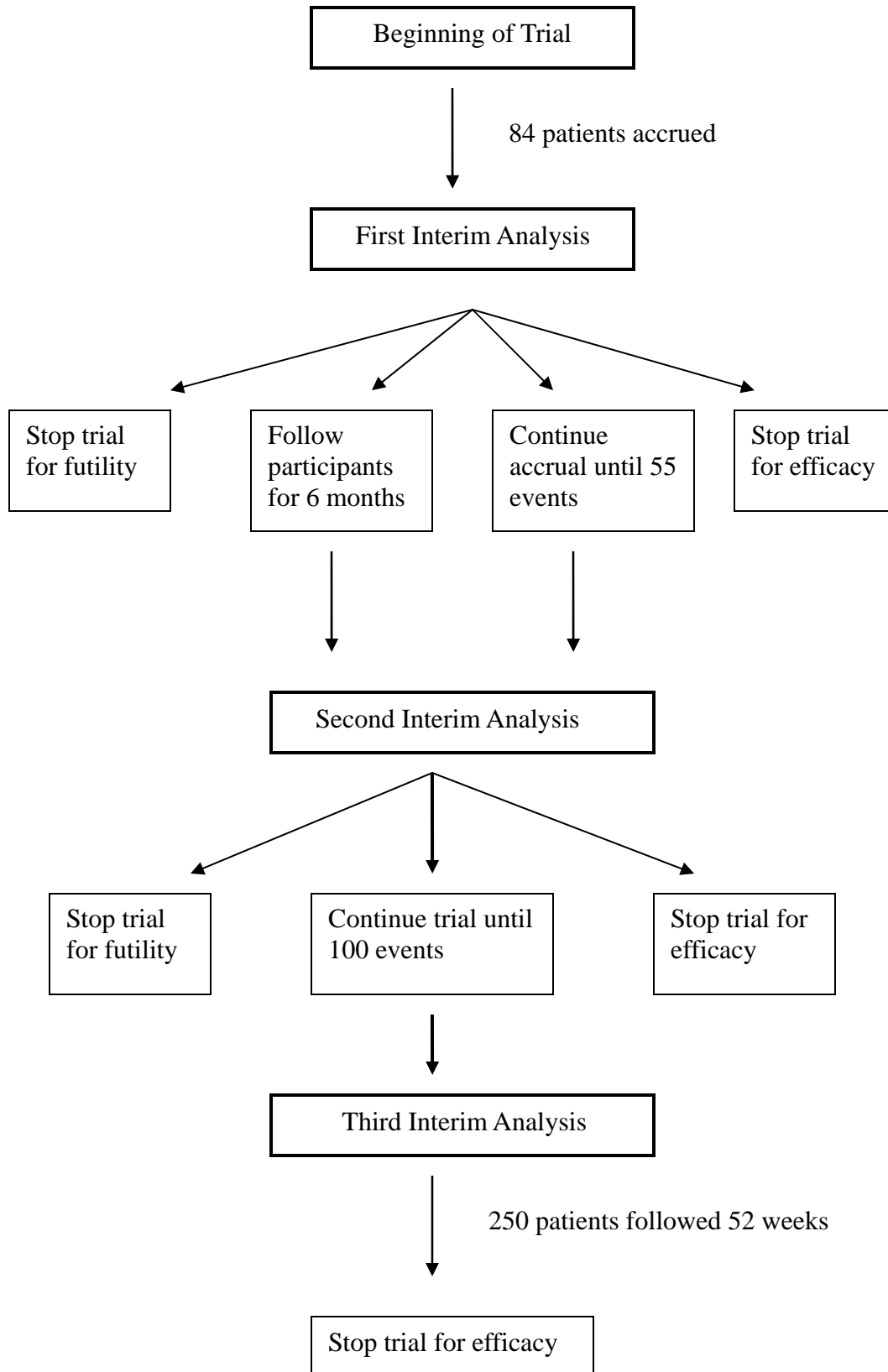
The stopping rules (used for determining how to proceed at each interim analysis) will be based on the monitoring method described in "Group Sequential Methods with Applications to Clinical Trials", Jennison C, Turnbull BW, Chapman & Hall/CRC, NY 2000. pp 145-169. At each interim analysis, the log-rank statistic is calculated based upon the number of events that have occurred so far. The stopping rules (for efficacy and futility) are then determined from two mathematical functions called “alpha spending function” and the “beta spending function” respectively. The alpha function determines the cutoff p-value at each interim analysis for declaring the efficacy of the trial, and specifies how the type I error of the study (i.e. the probability of stating an effect as significant when it is not) will be spent over the course of the trial in the interim analyses. The beta function is used to determine the futility of the trial and specifies how the type II error of the study (i.e. finding no difference between the two groups when in fact there is a difference) should be spent over the course of the trial in the interim analyses. These methods allow a great deal of flexibility in determining when the interim analyses are to be carried out.

