Safety of Urate Elevation in ALS – Phase II (SURE-ALS2)

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Funding Sponsor:	The Salah Foundation MGH cure ALS Fund
Study Product:	Inosine
Draft or Version Number:	V3.0

July 1, 2019

SITE INVESTIGATOR AGREEMENT

Protocol #: Version 3.0
July 1, 2019
IND #124,653

Title: Safety of Urate Elevation in ALS – Phase II (SURE-ALS2)

I have carefully read this protocol including all appendices and agree that it contains all the necessary information for conducting the study safely.

I will conduct this study in strict accordance with this protocol and according to the current Good Clinical Practice (GCP) regulations and guidelines and local and national regulatory requirements. Any changes in procedure will only be made if necessary to eliminate immediate hazards or to protect the safety, rights or welfare of subjects.

I will provide copies of the protocol and all other information relating to the pre-clinical and prior clinical experience, which were furnished to me, to all physicians and other study personnel responsible to me who participate in this study. I will discuss this information with them to assure that they are adequately informed regarding the study drug and conduct of the study.

I will ensure that the drugs supplied to me for this study will be used only for administration to subjects enrolled in this study protocol and for no other purpose.

I agree to keep records on all subjects and study information (case report forms, informed consent statements, drug shipment, drug return forms, and all other information collected during the study) in accordance with the current GCP, local and national regulations.

Print Site Name: _____

Print Site Investigator Name: _____

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

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LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
ALS	Amyotrophic Lateral Sclerosis
ALSFRS-R	Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised
CFR	Code of Federal Regulations
CFR	Code of Federal Regulations
CIB	Clinical Investigator's Brochure
CNS	Central Nervous System
CRF	Case Report Form
CSF	Cerebrospinal Fluid
C-SSRS	Columbia Suicide Severity Rating Scale
DBP	Diastolic Blood Pressure
DSMB	Data and Safety Monitoring Board
eCRF	Electronic Case Report Form
FDA	Food and Drug Administration
FRAP	Ferric Reducing Antioxidant Power
FWA	Federal-wide Assurance
GCP	Good Clinical Practice
GSH	Glutathione
HIPAA	Health Insurance Portability and Accountability Act
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IDE	Investigational Device Exemption
IND	Investigational New Drug Application
IRB	Institutional Review Board
ITT	Intent to treat
KO	Knockout
MOP	Manual of Procedures
MS	Multiple Sclerosis
N	Number (typically refers to subjects)
NDA	New Drug Application
NIH	National Institutes of Health
Nrf2	Nuclear factor erythroid 2–related factor 2
OHRP	Office for Human Research Protections
OHSR	Office of Human Subjects Research
PD	Parkinson's disease
PHI	Protected Health Information
PI	Principal Investigator

QA	Quality Assurance
QC	Quality Control
ROS	Reactive Oxygen Species
SAE	Serious Adverse Event/Serious Adverse Experience
SBP	Systolic Blood Pressure
SI	Site Investigator
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
UOx	Urate Oxidase
US	United States
VC	Vital Capacity
WOCBP	Women of Childbearing Potential

PROTOCOL SUMMARY

Study Title
SAFETY OF URATE ELEVATION IN ALS – PHASE II (SURE-ALS2)

Version Number
3.0

Study Indication
Amyotrophic Lateral Sclerosis (ALS)

Phase of Development
2

Rationale for the Study
Amyotrophic lateral sclerosis (ALS) is a fatal, neurodegenerative disease for which there is no cure. Multiple lines of evidence have implicated oxidative stress in the pathophysiology of ALS. Urate (uric acid) is an endogenous antioxidant system, and urate may serve as a major defense against oxidative stress. Urate has emerged as a promising neuro-protectant and therapeutic target based on convergent epidemiological, laboratory, and clinical data in multiple neurodegenerative diseases, most notably Parkinson's disease (PD). In PD, urate elevation has been pursued as a potential therapy by administration of inosine, a urate precursor that is available as an over-the-counter supplement. Administration of inosine results in a predictable elevation of urate levels and has been shown to be safe and well tolerated in PD.

Analysis of ALS databases revealed that higher urate levels are an independent predictor of slower progression and prolonged survival in ALS. However, whether elevating urate in people with ALS would result in better outcomes is unknown.

We have recently concluded a Pilot Study of Inosine in ALS, which was a short, open label, single center study involving 25 subjects. The study showed safety and feasibility of urate elevation in patients with ALS. We are now pursuing a multi-center Phase II trial to confirm these findings with longer exposure time.

Study Design
This is a multi-center, 20-week study of inosine treatment.

Study Objectives and Endpoints

The primary objective of the study is to determine the safety and tolerability of oral administration of inosine (administered daily) dosed to moderately elevate serum urate over 20 weeks.

The primary outcome measures will be

1- safety, as measured by adverse events
2- tolerability, defined as the ability of subjects to complete the entire 20-week study.

As an exploratory objective, we will test the feasibility and utility of a smartphone application for monitoring symptoms and disease progression in patients with ALS.

Study Location

Up to Three (3) Northeast ALS Consortium (NEALS) Centers in the United States

Number of Planned Subjects

It is expected that approximately 60 subjects will be consented and 30 subjects will be treated in the study.

Study Population

This study will be conducted in subjects who meet the El Escorial criteria of possible, laboratory-supported probable, probable, or definite criteria for a diagnosis of ALS. At screening, eligible subjects must be at least 18 years old and must provide written informed consent prior to screening. Subjects on a stable dose of riluzole and those not taking riluzole, and women of child-bearing age at screening are eligible for inclusion as long as they meet specific protocol requirements. Detailed criteria are described in the body of the protocol.

Treatment Plan

Subjects will be administered oral inosine daily. The dose of inosine will be titrated to obtain serum urate levels of 7 - 8 mg/dL.

Duration of Treatment and Follow-up

Subjects will remain on treatment until the Week 20 visit. Each subject will also have a Week 24 Follow-up Telephone Interview to assess for adverse events (AEs), changes in concomitant medications and to administer the ALSFRS-R.

SCHEDULE OF ACTIVITES

Activity	Screening Visit[1]	Baseline	Week 3, 6 and 9 Phone Call and Urate Test	Week 12	Week 16 Phone Call and Urate Test	Week 20	Week 24 Phone Call	Final Safety Visit (for early study drug discontinuation only)
Visit Names	Visit 1	Visit 2	Visits 3, 4, and 5	Visit 6	Visit 7	Visit 8	Visit 9	
Visit Window	-21 days from Day 0	Day 0	± 3 days	± 3 days	± 3 days	± 3 days	± 3 days	
Written Informed Consent	X							
Inclusion/Exclusion Review	X	X						
Medical History/Demographics	X							
ALS Diagnosis History	X							
Physical Examination	X							
Neurological Exam	X							
Vital Signs[2]/ Height & Weight[3]	X			X		X		X
Screening Labs[4]	X							
C-SSRS		X		X		X		X
Uric acid level[5]		X	X	X	X	X		X
Urine alkalinization protocol (if needed per protocol)		X		X				
Urinalysis[6]		X		X		X		X
Basic Metabolic Panel	X							
Safety Labs[7]		X		X		X		X
ALSFRS-R[8]		X		X		X	X	X
Pulmonary Function Testing[8]	X			X		X		X
Concomitant Medication Review	X	X	X	X	X	X	X	X
Adverse Event Review	X	X	X	X	X	X	X	X
Dispense Study Drug		X		X				
Drug Accountability				X		X		X
Exit Questionnaire						X		X
Mobile app installation[10]		X						
Mobile app un-installation[10]						X		X

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

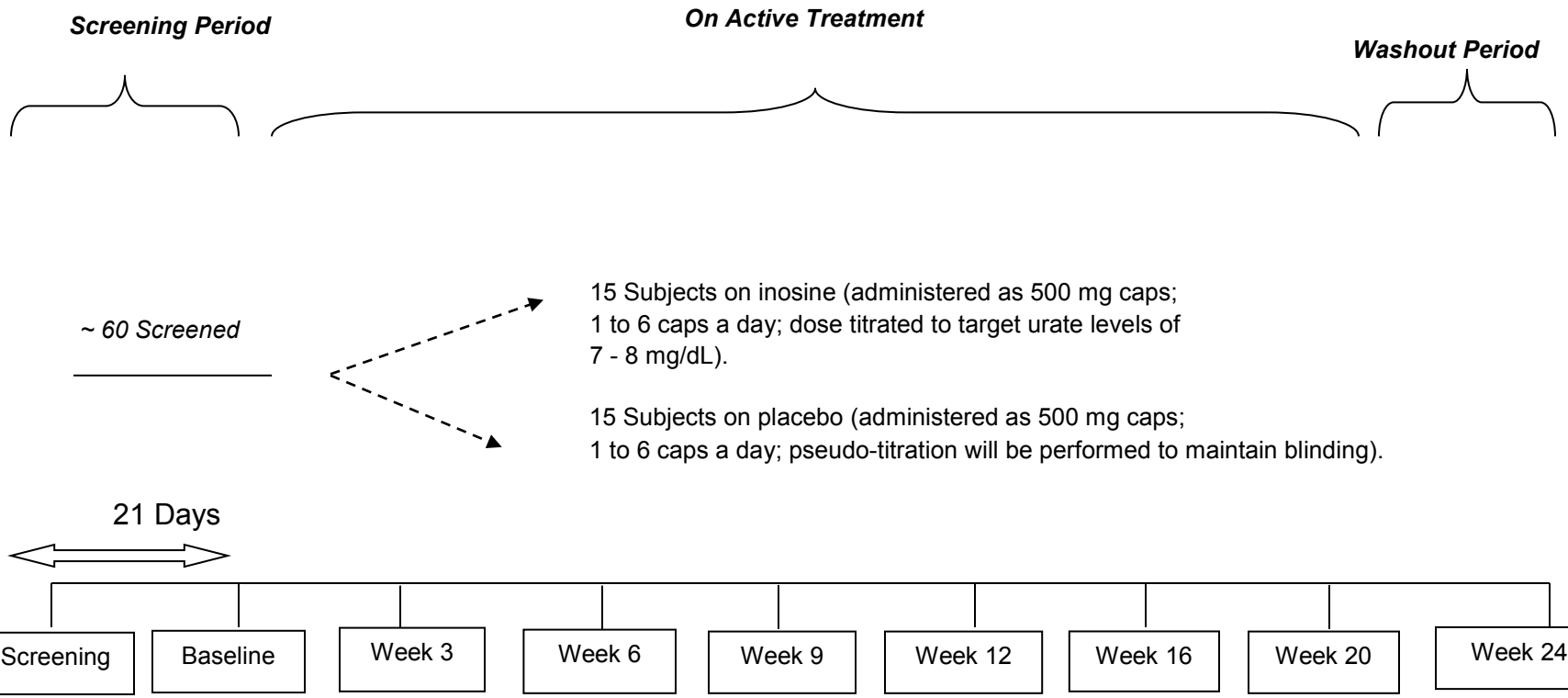
[2] Vital signs include systolic and diastolic pressure in mmHg, respiratory rate/minute, heart rate/minute and temperature.

[3] Height measured at Screening Visit only.

[4] Screening labs include uric acid, urinalysis and Serum pregnancy test (WOCBP) and will be performed at local institution.

[5] Uric acid level will be measured just before the first dose (Baseline visit) or just before scheduled morning dose (trough) (Weeks 3, 6, 9, 12, 16, 20) (Quest Diagnostics)
[6]Urinalysis with sediment (Quest Diagnostics)
[7]Safety labs include Hematology (CBC with differential), Basic Metabolic Panel, Liver Function Tests and will be performed at local institution.
[8]Pulmonary Function Testing includes Slow Vital Capacity (SVC)
[9]Adverse events that occur AFTER signing consent form will be recorded.
[10]App installation and un-installation procedures include a brief survey about phone use

STUDY WORKFLOW



Subjects 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 28 days (+5 days) after taking their last dose of study drug.

1 ETHICS/PROTECTION OF HUMAN SUBJECTS

1.1 Institutional Review Board (IRB)

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

1.2 Ethical Conduct of Study

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

1.3 Subject Information and Consent

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

2 INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

2.1.1 Clinical Features and Epidemiology of ALS

Amyotrophic lateral sclerosis (ALS) is a rare degenerative disorder of large motor neurons of the cerebral cortex, brain stem and spinal cord that results in progressive wasting and paralysis of voluntary muscles[1]. The incidence of ALS is currently approximately 2/100,000[2]. The lifetime ALS risk is 1 in 600 to 1 in 1000[2]. Even though the incidence of ALS is similar to that of multiple sclerosis, the prevalence is only 4- 6/100,000 (about 25,000-30,000 subjects in the United States), due to the higher mortality rate. Fifty per-cent of ALS cases die within three years of onset of symptoms and 90% die within five years[3]. The median age of onset is 55 years[3]. The cause in most cases is unknown[4]. Age and male gender are the only risk factors repeatedly documented in epidemiological studies. No treatment prevents, halts or reverses the disease, although the use of riluzole (the only FDA-approved therapy for ALS) is associated with a slight prolongation of survival[5].

2.1.2 Role of oxidative stress in ALS Pathogenesis

Despite decades of focused research, a unifying and well-tested theory of ALS disease pathophysiology remains enigmatic[4]. At the same time, the body of knowledge has expanded dramatically as research tools have improved. Research increasingly implicates oxidative stress as one of the major molecular mechanisms leading to neuronal death in ALS[6].

Oxidative stress has been implicated in the pathophysiology of ALS since the discovery that mutations in an antioxidant system, the copper/zinc superoxide dismutase (Cu/Zn SOD, SOD1) gene, are associated with 20% of cases of familial ALS[7, 8]. Identification of SOD1 as the first gene linked to ALS pathophysiology has enabled the development of animal models from which much of our understanding of the mechanisms of neurodegeneration in ALS has emerged. These studies have shown that the neurodegenerative processes involved in this disease are diverse and complex including not only oxidative stress, but also excitotoxicity, mitochondrial dysfunction, defects in RNA processing, inflammation, protein aggregation, disruption of neurofilaments and intracellular trafficking along microtubules, as well as involvement of non-neuronal cells in the vicinity of motor neurons[8, 9]. Nevertheless, oxidative stress, whether as a primary cause of disease or a secondary consequence, has been implicated in many of these processes and appears as a central mechanism by which motor neuron death occurs[7].

Despite the compelling evidence supporting a pathogenic role of oxidative stress in ALS, the agents with antioxidant properties studied to date (such as CoQ10 and Vitamin E)[10, 11] in randomized placebo-controlled clinical trials have failed to show benefits on disease progression. This observation highlights the fact that it is important to include assessment of measures of target engagement in the study design of pilot, proof-of-concept studies of candidate compounds in order to demonstrate the intended biological effect in early studies prior to proceeding along the drug development process.

2.2 Rationale for choosing inosine for ALS

Inosine is an intermediary metabolite of purine metabolism, is available as an over-the-counter oral supplement. Inosine is converted into urate, the anionic form of uric acid (Fig. 1).

Urate may be one of our major endogenous defenses against oxidative stress. As an apparent consequence of multiple independent mutations in the *urate oxidase* gene (*UOx*) during primate evolution[12-14], urate circulates at high concentrations near the limits of its solubility (accounting for our susceptibility to gout) and constitutes the end product in the metabolism of purines in humans and apes (Fig. 1). The demonstration that urate possesses antioxidant properties comparable to those of ascorbate[13, 15] and accounts for most of the antioxidant capacity in human plasma[16, 17], supports the hypothesis that our ancestors gained antioxidant benefits from *UOx* mutations and the resulting urate elevation[18, 19].

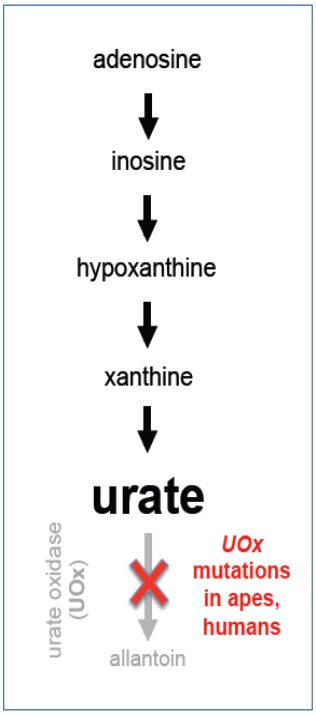


Figure 1.
Purine metabolism

Interestingly, urate has been gaining momentum as a promising agent for neuroprotection based on rapidly accumulating epidemiological observations, laboratory data, and early clinical trial results in multiple neurologic diseases, most notably Parkinson disease (PD)[20, 21]. These lines of evidence (summarized below) demonstrate the potential therapeutic significance of unraveling the molecular mechanisms of urate actions in the CNS and have already prompted clinical development of urate-elevating strategies for PD[21] and stroke[22].

2.2.1 Urate as neuroprotective agent: epidemiological evidence

Urate is a strong molecular predictor for both reduced risk and favorable progression in PD. A meta-analysis of prospective epidemiological data on urate and PD risk suggested a 20% reduction in the pooled rate ratio of PD for each 1.3 mg/dL increase in blood urate concentration[23]. Further, PD disability progressed more slowly among early PD patients with higher urate levels at baseline (Fig. 2). In the PRECEPT clinical trial, the risk of PD disability progressing to the need for dopaminergic therapy among those in the highest quintile of serum urate concentration was half that of the lowest quintile[24]. Similarly, in the DATATOP clinical trial, risk of PD progression was reduced by 18% for each 1.5 mg/dL increase in serum urate concentration with a similar inverse correlation between cerebrospinal fluid (CSF) urate and progression[25].

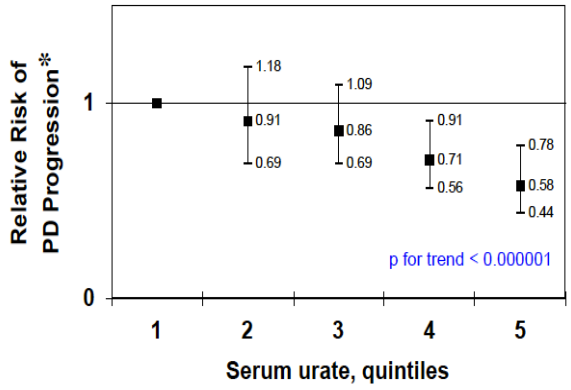


Figure 2. Higher serum urate as a predictor of favorable clinical progression in early PD. Risk of progression is defined as *the hazard ratio of reaching disability sufficient to require dopaminergic therapy among 1,578 *de novo* PD subjects of the DATATOP and PRECEPT clinical trial cohorts. Values (mean and SD) are shown for increasing quintiles of serum urate measured at baseline, and are relative to the lowest quintile. Data are pooled (unpublished data, Schwarzschild MA *et al.*) form our prior publications on PRECEPT and DATATOP cohorts. The 1st, 3rd and 5th quintiles correspond to serum urate values of <4.1, 4.9-5.5 and>6.4 mg/dL, respectively.

Further epidemiological support to a potential pathogenic association between urate and PD is provided by studies showing that both dietary and genetic factors that affect urate levels modify susceptibility to PD. Thus, a higher dietary urate index was associated with a lower risk of PD (top quintile vs. bottom: relative risk = 0.47, p-trend = 0.0008), after adjustment for potential confounders[26] and genetic variability in nine genes known to influence urate levels was found to be associated with PD risk[27]. Higher urate levels have also been correlated with slower clinical progression in Huntington's disease, multiple system atrophy, and mild cognitive impairment[28-30] raising the hypothesis of a broad neuroprotective effect of urate in multiple CNS neuronal populations.