At the first interim analysis, the following alpha and beta spending functions will be used:

$$\begin{aligned}\text{alpha spending function} &= (\text{number of events}/167)^2 * 0.025, \\ \text{beta spending function} &= (\text{number of events}/167) * 0.2\end{aligned}$$

The alpha spending function is used as a p-value to determine if the trial should be stopped for efficacy. The stopping rule for futility is chosen so that the probability of stopping for futility is at most the beta spending function described above, under the assumption that the treatment is effective (produces a 40% improvement in the rate of decline of the ALSFRS-R). For the second interim analysis, the stopping rules, are based on the same alpha and beta spending functions, that specify the probability of stopping on or before the second interim analysis for efficacy or futility respectively. However they are dependent on the results from the first interim analysis, and more specialized calculations are required to calculate these. At the third (and final) interim analysis, we choose a final p-value such that the overall chance of declaring efficacy is 0.025 that is equivalent to a two-sided p-value = 0.05.

Figure 9.8.9.1: Interim Analysis Plan



10.0 INVESTIGATOR REQUIREMENTS

10.1 Study Initiation

The following documentation must be received by the respective Site Management Center or their representative prior to initiation of the trial at each site:

- Current (signed and dated) curricula vitae of the site Principal Investigator and all Sub-Investigators.
- Institutional Review Board (IRB) membership list and/or Department of Health and Human Services number.
- Written documentation of IRB approval of protocol (identified by protocol number or title and date of approval) and informed consent document (identified by protocol number or title and date of approval).
- A copy of the IRB-approved informed consent document.
- Written documentation of IRB approval of any advertising materials to be used for study recruitment and all documents to be provided to volunteers must be provided to the Site Management Centers or their representative. The informed consent form and any advertising materials must also be reviewed and approved by the Site Management Centers or their representative.
- Current laboratory certification of the central laboratory performing the analysis (issuing agency and expiration date), as well as current normal laboratory ranges for all laboratory tests.
- A signed Clinical Research (Protocol) Agreement.
- Certified translations of IRB approval letters, pertinent correspondence, and approved informed consent document (when applicable).
- Original signed and dated Financial Disclosure Forms for the site Principal Investigator and all Sub-Investigators.
- NEALS certification for all site Evaluators for ALSFRS-R and VC.

10.2 Study Completion

The following data and materials are required by the Site Management Centers or their representative before the study can be considered complete or terminated:

- All available test results from screening through the end of the study (e.g., clinical data, all special test results).
- eCRFs properly completed by appropriate study personnel.
- Completed drug accountability records.
- Copies of protocol amendments and IRB approval/notification, if appropriate.
- A summary of the study prepared by the site Principal Investigator (an IRB summary closure letter is acceptable).

10.3 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 volunteers.
- Sponsor terminates the study.
- Study volunteer enrollment is unsatisfactory.
- Data recording is inaccurate or incomplete.

10.4 Informed Consent

Sample informed consent documents will be provided to the sites. No major deviations should be made from the sample informed consent form. The site-specific informed consent form shall be reviewed and approved by the respective Site Management Center or their representative prior to submission to the site IRB. The final IRB-approved document must be provided to the respective Site Management Center or their representative for regulatory purposes.

Only a licensed physician site Principal Investigator/Sub-Investigator may obtain informed consent for this study. Study staff members who are not licensed physicians will not be permitted to obtain consent.

The informed consent form must be signed and dated by the study volunteer or the study volunteer's legal guardian before starting his or her participation in the study. A copy of the informed consent document must be provided to the study volunteer or the study volunteer's legal guardian. If applicable, it will be provided in a certified translation of the local language (English, Spanish or French (Canadian) only).

Original signed and dated consent forms must remain in each volunteer's study file and must be available for verification by Study Monitors at any time.

10.5 Adverse Events

The adverse event (AE) definitions and reporting procedures provided in this protocol comply with all applicable U.S. FDA, Health Canada and ICH guidelines and regulations. The Medical Monitor assigned by the Coordination Centers shall promptly review all information relevant to the safety of an investigational new product received from any source. The Site Investigator will carefully monitor each volunteer throughout the study for possible adverse events. 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 drug being studied, whether serious or non-serious.

10.5.1 Definitions

An AE is any untoward medical occurrence in a volunteer administered a pharmaceutical product, regardless of causality assessment. An AE can therefore be any unfavorable and

unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product. Any worsening (i.e., any clinically significant adverse change in frequency and/or intensity) of a preexisting condition, which is temporally associated with the use of the investigational product, is also an adverse event.

For the purposes of this study, symptoms of progression/worsening of ALS, including 'normal' progression, will be recorded as adverse events. Whether or not an AE is ALS related will be recorded on the AE CRF. The following measures of disease progression will not be recorded as adverse events even if they worsen (they are being recorded and analyzed separately): vital capacity results, ALSFRS-R ratings, ALS QOL questionnaire ratings and QIDS-SR₁₆ ratings. Elective PEG tube/g-tube/feeding tube placement will not be considered an SAE even if the hospitalization is 24 hours or greater, as elective surgery is not considered an 'untoward' medical occurrence. Elective feeding tube placements will be recorded as 'Key Study Events' in the EDC.