Based on this convergence of data linking higher urate levels with improved outcomes in multiple neurodegenerative diseases, we examined ALS clinical trial data and found that urate levels are an independent predictor of progression and survival in ALS, where higher levels are associated with improved outcomes[31] (Figure 3). In a clinical trial database study of 251 ALS subjects, we found a 39% reduction in risk of death during the study for men, but not women, with each 1 mg/dL increase in urate levels[31].

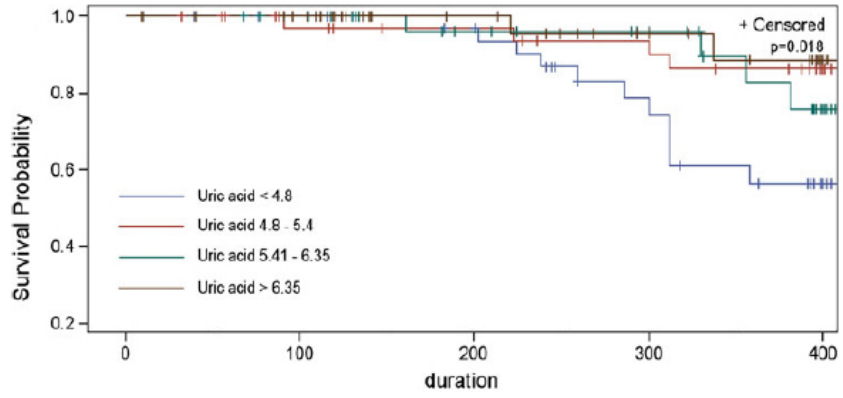


Figure 3. Kaplan–Meier survival curves stratified by uric acid levels.

Censored patients are indicated by crosses on the corresponding survival curve. Survival curves have been truncated at 400 days (when less than five subjects were at risk). Blue line uric acid <4.8 mg/dl; red line uric acid 4.8-5.4 mg/dl; green line uric acid 5.41-6.35 mg/dl; brown line uric acid > 6.35 mg/dl (log-rank p = 0.018).

Similar results were reported by other groups[32-34] and were confirmed and expanded by our recent analysis of individuals from PRO-ACT, the largest available ALS clinical trial dataset (Pooled Resource Open-Access ALS Clinical Trials database)[35]. In PRO-ACT, urate levels at trial entry were available for almost 1,800 individuals and were found to be one of the strongest predictors of disease progression and survival[35]. Thus, higher levels of urate at trial entry were predictive of a slower drop in ALS functional rating scale-revised [ALSFRS-R (p=0.01)] and vital capacity [VC (p<0.0001)], and longer survival (p=0.02) in both men and women ([35] and Paganoni et al., unpublished data).

2.2.2 Urate as neuroprotective agent: biological evidence

Pre-clinical evidence from multiple labs further supports urate as a neuroprotective agent.

In vitro, across a range of PD models, urate has prevented spontaneous degeneration of cultured nigral neurons as well as dopaminergic cell death induced by oxidative and mitochondrial toxins[36-45]. Urate protected dopaminergic neurons in cellular models of PD[36-38]. Interestingly, urate-mediated effects required its accumulation in astrocytes, suggesting a non cell-autonomous mechanism for urate's activity[38, 39]. Urate confers protection in various cellular models of neurotoxicity beyond that of PD. Urate protected cultured spinal cord neurons from glutamate toxicity[39] and from peroxynitrite-mediated death[40] in models of spinal cord injury and stroke[40, 41].

Recently, the Schwarzschild lab has also characterized the effect of urate manipulation *in vivo* in animal models of PD. In mice, administration of urate or a urate precursor does not lead to appreciable increase in urate CNS concentration as urate is metabolized peripherally by the enzyme urate oxidase (UOx). Therefore, mice with a knockout (KO) of the *UOx* gene (effectively were employed. These mice have increased concentrations of urate in the central nervous system (CNS)[36]. Effects of *UOx* manipulation were then assessed in a model of hemiparkinsonism induced by unilateral intrastriatal injection of 6-hydroxydopamine (6-OHDA) (Fig. 4).

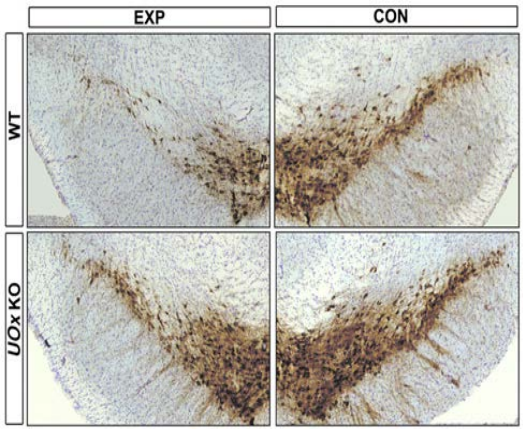


Figure 4. Evidence for neuroprotective effects of urate in an animal model of PD.

UOx KO mice (bottom panels), which have elevated brain urate levels, are resistant to loss of nigral tyrosine hydroxylase (TH)-positive neurons induced by intrastriatal 6-OHDA (EXP panel, bottom left) compared to lesioning in wild-type (WT) littermate mice (upper left panel).

UOx KO mice exhibited attenuated neurotoxic effects of 6-OHDA on nigral dopaminergic cell counts (Fig. 4), striatal dopamine content and rotational behavior[36]. These results strengthened the evidence supporting a neuroprotective role of endogenous urate in neurodegeneration.

2.2.3 Urate as neuroprotective agent: early clinical trial evidence

Indeed, based on the strong pre-clinical evidence of a neuroprotective role for urate in the CNS, recent clinical trials have begun to examine the feasibility of urate manipulation in a clinical setting with promising results[21, 22].

The Safety of Urate Elevation in Parkinson Disease (SURE-PD) trial, a phase II randomized, double-blind, placebo-controlled, dose-ranging trial of oral inosine in PD[21] recently demonstrated that inosine supplementation leads to a dose-dependent increase in blood and CSF urate levels in patients with PD. Inosine is available as an over-the-counter supplement. Although the study was not powered to test efficacy, secondary analyses demonstrated non-futility of inosine treatment for slowing disability[21]. Encouraging clinical results were also recently obtained by the Safety and Efficacy of Uric Acid in Patients with Acute Stroke (URICO-ICTUS) study: a randomized, double-blind trial of intravenous urate administration within a few hours of stroke onset[22].

A follow-up. NIH-funded, Phase 3 study of inosine in PD (SURE-PD3) is currently ongoing).

While urate is a mature target in PD and experience on inosine pharmacology is rapidly accumulating, urate manipulation is a new target for ALS. With this Phase 2 study, we seek to confirm the safety of inosine when administered to people with ALS as well as gather data on target engagement as measured by neuroimaging biomarkers.

2.2.4 Biomarkers of oxidative stress and damage

Oxidative stress arises from an imbalance between the production of oxidizing agents (such as reactive oxygen species, ROS) and the ability of the system to remove the damage they cause and to restore the prevailing reducing environment. Most ROS arise as by-products of aerobic metabolism and central nervous system (CNS) neurons are known to be especially susceptible to oxidative damage[42]. Oxidative stress and the capacity to manage it can be tested in several ways, ranging from direct measurement of the ROS; measurement of the resulting oxidative damage to biomolecules (such as nucleic acids, proteins or lipids); and detection of antioxidant levels (e.g., by measuring a composite index such as ferric reducing antioxidant power [FRAP], or individual antioxidants such as urate, ascorbate or glutathione). Elevated levels of markers of oxidative damage to different biomolecules have been repeatedly demonstrated in postmortem tissue and/or the cerebrospinal fluid (CSF) from ALS patients[1], including elevated protein carbonyl levels, a marker of oxidized proteins[43], and increased 3-nitrotyrosine levels, a marker for nitrosative damage[44] resulting from reactive nitrogen species comparable to ROS. As demonstrated by Dr. Cudkowicz and colleagues at MGH elevated levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG), a well-established marker of oxidized DNA, correlated with disease progression, suggesting it may represent a biomarker of disease severity[45]. Among antioxidant systems, FRAP and urate were found to be lower in ALS subjects compared to controls[34, 46, 47].

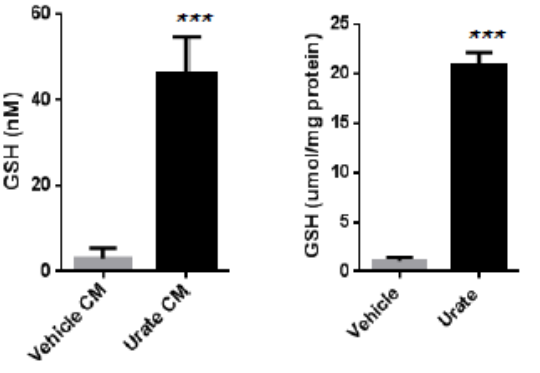
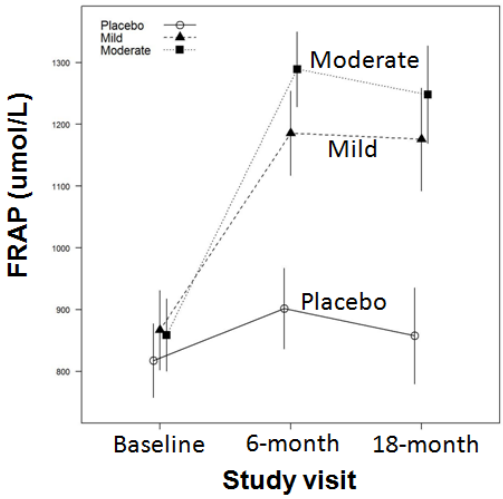


Figure 5. Marked elevation of GSH content in conditioned medium (CM) (**A**) and cell lysates (**B**) from urate- (versus vehicle-) treated astrocyte.*** denotes p value < 0.001; (n=4).