A serious adverse event (SAE) is any untoward medical occurrence that occurs at any dose (including overdose):

- Is fatal.
This serious criterion applies if the study volunteer's death is a direct outcome of a reported AE.
- Is life threatening.
This serious criterion applies if the study volunteer, in the view of the Site Investigator, is at substantial risk of dying from the AE as it occurs. It does not apply if an AE hypothetically might have caused death if it were more severe.
- Requires or prolongs inpatient hospitalization.
This serious criterion applies if the reported AE requires at least a 24-hour inpatient hospitalization or, if in the opinion of the Site Investigator, prolongs an 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 a "procedure" or a "treatment" is not an untoward medical occurrence.
- Results in permanent or significant disability/incapacity.
This serious criterion applies if the "disability" caused by the reported AE results in a substantial disruption of the volunteer's ability to carry out normal life functions.
- Is a congenital anomaly/birth defect.
This serious criterion applies if a study volunteer exposed to a medicinal (investigational) product gives birth to a child with a congenital anomaly or birth defect.

Medical and scientific judgment should be exercised in deciding whether expediting reports is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize

the volunteer or may require intervention to prevent one of the other outcomes listed in the definition above. These will usually be considered serious.

Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

A non-serious AE is any untoward medical occurrence that does not meet any of the aforementioned criteria for SAEs.

10.5.2 Evaluating and Recording of Adverse Events

At each visit all adverse events that are observed, elicited by the Site Investigator, or reported by the study volunteer, will be recorded in the appropriate section of the Adverse Event CRF and evaluated by the Site Investigator.

Minimum information required for each AE includes type of event, duration (start and end dates), severity, seriousness, causality to study drug, action taken, and outcome.

The Site Investigator using the following criteria as a guideline will grade the severity of AEs:

1. Mild: Nuisance, barely noticeable.
2. Moderate: Uncomfortable, troublesome symptoms not significantly interfering with daily activities or sleep.
3. Severe: Symptoms significantly interfere with daily activities or sleep.

The relationship of the AE to the study drug should be specified by the Site Investigator, 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 study drug, and/or a more likely alternative etiology exists.
3. Possibly Related: The reaction follows a reasonably temporal sequence from administration of the drug and follows a known response pattern to the suspected drug; the reaction could have been produced by the study drug or could have been produced by the volunteer's clinical state or by other modes of therapy administered to the volunteer.
4. Probably Related: The reaction follows a reasonable temporal sequence from administration of study drug; is confirmed by discontinuation of the study drug or by rechallenge; and cannot be reasonably explained by the known characteristics of the volunteer's clinical state.
5. Definitely Related: The reaction follows a reasonable temporal sequence from administration of study medication; that follows a known or

expected response pattern to the study medication; and that is confirmed by improvement on stopping or reducing the dosage of the study medication, and reappearance of the reaction on repeated exposure.

If discernible at the time of completing an AE CRF, a specific disease or syndrome rather than individual associated signs and symptoms should be identified by the Site Investigator and recorded on the appropriate AE CRF. However, if an observed or reported sign, symptom, or clinically significant laboratory anomaly is not considered by the Site Investigator to be a component of a specific disease or syndrome, then it should be recorded as a separate AE on the appropriate AE CRF (clinically significant laboratory abnormalities are those that are identified as such by the Site Investigator and/or those that require intervention). The only exception to this will be ALS progression symptoms as previously noted.

10.5.3 Reporting of Adverse Events

1. Serious Adverse Events

Any SAEs, including death due to any cause, which occur to any volunteer entered into treatment in this study, whether or not considered related to the investigational product (IP), must be reported within 24 hours of awareness to the MGH Project Management Center. Site Investigators must record and report SAEs that occur in all volunteers, including those who discontinue study medication early, up until the final Follow-Up Telephone Call; the Week 56 Follow-Up Telephone Call (13 months \pm 5 days). The Medical Monitor and DSMB will review all SAE reports. Sites will be instructed to submit these to their IRBs as well. Any SAE that results in the study being put on hold at any site will be reported to all site IRBs, the NIH, the FDA, and Health Canada, as applicable. All unexpected and IP related SAEs will be reported to Health Canada by the Canadian Site Management Center in an expedited manner, as required by Health Canada and ICH guidelines. Safety reporting in the U.S. will be in accordance with all applicable FDA and ICH Guidelines for marketed drugs under an IND-exempt clinical investigation. All sites will follow their respective IRB policies with respect to safety reporting. Any adverse experiences will be followed for resolution until the final follow-up visit (the Week 56 Telephone Call for volunteers who complete the study).

2. Other Reportable Events

- Pregnancy

Although not considered an AE, it is the responsibility of the Site Investigator or their designees to report any pregnancy in a volunteer (spontaneously reported to them) that occurs during the study or within 14 days of completing the study. All volunteers who become pregnant must be followed to the completion/termination of the pregnancy. If the pregnancy continues to term, the outcome (health of infant) must also be reported to the MGH Project Management Center.

10.6 Case Report Forms

The CRFs will be supplied by the Data Management Center and should be handled in accordance with the instructions provided.

10.7 Study Drug Accountability

The Central Research Pharmacy at the University of Rochester will provide all study drug required for this study. The recipient will acknowledge receipt of the drug indicating shipment content and condition. Damaged supplies will be replaced. Accurate records of all study drug dispensed from and returned to the study site will be maintained.

All partially used or empty containers should be retained until drug monitoring is completed. Unopened, expired, or unused study drug shall be returned as directed by the Coordination Centers or their representative.

10.8 Confidentiality of Data

Study volunteer medical information obtained by this study is confidential, and disclosure to third parties other than those noted below is prohibited. Upon the volunteer'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 volunteer confidentiality of data will be adhered to.

Data generated by this study must be available for inspection by representatives of the US FDA, Health Canada, NIH, all pertinent national and local health authorities, the Coordination Centers or their representative, and the IRBs.

10.9 Retention of Records

US FDA regulations (21 CFR 312.62[c]) require that records and documents pertaining to the conduct of this study and the distribution of investigational drug, including CRFs (if applicable), consent forms, laboratory test results, and medical inventory records, must be retained by the site Principal Investigator for 2 years after marketing application approval. If no application is filed, these records must be kept for 2 years after the investigation is discontinued and the US FDA and the applicable national and local health authorities are notified. The Coordination Centers or their representative will notify the site Principal Investigator of these events. The Principal Investigator should retain all study documents and records until they are notified in writing by the Study Sponsor or their representative.

10.10 Protocol Adherence

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

11.0 DATA COLLECTION AND SITE MONITORING

11.1 Project Organization

The study is a collaboration between the NEALS and CALS consortiums. The study will be conducted at multiple sites across the United States and Canada. The Coordination Centers for this study consist of a Project Management Center, and two Site Management Centers. The Project Management Center led by Dr. Swati Aggarwal will be located at the Neurology Clinical Trials Unit at Massachusetts General Hospital and will be in charge of IND application and communication with the FDA and sponsor, trial oversight and SAE management. There will be individual Canadian and United States Site Management Centers that will work closely for seamless project management. The Canadian center led by Dr. Lorne Zinman (University of Toronto) will be responsible for oversight of Canadian site recruitment, communication, Health Canada approval, site IRB approval and assisting with volunteer recruitment and retention. The United States site management center, led by Dr. Petra Kaufmann (Columbia University) will be responsible for oversight of US site recruitment, communication, and assisting with volunteer recruitment and retention. The NEALS Data Management Center will be the Data Management Center for the entire study, including creation and management of the Electronic Data Capture System (EDC). Outcome measure training, compliance, and study monitoring for the entire study will be provided by the NEALS Outcome Measures Center at SUNY Upstate Medical University under the direction of Dr. Jeremy Shefner. The three Study Leaders will communicate via telephone conference approximately weekly.

Drs. Cudkowicz and Shefner, as members of the Steering Committee, and Directors of the Coordination Center/ Data Management Center and Outcome Measures/ Study Monitoring Centers respectively, will be available to advise and provide mentorship to the leaders of the trial. They will also participate in the leadership conference calls and Steering Committee meetings.

The Steering Committee is composed of a diverse group of ALS experts across the US and Canada. Steering Committee members will participate in monthly conference calls during the study start-up period and then approximately every 3 months once the study begins.

A contact information database will be created and maintained that will be used for group/individual mailings of paper documents and to facilitate telephone and fax communications between the Project Management Center and Site Management Centers and clinical sites. A Web portal will be established to provide the study personnel with an easily accessible repository of documents, which is essential for sharing information across multiple clinical trial sites. The secure, password-protected portal will contain role-based resources for study personnel. For example, the portal provides personnel lists with contact information, the complete study protocol, model informed consent forms and other study-related documents, a list of subject accrual figures that is updated continuously to allow anyone in the group to monitor study progress, a list of frequently asked questions for quick reference, and other resources that the Clinical Investigators and Coordinators might find useful.

11.2 Role of Data Management

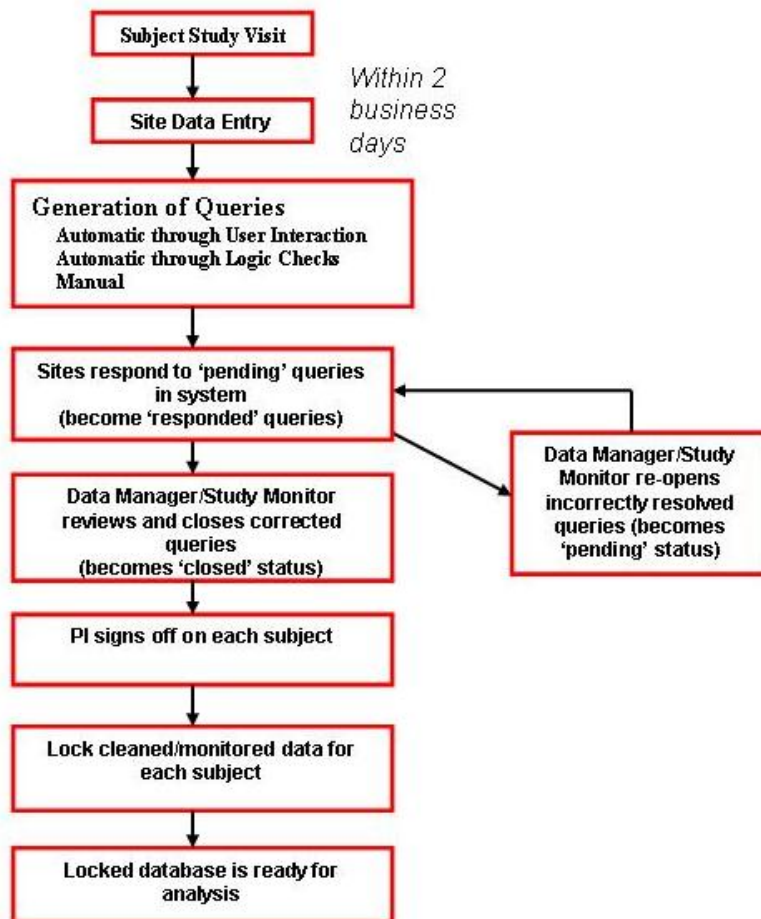
11.2.1 Data Flow and Management Overview