More recently, MRS has enabled the measurement of various metabolites *in vivo* and has been utilized to show that the levels of glutathione are reduced in the motor cortex of ALS patients by about 30%[48]. Altogether, these results suggest that antioxidant capacity is reduced in ALS.

The mechanisms of urate-based neuroprotection are not completely clear. However, recent evidence from the Schwarzschild lab[38] and other labs[49] suggests a role for nuclear factor erythroid 2–related factor 2 (Nrf2) signaling pathway in urate-mediated neuroprotection. In primary astrocytic cultures, urate

treatment induced expression of key Nrf2 target genes[50] including expression of the first enzyme of the glutathione (GSH) biosynthetic pathway. Consistently, urate treatment resulted in up-regulation of GSH synthesis and release (Fig. 5).



Further, results from the SURE-PD trial demonstrated that inosine supplementation leads to increased plasma antioxidant capacity as measured by FRAP (Fig. 6). These findings provide evidence of an intended biological effect of potential therapeutic relevance and suggest that homeostatic mechanisms do not counteract the plasma antioxidant effects of urate elevation.

2.3 Inosine

Elevation of urate levels in humans cannot be achieved by oral administration of urate, possibly due to its degradation by the activity of uricase expressed by the intestinal flora before reaching the circulation[51]. On the other hand, predictable elevation of urate levels can be obtained by administration of the urate precursor inosine.

Inosine (chemical name: hypoxanthine 9-β-D-ribofuranoside) is an intermediary metabolite of purine metabolism (see Fig. 1). Inosine is available as an over-the-counter supplement for oral use.

2.3.1 Potential Risks and Benefits

Inosine's reputation as an athletic performance enhancer has led to its widespread marketing and use as a nutritional supplement. Potential side effects of inosine relate to the resulting elevated urate (uric acid) levels (hyperuricemia). Hyperuricemia has been linked to gout. Gout generally develops in people with serum urate concentrations above 8 mg/dL (the upper limit of the normal range). Hyperuricemia has also been linked to the formation of urate stones in the kidneys or ureters (urolithiasis). Urate urolithiasis account for 5 to 10 percent of urinary tract stones in the United States and Europe. In most urate stone formers the primary pathophysiologic defect, however, is an excessively acidic urine pH rather than hyperuricemia and hyperuricosuria[52]. High urate levels have also been associated with high blood pressure and increased risk for cardiovascular events. However, whether a causal relationship between high urate and cardiovascular disease exists is unclear[53].

Considerable non-clinical human experience on inosine has been gained from the widespread use of inosine as a nutritional supplement and a purported (but unsubstantiated) athletic performance

enhancer. Although inosine use has been generally considered safe in these contexts, exposures are variable in size and poorly documented.

Three exercise physiology studies of oral inosine on athletic performance in humans have been published[54-56]. All three reported that multi-gram doses (up to 10 gm/day) were well tolerated short-term (up to 10 days), but had no demonstrable benefit on athletic performance.

Of relevance to the proposed study, multi-year clinical trials of oral inosine in multiple sclerosis (MS) and Parkinson's disease (PD) at doses comparable to those planned here have been conducted. In these studies, inosine at doses sufficient to chronically elevate urate into the ranges targeted here was well tolerated, with the most consistent AE being the rare development of uric acid urolithiasis.

The MS literature includes 4 peer-reviewed published studies on the use of inosine[51, 57-59]. Inosine has been administered in this population for prolonged periods (up to 1 year) at up to 3 gm/day (divided into 2 or 3 doses daily).

In the study by Spitsin et al.[51], 11 subjects with advanced MS received increasing doses of inosine from 1 to 3 gm/day. Urate levels in blood were monitored every 2 weeks and the daily inosine dose was increased every other week until serum urate levels reached approximately 8 mg/dl. Thereafter, each patient received a daily dose of inosine sufficient to maintain serum urate levels within a range of 7 ± 9 mg/dL for up to a year. No patients experienced symptomatic hyperuricemia or gouty arthritis, and no urate crystals or other abnormalities were reported in urine.

Gonsette et al[57] performed a placebo-controlled trial of inosine in MS. Seventy-nine subjects with MS received inosine for 24 months. The treatment regimen consisted of 1 gm of inosine administered three times a day (initial total daily dose 3 gm daily). Inosine dose was then adapted by the treating neurologist after 1 month and then every 3 months to keep serum urate levels lower than 9 mg/dl in women and lower than 10 mg/dl in men. Particular attention was paid to side effects possibly related to a chronic, asymptomatic hyperuricemia. There were no reports of gout, stroke, or coronary heart disease. Symptoms of renal or ureteral stones were reported in three cases in the experimental group (3.8%) and one case in the placebo group (1.3%). Spontaneous elimination occurred in three cases and one patient had lithotripsy. Of note, in this study there were no efforts to reduce the risk of stone formation through screening of low urine pH, a major risk factor in the formation of urate urolithiasis[52]. In addition, subjects with baseline urate up to 7 mg/dL were allowed into the study with target urate levels up to 9-10 mg/dL (both entry cut-off and target urate levels in the MS study were higher than in the planned study for ALS).

Spitsin, Markowitz and colleagues[58, 59] administered inosine to a group of 16 subjects with MS for one year. The goal of the study was to investigate the effect of chronically elevated urate levels on

blood pressure as well as collect MS-related inflammatory and imaging biomarkers. The inosine dosage was initiated at 1–2 gm per day, and increased by 0.5 g per day at biweekly intervals until the target serum UA levels of 6–9 mg/dL were achieved. For most patients the inosine dose required to maintain target UA levels was between 2 and 3 g per day. Inosine treatment did not affect blood pressure over a one-year period[58]. Four subjects (25%) developed kidney stones which were treated with conservative measures including termination of inosine administration, improved hydration, and pain medication. Exceptionally high serum urate levels were recorded for at least two of these subjects (10–15 mg/dL)[58]. In all patients in whom inosine treatment was stopped, serum UA levels returned to pretrial levels within 3–5 days and no re-occurrence of kidney stones was reported. This included a subject who resumed inosine treatment 2 months after developing a kidney stone[59]. In 1 patient who developed kidney stones, an ultrasound scan revealed the presence of kidney cancer in the other kidney, which was successfully treated surgically[59]. Similar to the study by Gonsette et al.[57], this study did not exclude subjects with acidic urine at baseline[52].

In the recent SURE-PD trial[21], a total of 75 subjects with Parkinson's Disease (mean age, 62 years; 55% women) were randomized to either placebo or inosine titrated to produce mild (6.1-7 mg/dL) or moderate (7.1-8 mg/dL) serum urate elevation and were followed for up to 24 months. Individuals at greatest risk of known effects of increased urate (i.e., those with a history of gout or urolithiasis, or with urine pH ≤5.0, a major risk factor for uric acid urolithiasis) were excluded from enrolling. Treatment was initiated gradually with 1 capsule (500 mg of inosine per capsule) taken 2 times daily for 2 weeks. After the 2- and 4-week visits, participants received up to 2 capsules 2 to 3 times daily, as algorithmically determined by serum urate concentration and treatment group assignment. Target urate levels were achieved between 2 and 4 weeks after initiation of inosine treatment[21]. Serious adverse events (17), including infrequent cardiovascular events, occurred at the same or lower rates in the inosine groups relative to placebo. No participant developed gout and 3 receiving inosine developed symptomatic nephrolithiasis (6%); two were determined to be calcium oxalate stones and one was believed to be a uric acid stone. Despite the increased frequency of nephrolithiasis while receiving inosine, there were no other renal SAEs and renal function measures of glomerular filtration rate and serum creatinine remained unchanged from baseline in all groups. Serial vital signs, serum assays, and electrocardiograms showed no effect of inosine on blood pressure, body mass index, serum glucose and cholesterol levels or electrocardiographic parameters. Treatment was tolerated by 95% of participants at 6 months, and no participant withdrew because of an adverse event. Serum urate rose by 2.3 and 3.0 mg/dL in the 2 inosine groups ($P < .001$ for each) vs. placebo, and cerebrospinal fluid urate level was greater in both inosine groups ($P = .006$ and $<.001$, respectively). Secondary analyses demonstrated non-futility of inosine treatment for slowing disability.

The Phase III SURE-PD3 trial is ongoing and the goal is to enroll 270 subjects with Parkinson's Disease by December 2017.

2.3.2 SAFETY SUMMARY

Inosine is available over-the-counter as an athletic performance enhancer. Considerable non-clinical human experience on inosine has been gained from its widespread use as a nutritional supplement. In this context, inosine has been considered safe, although exposures are variable in size and poorly documented.

Potential side effects of inosine relate to the resulting elevated urate (uric acid) levels (hyperuricemia). Hyperuricemia has been linked to gout (which generally develops in people with serum urate concentrations above 8 mg/dL) and to the formation of urate stones in the kidneys or ureters (urolithiasis). In most uric acid stone formers the primary pathophysiologic defect, however, is an excessively acidic urine pH rather than hyperuricemia[52]. High urate levels have also been associated with high blood pressure and increased risk for cardiovascular events. However, whether a causal relationship between high urate and cardiovascular disease exists is unclear[53].