The Data Management staff at the Neurology Clinical Trials Unit (NCTU) at MGH will be responsible for all aspects of data acquisition and processing, from the design of instruments used to collect data through the delivery of an accurate and timely database to the Biostatistics Center. The Data Management staff at MGH will design the electronic case report forms (eCRFs) that will capture all the data collected as part of the protocol. Interactive computer modules for real-time capture of all important study events including enrollments, serious adverse events (SAEs), reportable incidents and premature withdrawals will be created. The Electronic Data Capture and Data Management System (EDC & DM) will be tested and validated to ensure accuracy, reliability and consistent intended performance. The system conforms to the 21 CFR Part 11 and other guidance documents on computerized systems in clinical trials. The system will allow the study sites to single-enter the data via an Internet browser-based interface. The entered data will be saved in a Microsoft SQL Server database located on a server maintained by Partners Healthcare Systems IS department. Necessary documentation on the validation procedure will be maintained by Data Management staff at the MGH Project Management Center. A Data Manager will be responsible for the data acquisition and management at the MGH Project Management Center.

The flow of data and the data clarification process is summarized in Figure 10.1. The site personnel are instructed to enter information within 48 hours of the visit. 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 Web-based forms (eCRFs) to provide immediate error checking of the data entered. The system will automatically create electronic queries on behalf of the Data Manager if saved electronic Case Report Forms (eCRFs) contain data that are out of range, out of window, missing or not calculated correctly. The Data Manager identifies the errors in the EDC system by using electronic logic checks and the Study Monitor identifies errors by direct visualization and comparison of data entered into the system with the source documents. In addition, a medical reviewer will be appointed and will be responsible for reviewing all adverse events and corresponding concomitant study data remotely via EDC for accuracy and consistency. Any inconsistent or questionable data points are queried to the sites and followed up on by both the Study Monitor and Data Manager as needed. Once the site addresses the query, the Data Manager or the Study Monitor can verify that the response is satisfactory, and that the value in the data field has been corrected. The Data Manager or Study Monitor can then “close” the query in the database. The sites and Study Monitor will only have access to the queries concerning their subjects. The Data Manager will be able to see the queries for the entire subject population. The sites will have the ability to write a comment for a form or field. Each comment has its own history, which is recorded in a log. The log records the information that has been entered in the comment, who entered it, and when it was entered. The comment field is treated as a regular data field; hence all changes to the comments are tracked in the audit trail. We propose to use the National Cancer Institute’s (NCI) Common Terminology Criteria for Coding Adverse Events (CTCAE) version 3.0. This is a descriptive terminology, organized by body system and including specific criteria for

grading severity of Adverse Events. This system will allow study staff to quickly search for the most relevant term for each event and will give specific criteria governing the reporting of severity for each term. With this system, the event will be coded at the site and subsequently checked by the Data Manager. The system will have predefined roles and system Administrators will assign them to the system users. Depending on the role assigned, the users will differ not only in their rights to enter or view certain data, but also in their rights to access certain forms and views. These will be described fully in the EDC system functional specification document section of the Manual of Operations.

Figure 11.1 Data Flow and Clarification



11.2.2 Database Security

The MS SQL Server database is located on a secure database server. This server is located in a restricted area of the Partners Healthcare server farm and physical access to it is limited to authorized personnel only. Both database and Web servers are located on the Partners Healthcare network behind the firewall. Access to the data at the clinical site will be restricted and monitored through the system's software with its required log-on,

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

In addition, the EDC system will have an extra level of password security. At study initiation, the Data Manager will set default passwords for the relevant study personnel at the MGH Project Management Center, the two Site Management Centers, the Outcome Measures and Study Monitoring Center, and at the study sites. When a new user logs in with the assigned username and default password for the first time, he or she will be forced to change the password to a unique one (at least six characters long), known only to the user. An ongoing paper log will be kept showing when usernames and passwords are set up, for whom, in what user capacity and when usernames are disabled. In case an employee forgets her/his password or a new user is added, they will submit a password request form via email to the Data Manager, who will issue a new default password. They must then go through the Change Password process. The passwords will expire every three months, then users will be required to go through the Change Password process. To avoid password-based software attacks, the system will lock a user for 1 minute if an incorrect password is provided 3 times in a row. A user will also be able to change the password at will if he or she feels that it may have been compromised.

11.2.3 Data Lock Process

The application will have the ability to lock the database to prevent any modification of data once the study is closed. Once this option is activated, every user will have Read-Only access to the data. Throughout the study, the Study Monitors will be verifying the source documents against the database. The Study Monitors will review source documents and electronic case report forms for accuracy and completeness as described in the study-monitoring plan. The Data Manager can only lock the database once the following steps occur: the Site Investigator has signed off on each subject, the Study Monitor has verified the subject's data, and all queries have been resolved. The database will be transferred to the Biostatistics Center by unloading the relational MS SQL Server database to a SAS format for statistical analysis. The database will also be accessible to biostatisticians for reporting and statistical analysis during the trial.

11.3 Quality Assurance

Steps to be taken to assure the accuracy and reliability of data include the selection of qualified Site Investigators and appropriate study centers, review of protocol procedures

with the Site Investigator and associated personnel prior to the study, and periodic monitoring by the Study Monitors. Case report forms will be reviewed for accuracy and completeness by Study Monitors during on-site monitoring visits and any discrepancies will be resolved with the Site Investigator or designees, as appropriate. The data will be entered by site personnel into the clinical trial database and verified for accuracy.

11.4 Study Monitoring

Study Monitors, supervised by the Director of Monitoring (Dr. Jeremy Shefner), will visit each study site at least twice annually to review source documentation materials, informed consent forms, and confirm entered data and that data queries have been accurately completed. Study Monitors will also verify that Serious Adverse Events and protocol violations have been reported appropriately to the respective Coordination Centers and their local IRB as required. [Serious Adverse Events will be reported to the MGH Project Management Center and protocol exceptions and violations will be reported to the respective Site Management Centers]. The Study Monitors will also review clinical facilities resources and procedures for evaluating study participants and study medication dispensing. Subsequently, the Study Monitors will provide reports of protocol compliance to the Study Leaders and the Steering Committee. Completed informed consent forms from each participant must be available in the participant's file and verified for proper documentation. A document outlining the monitoring plan will be provided to each Study Monitor.

11.5 Drug Monitoring

The Drug Monitor at the NEALS Outcome Measure Center, SUNY Upstate Medical University will closely monitor the lithium levels. The Drug Monitor will receive all lithium levels from the central laboratory, determine appropriate dosage adjustments if serum lithium levels fall outside of the target window of 0.4-0.8 mEq/L, and communicate with the Site Investigators. If serum lithium levels exceed 1.5 mmol/L the Drug Monitor will contact the Site Investigator, who will contact the participant and instruct him/her to hold study drug for at least 2 days after which the daily dosage will be reduced by 300 mg or more, or permanently discontinued if needed, per the instructions of the Drug Monitor.

Any adjustments to the dosage will not be made without approval from the Drug Monitor. The Drug Monitor should always be consulted for any dosage adjustments or re-challenge of a participant in relation to AEs considered to be related to study drug.

Each placebo treated participant will be paired with a participant on lithium at another site. For every dosage adjustment for a participant assigned to lithium, equivalent sham dosage modifications shall be made to the placebo treated participant paired with this particular lithium treated participant to maintain blinding. The Drug Monitor will be alerted via email through the EDC if participants' ALSFRS-R scores drop by 6 points or more. The Drug Monitor will then advise the Drug Distributor regarding the switch from placebo to lithium for participants initially assigned to placebo.

12.0 HUMAN SUBJECTS

12.1 Inclusion of Women

The gender distribution for subjects with sporadic ALS is approximately 60% male and 40% female. The study goal is to recruit men and women with ALS in a 3:2 ratio. The MGH patient population includes 53% men and 47% women. Subjects at participating sites will be recruited from those site-specific areas and their surrounding communities. Special efforts will be employed to recruit female subjects. Advertising the study with several ALS and motor neuron disorder (MND) foundations will aid in the recruitment process, and in particular with the recruitment of female subjects.

The sites in the proposed study have demonstrated the ability to enroll females in prior NEALS and industry-sponsored studies. In the NEALS multi-center topiramate trial in ALS, 36% of the subjects were female. Similarly in the NEALS clinical trial of creatine in ALS and in the clinical trial of celebrex in ALS, 39% and 36% of the subjects were female, respectively. Based on this information, we do not anticipate difficulty enrolling the number of females for this study that represents the percentage of females with ALS in the total population.

12.2 Inclusion of Minorities

ALS is a relatively rare disease and there is mixed data on its incidence in minority groups. Most epidemiologic studies of ALS have investigated homogeneous populations. One of the only mortality studies conducted within a large, multi-ethnic population by Annegers et al. (1991) found that age- and sex-adjusted mortality did not differ among ethnic groups [119]. Some studies suggest that ALS may be less frequent in non-white individuals. An incidence study in the state of Washington found rates in white males to be 1.8 per 100,000 compared to 0.74 per 100,000 per year for black males, although the difference was not statistically significant due to the small numbers of incident cases [4]. Dr. Kasarskis recently determined the rates of ALS in whites, blacks and other racial groups in a cohort of US soldiers serving during the Gulf War I era. They concluded that the rate for ALS/MND in "Other racial groups" (mainly Hispanic) was significantly elevated compared to whites in this young, predominantly male cohort whereas the overall rate for blacks was 33% lower than the specific rates estimates [120].