The safety profile of chronic inosine administration in a human clinical research setting has been well characterized by research studies in MS[51, 57-59] and PD[21]. At doses comparable (or higher) to those planned here, inosine was well tolerated up to 2 years. The most consistent AE was the development of uric acid urolithiasis. In the PD study[21], where people with acidic urine were excluded and target urate levels did not exceed 8 mg/dL (as planned here), the prevalence of urolithiasis was 6%. There were no reports of gout and no excess cardiovascular events relative to placebo in any of these studies.

3 OBJECTIVES

3.1 Study Objectives

The primary objective of the study is to determine the safety and tolerability of oral administration of inosine (administered daily) dosed to moderately elevate serum urate over 20 weeks.

An exploratory objective of the study is to test the feasibility and utility of a smartphone application, for monitoring symptoms and disease progression in patients with ALS.

3.2 Study Outcome Measures

The primary outcome measures will be:

1- Safety, as measured by adverse events;
2- Tolerance will be defined as completion of the 20-week study without permanently discontinuing study drug or suspending study drug for greater than 28 days.

Exploratory outcome measures will be:

1- feasibility of a smartphone application for monitoring ALSFRS-R in patients with ALS;

4 STUDY DESIGN

4.1 Overall Study Design and Plan

During the enrollment period, approximately 60 subjects will be screened at up to three (3) Northeast ALS Consortium (NEALS) Centers in the United States. Approximately 30 of these subjects are expected to be found eligible for study enrollment and will be randomized 2:1 to receive inosine or placebo. Per study protocol, the aim is to elevated serum urate levels only in those subjects whose baseline urate level falls below the predicted median for this population. Approximately 30 subjects will receive inosine or placebo for 20 weeks (administered in the form of 500 mg capsules, 1 to 6 capsules a day for a total daily dose of up to 3 gm). The dose of inosine will be titrated to target urate levels of 7-8 mg/dL based on urate level measurement that will occur at the Week 3, Week 6, Week 9, Week 12 and Week 16 visits. Clinic visits will occur at Screening, Baseline, Week 12, Week 20 and Unscheduled Safety Visits. Telephone visits and remote serum urate testing will occur at Week 3, Week 6, Week 9 and Week 16. There will be a window of ± 3 days for each visit. The Screening Visit must be completed within 21 days of the Baseline Visit. All visit windows are consecutive calendar days and are calculated from the day the participant starts study treatment (Day 0, the day of the Baseline Visit).

4.2 Study Centers

This study will be conducted as a multi-center study at up to three (3) Northeast ALS Consortium (NEALS) Centers in the United States.

4.3 Study Duration

Subjects will remain on treatment until the Week 20 visit. Each subject will also have a Week 24 Follow-up Telephone Interview to assess for adverse events (AEs), changes in concomitant medications and to administer the ALSFRS-R.

4.4 Protocol Adherence

The Site Investigators agree to adhere to the protocol detailed in this document and agree that any changes to the protocol must be approved by the site Institutional Review Board (IRB). The Site Investigator (SI) will be responsible for enrolling only those study subjects who have met protocol eligibility criteria.

5 SELECTION AND WITHDRAWAL OF STUDY POPULATION

5.1 Number of Study Subjects

Approximately 60 subjects will be consented for the study. Any subject who signs consent will be considered enrolled in the study. Screening study procedures will begin after informed consent.

We expect a 50% screen fail rate based on our goal of elevating serum urate levels only in those subjects whose serum urate level falls below the population median. This subpopulation is at greater risk of faster disease progression and can also accommodate an increase in serum urate to higher, but still normal levels, which are known to be associated with slower disease progression[21, 31, 35].

Thirty (30) subjects will receive inosine or placebo for 20 weeks per study protocol and will be followed up to Week 24.

If a subject fails screening, at a minimum, the following information will be captured and entered in the data capture system: inclusion/exclusion criteria, demographics, screening lab results, reason for screen failure.

5.2 Inclusion and Exclusion Criteria

5.2.1 Inclusion Criteria

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

1. Age 18-85.
2. Sporadic or familial ALS diagnosed as possible, laboratory-supported probable, probable, or definite as defined by revised El Escorial criteria (Appendix 1).
3. Slow vital capacity (SVC) ≥ 60% of predicted for age, height, and gender at the Screening Visit.
4. Capable of providing informed consent and following trial procedures.
5. Serum urate < 5.5 mg/dL at screening (i.e. below the population median serum urate levels).
6. Women must not be able to become pregnant (e.g. post menopausal, surgically sterile, or using adequate birth control methods) for the duration of the study and 3 months after study completion. Adequate contraception includes: abstinence, hormonal contraception (oral contraception, implanted contraception, injected contraception or other hormonal (patch or contraceptive ring, for example) contraception), intrauterine device (IUD) in place for ≥ 3 months, barrier method in conjunction with spermicide, or another adequate method.
7. Is able and willing to participate in the Mobile app study procedures.

5.2.2 Subject Exclusion Criteria

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

1. History of urolithiasis.
2. Urine pH < 5.5 at screening (as acidic urine is a major determinant of uric acid urolithiasis).
3. History of gout.
4. History of stroke or myocardial infarction.
5. History of symptomatic coronary artery disease (e.g. angina pectoris) or symptomatic peripheral arterial disease within 1 year prior to Screening.
6. Symptomatic congestive heart failure with a documented ejection fraction below 45%.
7. Poorly controlled arterial hypertension (SBP>160mmHg or DBP>100mmHg at Screening).
8. Women who are pregnant or lactating.
9. The presence of unstable psychiatric disease, cognitive impairment, or dementia that would impair ability of the subject to provide informed consent, according to Site Investigator judgment, or a history of active substance abuse within the prior year.
10. Anything that, in the opinion of the Site Investigator, would place the subject at increased risk or preclude the subject's full compliance with or completion of the study.
11. Use of the following within 30 days prior to Screening: inosine, allopurinol, probenecid, more than 300mg vitamin C daily (note that a subject may take a standard multivitamin up to one tablet or capsule daily). Use of thiazides is permissible as long as the subject is on a stable dose from 1 week prior to Screening.
12. Known hypersensitivity or intolerability to inosine.
13. Renal insufficiency as defined by eGFR < 60 mL/min/1.73m^2 at Screening.

Participation in other investigational trials for the treatment of patients with ALS is allowed.

5.3 Withdrawal

Study drug will be discontinued if:

- Any clinical adverse event (AE), laboratory abnormality, concurrent illness, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the subject.

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

Subjects who withdraw from the study will not be replaced.

5.3.1 Handling of Withdrawals

A subject may choose to discontinue participation in the study at any time. However, the Site Investigator or designee will encourage subjects to continue with follow-up, regardless of their compliance with study drug. If a subject who initiated study drug permanently discontinues study drug, the SI or designee should still encourage subjects to follow the study protocol under the

modified intent-to-treat principle (ITT). These subjects will be encouraged to follow the study visits, off drug, up to the final visit. Loss to follow-up should be prevented whenever possible.

Any subject who is on study drug and needs to begin the use of any prohibited medication, must immediately discontinue use of study drug. Subjects who permanently discontinue study drug should return any unused study drug and complete early study drug discontinuation procedures per protocol.

If a subject wishes to withdraw consent, i.e., withdraw his or her participation in future study procedures, the subject will be asked to delay consent withdrawal to allow for a final safety visit and final safety telephone call. The subject will be asked to return to the study site for a final safety visit within 14 days of asking to withdraw consent. The subject will also be asked to have a final telephone call 28 days (+ 5 days) after taking their last dose of study drug.

In the event a subject no longer wishes to have their personal health information used for the analysis of this study, he or she will notify the site through an authorized letter and his or her data will no longer be included as part of the results for this study.

5.3.2 Termination of Study

This study may be prematurely terminated if, in the opinion of the Principal Investigator or Medical Monitor, there is sufficient reasonable cause.

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

- Determination of unexpected, significant, or unacceptable risk to subjects.
- Enrollment is unsatisfactory.
- Insufficient adherence to protocol requirements.
- Data that is not sufficiently complete and/or evaluable.

6 TREATMENTS ADMINISTERED

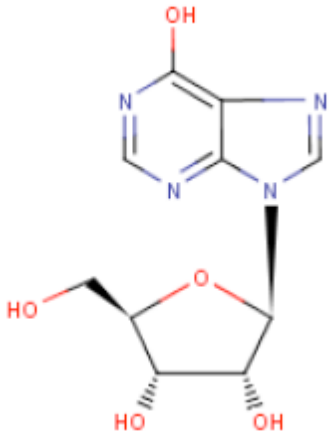
6.1 Treatments

6.1.1 Study Product Description

Inosine (chemical name: hypoxanthine 9-β-D-ribofuranoside) is an intermediary metabolite of purine metabolism (structure shown below) that is ultimately converted into urate.

Chemical Formula: C10H12N4O5
Molecular Weight: 268.23

adenosine → **inosine** → hypoxanthine → xanthine → urate.



Inosine is a white powder. It is provided by a commercial vendor as 500 mg capsules for oral use.

6.1.2 Treatments administered

Inosine will be administered as one capsule twice a day for the first 3 weeks of the study. Inosine dose will be titrated at Weeks 3, 6, 9, 12 and 16 to target urate levels (7 – 8 mg/dL). Inosine will be provided to each subject in individually labeled containers by the site research pharmacy.

The following pre-specified titration algorithm will be used to adjust inosine dose based on urate levels measured at Weeks 3, 6, 9, 12 and 16.

1- Serum urate < 67% increase from most recent level to lower limit of target range (7 mg/dL) ==> increase by 2 caps/day, except:

   - If subject is already on 5 caps/day, then increase by 1 cap/day up to 6 caps/day.
   - If subject is already on 6 caps/day, then continue at this dose.

2- Serum urate 67-less than 100% increase from most recent level to lower limit of target range (7 mg/dL) ==> increase by 1 cap/day, except:

   - If subject is already at 6 caps/day, then continue at this dose.

3- Serum urate within target range (7-8 mg/dL) ==> no change.