Approximately 17% of the national population is of a non-white racial background. For the purposes of recruitment in this study we will assume a racial distribution similar to the overall US population. Ethnically, 12.5% of the national population is Hispanic or Latino (<http://www.census.gov>). Thus, the study goal is to enroll at least 12.5% of the subjects from this ethnic group. Approximately 13% of our nation's population is Black, 0.3% is Hawaiian/Pacific Islander, 4.2% is Asian, and approximately 1.5% is American Indian. Our goal is to enroll ALS subjects that are representative of the nationwide demographics. The racial composition of MGH is 82% White, 3% Black, 1% Asian, and 12% unknown. The ethnic population of MGH is 3% Hispanic and 97% non-Hispanic. For geographical reasons, much of the local minority population does not have the MGH as its primary care facility in Boston.

To reach the recruitment goal, efforts will be focused on enrollment of individuals with ALS who are members of underrepresented minorities. Recruitment of members of minority groups will be closely monitored. Participants will be recruited from the clinic population of the participating sites and through contact with support groups and healthcare providers from surrounding areas. IRB approval will be obtained for all planned letters, newsletters and web advertisements.

Potential study participants will not be excluded from this study for reasons of race or gender and efforts will be made to enroll in representative numbers with respect to both gender and race. In particular, no racial discrimination will be made in participant enrollment. The participation of minority subjects will be actively encouraged throughout the study.

The Study Leaders will emphasize the significance of a balanced recruitment effort with each Site Investigator, as well as conduct reviews of recruitment rates. The Study Leaders will contact sites with low minority enrollment and provide methods of improved recruitment.

13.0 PUBLICATION OF RESEARCH FINDINGS

The Study Leaders, Drs. Swati Aggarwal, Lorne Zinman, Petra Kaufmann and the Steering Committee will be responsible for publication of results from this trial. The Study Leaders and Steering Committee will comply with NINDS guidelines on publication of NIH funded clinical trials. Their responsibilities 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

Submitted to Biomedical Journals (International Committee of Medical Journal Editors, 1997).

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15.0 APPENDICES

15.1 Appendix I: El Escorial World Federation of Neurology Criteria for the Diagnosis of Amyotrophic Lateral Sclerosis (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, electrophysiological or neuropathologic examination,

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

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

B - The absence of:

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

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

CLINICAL STUDIES IN THE DIAGNOSIS OF ALS

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

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

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

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

D. Clinically Possible ALS is defined when clinical signs of UMN and LMN dysfunction are found together in only one region or UMN signs are found alone in two or more regions; or LMN signs are found rostral to UMN signs and the diagnosis of Clinically 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

15.2 Appendix II: World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
and the 52nd WMA General Assembly, Edinburgh, Scotland, October 2000
Note of Clarification on Paragraph 29 added by the WMA General Assembly, Washington 2002
Note of Clarification on Paragraph 30 added by the WMA General Assembly, Tokyo 2004

A. INTRODUCTION

1. The World Medical Association has developed the Declaration of Helsinki as a statement of ethical principles to provide guidance to physicians and other participants in medical research involving human subjects. Medical research involving human subjects includes research on identifiable human material or identifiable data.
2. It is the duty of the physician to promote and safeguard the health of the people. The physician's knowledge and conscience are dedicated to the fulfillment of this duty.
3. The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."
4. Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.
5. In medical research on human subjects, considerations related to the well-being of the human subject should take precedence over the interests of science and society.
6. The primary purpose of medical research involving human subjects is to improve prophylactic, diagnostic and therapeutic procedures and the understanding of the aetiology and pathogenesis of disease. Even the best proven prophylactic, diagnostic, and therapeutic methods must continuously be challenged through research for their effectiveness, efficiency, accessibility and quality.

7. In current medical practice and in medical research, most prophylactic, diagnostic and therapeutic procedures involve risks and burdens.
8. Medical research is subject to ethical standards that promote respect for all human beings and protect their health and rights. Some research populations are vulnerable and need special protection. The particular needs of the economically and medically disadvantaged must be recognized. Special attention is also required for those who cannot give or refuse consent for themselves, for those who may be subject to giving consent under duress, for those who will not benefit personally from the research and for those for whom the research is combined with care.
9. Research Investigators should be aware of the ethical, legal and regulatory requirements for research on human subjects in their own countries as well as applicable international requirements. No national ethical, legal or regulatory requirement should be allowed to reduce or eliminate any of the protections for human subjects set forth in this Declaration.

B. BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH

10. It is the duty of the physician in medical research to protect the life, health, privacy, and dignity of the human subject.
11. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and on adequate laboratory and, where appropriate, animal experimentation.
12. Appropriate caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.
13. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol. This protocol should be submitted for consideration, comment, guidance, and where appropriate, approval to a specially appointed ethical review committee, which must be independent of the investigator, the sponsor or any other kind of undue influence. This independent committee should be in conformity with the laws and regulations of the country in which the research experiment is performed. The committee has the right to monitor ongoing trials. The researcher has the obligation to provide monitoring information to the committee, especially any serious adverse events. The researcher should also submit to the committee, for review, information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest and incentives for subjects.