4- Serum urate > upper limit of target range (8.1 mg/dL or higher) but ≤ 9.0 mg/dL ==> reduce by 1 cap/day the dose being taken at the most recent visit, except:

   - If subject was already down to 1 cap/day, s/he will continue at 1 cap/day.

5- Serum urate > 9.0 mg/dL ==> hold treatment (i.e., take 0 caps/day) x 7 days, and then resume treatment at 1 less cap/day than the prior dose (i.e., at the most recent visit), except:

   - If that reduction results in no caps being taken, then continue the hold (i.e., 0 caps/day) x 7 more days, then resume treatment at 1 cap/day.

6- If a subject returns 3 consecutive serum urate levels > 9.0 mg/dL, then the study drug will be permanently discontinued due to "excess sensitivity to study drug" (This will not be treated as an adverse event (AE)).

7- If a subject's baseline level is ≥ 7.0 mg/dL, study drug will be held until the Week 3 labs.

6.2 Receiving, Storage, Dispensing

6.2.1 Receipt of Drug Supply

Inosine will be shipped from the central pharmacy to the study research pharmacies.

6.2.2 Storage

The drug supply will be kept at the site research pharmacies in a locked, safe area at controlled room temperature with access limited to those directly involved in the study.

6.2.3 Dispensing of Study Drug

Inosine will be provided to each subject in individually labeled containers by the site research pharmacies. Subject compliance will be determined at the end of the study as detailed below.

6.3 Modification of Study Intervention/Investigational Product for a Subject

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

If the event is serious or life threatening, and deemed to be definitely drug related, the study drug will be discontinued immediately. Study subjects must remain off the study drug permanently. Subjects may not resume study drug. All AEs will be followed to resolution.

6.3.1 Precautionary suspensions

Investigators may approve a brief non-AE-triggered suspension of study drug employed as a medical precaution (e.g., as in a peri-operative suspension of study drug required by the subject's surgeon for an elective procedure, during a day of potential dehydration as in religious fasting, etc).

6.3.2. Dosage Discontinuation

Reasons for discontinuation of study medication may include an AE, Medical Monitor or Principal Investigator recommendation, protocol deviation, loss-to-follow-up, subject request, or death.

Study subjects who discontinue study drug prematurely (early termination from study) will be encouraged to return for a Final Safety Visit and participate in a Follow-Up Telephone Call 28 days (+ 5 days) after the last dose of study drug.

6.4 Accountability Procedures

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

6.5 Prior and Concomitant Therapy

Throughout the study, the subject may be prescribed concomitant medications or treatments deemed necessary to provide adequate supportive care provided that the medications are licensed in the United States. All concomitant medications and/or treatments received by a subject should be recorded on the appropriate source document and eCRF.

6.5.1 Prohibited Medications and Contraindications

Prohibited Medications

Throughout the course of the trial, study subjects should not be treated with the following medications. If, for safety or health reasons, or by subject choice, the prohibited medication cannot be stopped, then the study medication should be stopped.

Prohibited medications include:

- Inosine from a different source
- Allopurinol
- Probenecid
- More than 300mg Vitamin C daily (please note that a daily multivitamin containing vitamin C is allowed)

Pregnancy & Nursing Mothers

There are no adequate and well-controlled studies in pregnant women. Subjects or partners of male subjects should not become pregnant during the study or 3 months after stopping study drug. If a female subject becomes pregnant, study treatment must be discontinued immediately.

It is not known whether inosine is excreted in human milk. Caution should be exercised; therefore, no subject should nurse their infant while participating in this study.

7 STUDY SCHEDULE

No study procedures should be performed prior to the signing of the informed consent form (ICF). All subjects will sign an ICF prior to undergoing any study tests or procedures. The VC should be performed first at the visits so as not to fatigue the subject with other testing.

Visit windows are consecutive calendar days and the target visit dates are calculated from the Baseline Visit.

Subjects who withdraw consent or early terminate from the study will be asked to come in for a Final Safety Visit and will have a Final Telephone Interview 28 days (+5 days) after stopping study drug.

7.1 Screening Visit

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

- Obtain written informed consent from subject
- Assess inclusion and exclusion criteria
- Obtain medical history and demographics
- Obtain ALS diagnosis history
- Perform Pulmonary Function Tests (SVC)
- Perform physical examination
- Perform neurological examination
- Measure vital signs including height and weight
- Collect blood for screening labs
- Review and document concomitant medications and therapies
- Assess and document adverse events (AEs) that occur after subject signs informed consent form (ICF)

7.1.1 Screen Failures

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

- Demographics
- Inclusion and Exclusion criteria
- Reason for screen failure
- Screening labs results

7.2 Baseline Visit

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

- Review inclusion and exclusion criteria
- Review and document concomitant medications and therapies
- Assess and document AEs
- Collect blood samples for clinical laboratory assessments (urate level, safety labs)
- Collect urine for urinalysis
- Administer ALSFRS-R questionnaire
- Perform Pulmonary Function Tests (SVC)
- Columbia Suicide Severity Rating Scale (C-SSRS) – Baseline
- Administer first dose of study drug. The subject should be observed at the site for 15 minutes by an appropriate healthcare staff member according to the site's institutional/state regulations to assess medical status and any immediate reaction to the study drug.
- Dispense study medication and dosing diary
- Subjects will be given a written schedule of the Week 3, Week 6, Week 9 and Week 16 phone calls. They will also be provided with laboratory requisition forms for blood draws at a Quest Diagnostics laboratory. They will be instructed to have the blood draw performed on specific days prior to each phone call as detailed below. Subjects will also be provided with written address and contact information of the Quest Diagnostics laboratory that is closest to their place of residence.
- Mobile app installation.

7.3 Phone Calls

Subjects will be called sometime after the Baseline Visit to remind them to go to a Quest Diagnostics laboratory to have blood draw for urate level assessment per written schedule that they were given at the Baseline Visit. Subjects should have blood drawn in the morning just prior to scheduled dose (trough level).

Week 3 blood draw to monitor urate level will occur 21 ± 3 days after the Baseline Visit.

Week 6 blood draw to monitor urate level will occur 42 ± 3 days after the Baseline Visit.

Week 9 blood draw to monitor urate level will occur 63 ± 3 days after the Baseline Visit.

Week 16 blood draw to monitor urate level will occur 112 days ± 3 days after the Baseline Visit.

The Phone Calls will take place approximately one-seven days after each blood draw and the following procedures will be performed:

- Review results of the urate level test

- Instruct subject to continue the same dose of inosine or modify dose to target urate levels
- Review and document concomitant medications and therapies
- Assess and document AEs

7.4 Week 12 Visit

This visit will take place 84 ± 3 days after the Baseline Visit. This visit should occur in the morning and subjects should hold their morning dose to obtain uric acid trough level. The following will be performed:

- Measure vital signs, including weight
- Collect blood samples for clinical laboratory assessments (urate level, safety labs)
- Collect urine for urinalysis
- Administer ALSFRS-R questionnaire
- Perform Pulmonary Function Tests (SVC)
- Columbia Suicide Severity Rating Scale (C-SSRS) – Since Last Visit
- Review and document concomitant medications and therapies
- Assess and document AEs
- Dispense study medication and dosing diary
- Perform study drug accountability and collect all unused study drug and empty containers

7.5 Week 20 Visit

This visit will take place 140 ± 7 days after the Baseline Visit. This visit should occur in the morning and subjects should hold their morning dose to obtain uric acid trough level. The following will be performed:

- Measure vital signs, including weight
- Collect blood samples for clinical laboratory assessments (urate level, safety labs)
- Collect urine for urinalysis
- Administer ALSFRS-R questionnaire
- Perform Pulmonary Function Tests (SVC)
- Columbia Suicide Severity Rating Scale (C-SSRS) – Since Last Visit
- Review and document concomitant medications and therapies
- Assess and document AEs
- Collect study drug and dosing diary
- Perform study drug accountability and collect all unused study drug and empty containers
- Uninstall mobile app

7.6 Week 24 Phone Call

A follow-up phone call will take place 28 + 5 days after the subject's last dose of study drug. The following will be performed:

- Complete ALSFRS-R Questionnaire
- Review and document concomitant medications and therapies
- Assess and document AEs

7.7 Final Safety Visit

Subjects who early terminate from the study and do not wish to be followed per protocol off study drug will be asked to come in for a Final Safety Visit and will be asked to have a final Follow-Up Telephone Call (+ 5 days, but no earlier than 28 days, after subject's last dose of study drug).

The following will be performed at the Final Safety Visit:

- Measure vital signs, including weight
- Collect blood samples for clinical laboratory assessments (urate level, safety labs)
- Administer ALSFRS-R questionnaire
- Perform Pulmonary Function Tests (SVC)
- Columbia Suicide Severity Rating Scale (C-SSRS) – Since Last Visit
- Review and document concomitant medications and therapies
- Assess and document AEs
- Collect study drug and dosing diary
- Perform study drug accountability and collect all unused study drug and empty containers
- Uninstall mobile app.

The following procedures will be performed via telephone 28 + 5 days after the Final Safety Visit:

- Administer ALSFRS-R questionnaire
- Review and document concomitant medications and therapies
- Assess and document AEs

7.8 Protocol Deviations

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

All deviations from the protocol must be addressed in the subject's source documents. All major protocol deviations will be entered in the Protocol Deviations Log in the Electronic Data Capture (EDC) System and should be submitted to the site's local IRB per institutional guidelines.

7.8.1 Missed Visits and Procedures

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

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

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

7.9 Recording Deaths

Information on whether a subject has died may be obtained by the subject's family, clinic notes, or utilizing public means such as a reliable internet source such as the Centers for Disease Control and Prevention (CDC) National Death Index (*http://www.cdc.gov/nchs/ndi.htm*) or the Social Security Death Index (*http://ssdmf.info/*).

8 CLINICAL ASSESSMENTS AND OUTCOME MEASURES

8.1 Clinical Variables

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

8.1.1 Vital Signs, Height & Weight

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

8.1.2 Clinical Laboratory Assessments

The following laboratory tests will be performed during the study:

- Serum urate level (Screening, Baseline, Weeks 3, 6, 9, 12, 16 and 20)
- Urinalysis: albumin, bilirubin, blood, clarity, color, glucose, ketones, nitrate, pH, protein, specific gravity, urobilinogen and WBC screen (Screening, Baseline, Week 12 and Week 20 visits). Urine sediment analysis will be also performed at the Baseline, Week 12 and Week 20 visits.
- Serum human chorionic gonadotrophin (hCG) for women of childbearing potential (WOCBP) (Screening ONLY)
- Hematology with differential panel: complete blood count with differential (hematocrit, hemoglobin, platelet count, RBC indices, Total RBC, Total WBC, and WBC & differential) (Baseline, Week 12 and Week 20)
- Blood chemistry panel/Liver function tests (LFTs): alanine aminotransferase (ALT (SGPT)), aspartate aminotransferase (AST (SGOT)), albumin, alkaline phosphatase, bicarbonate, blood urea nitrogen, calcium, chloride, creatinine, glucose, magnesium, phosphate, potassium, sodium, total bilirubin and total protein (Screening, Baseline, Week 12 and Week 20)

Additional testing, may be ordered if needed, to further assess an adverse event (AE), or if there is any suspicion that a subject may be pregnant, throughout the course of the study.

8.1.3 Physical Examination

A physical examination will be performed and recorded. The following systems will be examined: head/neck, eyes, ears, nose/throat, cardiovascular, lungs, abdomen, musculoskeletal, central nervous system, extremities, and skin.

8.1.4 Neurological Examination

A neurological examination will be performed and recorded.

8.1.5 Adverse Events

Adverse events (AEs) will be documented at each study visit, including the Screening Visit once the informed consent form has been signed by the subject, and at all study visits, including the final telephone call 28 days after the last dose of study drug. Information on adverse effects of study medication and on inter-current events will be determined at each visit by direct questioning of the subjects and review of concomitant medications.

8.1.6 ALSFRS-R

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

8.1.7 Pulmonary Function Testing

Pulmonary Function Testing includes Slow Vital Capacity (SVC).

Slow Vital Capacity (SVC): The vital capacity (VC) (percent of predicted normal) will be determined, using the upright slow VC method. The VC can be measured using conventional spirometers that have had a calibration check prior to subject testing. A printout from the spirometer of all VC trials will be retained. 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.

8.1.8 C-SSRS

The US FDA recommends the use of a suicidality assessment instrument that maps to the Columbia Classification Algorithm for Suicide Assessment (C-CASA)[62]. The C-CASA was developed to assist the FDA in coding suicidality data accumulated during the conduct of clinical trials of antidepressant drugs. One such assessment instrument is the Columbia Suicide Severity Rating Scale (C-SSRS)[63]. The C-SSRS involves a series of probing questions to inquire about possible suicidal thinking and behavior.

Only investigators who have been fully trained in the administration of the C-SSRS will assess subject suicidality. As part of training, investigators are prepared to respond to and manage instances in which patients express suicidal ideation or exhibit suicidal behavior.

At the Baseline Visit, the C-SSRS *Baseline* version will be administered. This version is used to assess suicidality over the subject's lifetime and specifically for the previous 6 month time period.

At the Week 12 and the Final Safety visit, as applicable, the *Since Last Visit* version of the C-SSRS will be administered. This version of the scale assesses suicidality since the subject's last visit.

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

8.1.9 Mobile App Study

The objective of the proposed study is to test the feasibility and utility of a novel smartphone application ('app') for monitoring disease progression in patients with Amyotrophic Lateral Sclerosis (ALS). The cell phone app will collect two broad categories of data:

1) "Actively collected data," wherein the subject is instructed to complete selected activities, and data about these activities is recorded, and
2) "Passively collected data," wherein data is recorded as the subject performs everyday activities naturally using existing cell phone sensors

Data will be collected from the subjects' smartphones over the course of the study (20 weeks), and only pre-specified forms of data will be accessed and collected by the app. In addition, the subject will be asked about their general smartphone usage.

8.1.11 Exit Questionnaire

An Exit questionnaire will be collected at Week 20 and will include a trial satisfaction survey and a blindedness questionnaire.

9 SAFETY AND ADVERSE EVENTS

The adverse event (AE) definitions and reporting procedures provided in this protocol comply with all applicable regulations and International Conference on Harmonization (ICH) guidelines. The Principal Investigator will carefully monitor each subject 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 product being studied, whether serious or non-serious.

9.1 Definitions of AEs, Suspected Adverse Drug Reactions & SAEs

9.1.1 Adverse Event and Suspected Adverse Drug Reactions

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

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

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

Adverse events are generally detected in two ways:

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

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

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

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 and ALSFRS-R results.

If discernible at the time of completing the AE log, a specific disease or syndrome rather than individual associated signs and symptoms should be identified by the Site Investigator and recorded on the AE log. 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 AE log. Clinically significant laboratory abnormalities, such as those that require intervention, are those that are identified as such by the Site Investigator.

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

The following will be considered Adverse Events of Special Interest regardless of severity: nephrolithiasis, urolithiasis, and gouty arthritis.

9.1.2 Serious Adverse Events

A serious adverse event (SAE) is defined as an adverse event that meets any of the following criteria:

1. Results in death.
2. Is life threatening: that is, poses an immediate risk of death as the event occurred.
    a. This serious criterion applies if the study subject, in the view of the Site Investigator or Sponsor, is at immediate risk of death from the AE as it occurs. It does not apply if an AE hypothetically might have caused death if it were more severe.
3. Requires inpatient hospitalization or prolongation of existing hospitalization.
    a. Hospitalization for an elective procedure (including elective PEG tube/g-tube/feeding tube placement) or a routinely scheduled treatment is not an SAE by this criterion because an elective or scheduled "procedure" or a "treatment" is not an untoward medical occurrence.

4. Results in persistent or significant disability or incapacity.
    a. This serious criterion applies if the "disability" caused by the reported AE results in a substantial disruption of the subject's ability to carry out normal life functions.
5. Results in congenital anomaly or birth defect in the offspring of the subject (whether the subject is male or female).
6. Necessitates medical or surgical intervention to preclude permanent impairment of a body function or permanent damage to a body structure.
7. Important medical events that may not result in death, are not life-threatening, or do not require hospitalization may also be considered SAEs when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

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

The Site Investigator is responsible for classifying adverse events as serious or non-serious.

9.2 Assessment and Recording of Adverse Events

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

9.2.1 Assessment of Adverse Events

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

1. Type of event
2. Date of onset and resolution (duration)
3. Severity (mild, moderate, severe)
4. Seriousness (does the event meet the above definition for an SAE)

5. Causality, relation to investigational product and disease
6. Action taken regarding investigational product
7. Outcome

9.2.2 Relatedness of Adverse Event to Investigational Product

The relationship of the AE to the investigational product 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 investigational product, and/or a more likely alternative etiology exists.
3. Possibly Related:	The reaction follows a reasonably temporal sequence from administration of the investigational product and follows a known response pattern to the suspected investigational product; the reaction could have been produced by the investigational product or could have been produced by the subject's clinical state or by other modes of therapy administered to the subject. (Suspected ADR)
4. Probably Related:	The reaction follows a reasonably temporal sequence from administration of investigational product; is confirmed by discontinuation of the investigational product or by re-challenge; and cannot be reasonably explained by the known characteristics of the subject's clinical state. (Suspected ADR)
5. Definitely Related:	The reaction follows a reasonable temporal sequence from administration of investigational product; that follows a known or expected response pattern to the investigational product; and that is confirmed by improvement on stopping or reducing the dosage of the investigational product, and reappearance of the reaction on repeated exposure. (Suspected ADR)

9.2.3 Recording of Adverse Events

All clinical adverse events are recorded in the Adverse Event (AE) Log in the subject's study binder. The site should fill out the AE Log and enter the AE information into the Electronic Data Capture

(EDC) system within 48 hours of the site learning of a new AE or receiving an update on an existing AE.

Please Note: Serious Adverse Events (SAEs) must be reported to the Medical Monitor and Coordination Center within 24 hours of the site learning of the SAE.

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

9.3 Adverse Events and Serious Adverse Events - Reportable Events

The following are considered reportable events and must be reported to the Medical Monitor and Coordination Center within 24 hours of the site being notified of the event.

- All events that meet the above criteria for Serious Adverse Events (SAEs)

- Dosage Changes (Dose Management)
  - Investigational Product Suspension, Reduction or Re-challenge
  - Investigational Product Discontinuation

- Key Study Events:
  - Subject Final Disposition
  - Feeding Tube Placement
  - Permanent Assisted Ventilation (PAV)*
  - Tracheostomy
  - Mortality
  - Pregnancy
  - Diaphragm Pacing System (DPS) device implantation
  - Emergency or Accidental Unblinding Events

* Permanent Assisted Ventilation (PAV) is defined as more than 22 hours daily of non-invasive mechanical ventilation for more than one week (7 days). The date of onset of PAV is the first day of the seven days.

9.4 Known Adverse Event/ Experience Prophylaxis Plan

Acidic urine is a major risk factor for uric acid urolithiasis, which form from uric acid crystals. Accordingly, urine samples will be collected at in-person site visits for urinalysis to determine pH. A pre-specified algorithm will be followed for triggering urine alkalinization therapy for uric acid stone prophylaxis, starting at the Baseline visit. Because urate-elevating inosine treatment does not have an appreciable effect on urine pH as demonstrated in SURE-PD, the rate of

alkalinization due to low urine pH will be similar in placebo- and inosine-assigned subjects, mitigating any unblinding effect.