14. The research protocol should always contain a statement of the ethical considerations involved and should indicate that there is compliance with the principles enunciated in this Declaration.
15. Medical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given consent.
16. Every medical research project involving human subjects should be preceded by careful assessment of predictable risks and burdens in comparison with foreseeable benefits to the subject or to others. This does not preclude the participation of healthy volunteers in medical research. The design of all studies should be publicly available.
17. Physicians should abstain from engaging in research projects involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians should cease any investigation if the risks are found to outweigh the potential benefits or if there is conclusive proof of positive and beneficial results.
18. Medical research involving human subjects should only be conducted if the importance of the objective outweighs the inherent risks and burdens to the subject. This is especially important when the human subjects are healthy volunteers.
19. Medical research is only justified if there is a reasonable likelihood that the populations in which the research is carried out stand to benefit from the results of the research.
20. The subjects must be volunteers and informed participants in the research project.
21. The right of research subjects to safeguard their integrity must always be respected. Every precaution should be taken to respect the privacy of the subject, the confidentiality of the patient's information and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.
22. In any research on human beings, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail. The subject should be informed of the right to abstain from participation in the study or to withdraw consent to participate at any time without reprisal. After ensuring that the subject has understood the information, the physician should then obtain the subject's freely-given

informed consent, preferably in writing. If the consent cannot be obtained in writing, the non-written consent must be formally documented and witnessed.

23. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship with the physician or may consent under duress. In that case the informed consent should be obtained by a well-informed physician who is not engaged in the investigation and who is completely independent of this relationship.
24. For a research subject who is legally incompetent, physically or mentally incapable of giving consent or is a legally incompetent minor, the investigator must obtain informed consent from the legally authorized representative in accordance with applicable law. These groups should not be included in research unless the research is necessary to promote the health of the population represented and this research cannot instead be performed on legally competent persons.
25. When a subject deemed legally incompetent, such as a minor child, is able to give assent to decisions about participation in research, the investigator must obtain that assent in addition to the consent of the legally authorized representative.
26. Research on individuals from whom it is not possible to obtain consent, including proxy or advance consent, should be done only if the physical/mental condition that prevents obtaining informed consent is a necessary characteristic of the research population. The specific reasons for involving research subjects with a condition that renders them unable to give informed consent should be stated in the experimental protocol for consideration and approval of the review committee. The protocol should state that consent to remain in the research should be obtained as soon as possible from the individual or a legally authorized surrogate.
27. Both authors and publishers have ethical obligations. In publication of the results of research, the investigators are obliged to preserve the accuracy of the results. Negative as well as positive results should be published or otherwise publicly available. Sources of funding, institutional affiliations and any possible conflicts of interest should be declared in the publication. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

28. The physician may combine medical research with medical care, only to the extent that the research is justified by its potential prophylactic,

diagnostic or therapeutic value. When medical research is combined with medical care, additional standards apply to protect the patients who are research subjects.

29. The benefits, risks, burdens and effectiveness of a new method should be tested against those of the best current prophylactic, diagnostic, and therapeutic methods. This does not exclude the use of placebo, or no treatment, in studies where no proven prophylactic, diagnostic or therapeutic method exists. See footnote.
30. At the conclusion of the study, every patient entered into the study should be assured of access to the best proven prophylactic, diagnostic and therapeutic methods identified by the study. See footnote.
31. The physician should fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study must never interfere with the patient-physician relationship.
32. In the treatment of a patient, where proven prophylactic, diagnostic and therapeutic methods do not exist or have been ineffective, the physician, with informed consent from the patient, must be free to use unproven or new prophylactic, diagnostic and therapeutic measures, if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, these measures should be made the object of research, designed to evaluate their safety and efficacy. In all cases, new information should be recorded and, where appropriate, published. The other relevant guidelines of this Declaration should be followed.

Note: Note of clarification on paragraph 29 of the WMA Declaration of Helsinki

The WMA hereby reaffirms its position that extreme care must be taken in making use of a placebo-controlled trial and that in general this methodology should only be used in the absence of existing proven therapy. However, a placebo-controlled trial may be ethically acceptable, even if proven therapy is available, under the following circumstances:

- Where for compelling and scientifically sound methodological reasons its use is necessary to determine the efficacy or safety of a prophylactic, diagnostic or therapeutic method; or
- Where a prophylactic, diagnostic or therapeutic method is being investigated for a minor condition and the patients who receive placebo will not be subject to any additional risk of serious or irreversible harm.

All other provisions of the Declaration of Helsinki must be adhered to, especially the need for appropriate ethical and scientific review.

Note: Note of clarification on paragraph 30 of the WMA Declaration of Helsinki

The WMA hereby reaffirms its position that it is necessary during the study planning process to identify post-trial access by study participants to prophylactic, diagnostic and therapeutic procedures identified as beneficial in the study or access to other appropriate care. Post-trial access arrangements or other care must be described in the study protocol so the ethical review committee may consider such arrangements during its review.

The Declaration of Helsinki (Document 17.C) is an official policy document of the World Medical Association, the global representative body for physicians. It was first adopted in 1964 (Helsinki, Finland) and revised in 1975 (Tokyo, Japan), 1983 (Venice, Italy), 1989 (Hong Kong), 1996 (Somerset-West, South Africa) and 2000 (Edinburgh, Scotland). Note of clarification on Paragraph 29 added by the WMA General Assembly, Washington 2002.