9.4.1 Alkalinization Protocol

Site staff will receive notification from the Coordination Center of the need to initiate alkalinization within a few days of the urinalysis that triggers the protocol. The following steps should then be taken:

1- Site staff will prescribe "lower-dose" potassium citrate, which depends on subject weight (as recorded most recently on a study visit):
   - Less than 70 kg: 10 mEq, in tablet form, po tid (with meals)
   - 70 kg or greater: 10 mEq, in tablet form, po qid (with meals and at bedtime)

2- Staff will phone the subject to review the purpose, administration, potential adverse effects of potassium citrate, and plan rechecking urine pH and labs (CBC and BMP) in two weeks. In addition, staff will review recommendations for adequate hydration. The prescriber should review with the subject:
   - Any contraindications to potassium citrate, including a history of peptic ulcer disease, current use of an anticholinergic agent (e.g., trihexyphenidyl), hyperkalemia or renal insufficiency per most recent electrolyte measurements, or an active urinary tract infection.
     - If potassium citrate were deemed contraindicated (or later found insufficient) then site staff may work with the Medical Safety Monitor to employ an alternative dosing schedule or an alternative alkalinizing agent such as sodium bicarbonate.
   - Potential adverse effects of potassium citrate, including minor gastrointestinal complaints, such as abdominal discomfort, vomiting, diarrhea, loose bowel movements or nausea, and rarely gastrointestinal bleeding. The subject should be instructed to check with site staff or another doctor at once if tarry stools or other evidence of gastrointestinal bleeding is noticed.
   - The possibility of abnormal results of blood lab test that will be monitored regularly on subsequent visits.
   - The importance of taking each dose faithfully, and without crushing, chewing or sucking the tablet, and to check with physician if there is trouble swallowing tablets or if the tablet seems to stick in the throat. Subjects should not change dosing alkalinization medication until instructed to do so based on follow-up urinalysis results.

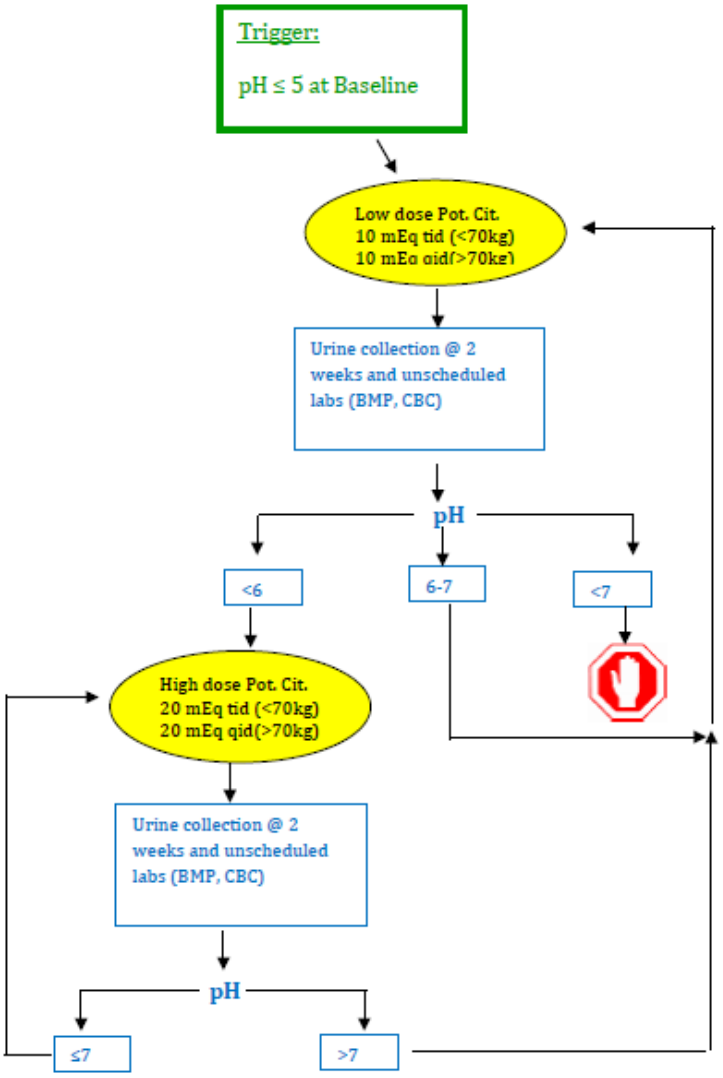
3- Site staff will order urine pH and labs (CBC and BMP) to be collected two weeks after the initiation of urine alkalinization and will follow the algorithm in the figure to adjust

alkalinization therapy. Urine pH and labs (CBC and BMP) will be collected every two weeks as needed per algorithm illustrated in the figure.

- For subjects on "lower-dose" potassium citrate who are instructed to increase it to "higher-dose" potassium citrate, they will be instructed to double their current dose. Depending on their weight:
    i. less than 70 kg: 20 mEq, in tablet form, po tid (with meals)
    ii. 70 kg or greater: 20 mEq, in tablet form, po qid (with meals and at bedtime)

4- For any deviations from compliance with the medication site staff will contact the CCC who may engage the Medical Safety Monitor to obtain guidance.

5- Subjects will be instructed to follow this algorithm for alkalinization for the duration of the treatment period of the trial. Any alkalinization therapy ongoing at the time of Week 20 visit will be discontinued immediately after that visit (or at the time of study drug discontinuation if occurring earlier) irrespective of urine pH results at the visit.



9.4.2 Lab Monitoring during Alkalinization Protocol

Current full prescribing information for potassium citrate indicates that laboratory tests should be checked every 4 months for:

- Electrolytes (to monitor for potassium elevation)
- Creatinine (to monitor for renal disease, a risk factor for hyperkalemic complications)
- CBC (to monitor for anemia as an indicator of gastrointestinal bleeding due to mucosal lesions, which have been observed rarely on solid potassium treatments – at an estimated frequency of approximately 1 lesion per 100,000 patient-years).

Under the study protocol labs will be monitored even more frequently as per algorithm above.

If hyperkalemia develops while a subject is taking potassium citrate, it should be suspended. Potassium testing should be repeated within 7 days of the suspension. If serum potassium falls back into the normal range, then the potassium citrate will be resumed at a reduced dosage in an effort to find a lower dosage that does not produce hyperkalemia but on which urine pH remains above 5. If hyperkalemia remains, the potassium citrate will be discontinued. An alternative, non-potassium-based alkalinization measure may be tried if the Medical Safety Monitor and Site Investigator believe such a measure is warranted. If a non-potassium-based alkalinization measure is not warranted, then the subject should discontinue study drug.

If a significant rise in serum creatinine or a significant fall in blood hematocrit or hemoglobin develops while a subject is taking potassium citrate, it should be suspended and the source of the abnormality determined. If these abnormalities are attributable to irreversible renal disease or to gastrointestinal bleeding, then the potassium citrate should not be resumed. Consideration may be given to an alternative, non-potassium-based alkalinization measure if the Medical Safety Monitor and Site Investigator believe such a measure is warranted.

10 SAFETY MONITORING AND STATISTICAL ANALYSIS PLAN

10.1 Safety Monitoring

An independent Medical Safety Monitor (MSM) will review receive a quarterly report of all AEs and safety laboratory tests. Recommendations for modification or termination of the trial based on safety data will be made by the MSM to the PI and the DSMB.

The Data and Safety Monitoring Board (DSMB) will periodically review blinded, and if necessary, unblinded medical event data. SAEs, drug suspensions/rechallenges, drug discontinuations, and premature withdrawals will be tracked in real time, and the DSMB alerted if any imbalances arise between treatment groups.

Unanticipated problems involving risks to subjects or others including adverse events will be reported to the local site IRB in accordance with institutional guidelines for reporting unanticipated problems including adverse events.

If serum urate is found to be above target range (i.e. 8.1 mg/dL or higher), inosine dose will be reduced or treatment will be stopped following the pre-specified titration algorithm described in Section 6.

10.2 Statistical Considerations

10.2.1 Analysis Samples

The As-treated (AT) sample will include all subjects who receive at least one dose of study drug classified according to the actual treatment received. An intention-to-treat (ITT) sample will include all randomized subjects classified according to their randomized treatment assignment without regard to compliance with their assigned treatment. The AT sample will be used as the primary sample for analyzing safety, tolerability, efficacy, and target engagement outcomes. Secondary analyses of safety outcomes will look at the AT sample with events censored after discontinuation of inosine among participants initiated on inosine and after initiation of inosine among participants initiated on placebo should any off-label use be detected. Secondary analysis of the ITT sample will be used to estimate efficacy in preparation for future phase 3 trials.

10.2.2 Outcomes

10.2.2.1 Safety outcomes

The proportion of subjects experiencing one or more adverse events (AE) prior to treatment, one or more treatment-emergent (TE, defined as occurring any time after first dose of study drug) AEs, one or more severe TE AEs, one or more serious TE AEs (SAE), one or more TE AEs of special interest (AESI, including gout, gouty arthritis, and kidney stones), and one or more AEs classified by MedDRA system organ class, higher level term, and preferred term over 24 weeks in the inosine group will be compared to the placebo group using one-tailed Fisher's exact tests at alpha = 0.05. The proportion of subjects experiencing one or more TE clinically significant abnormal labs for each safety assay performed or clinically significant abnormal vital signs for each vital sign monitored over 24 weeks in the inosine group will be compared to the placebo group using Fisher's exact tests. As a sensitivity analysis, we will also analyze event rates using negative binomial models for each type of event, adjusting for each participant's length of follow-up.

10.2.2.2 Tolerability

Tolerance will be defined as completion of the 20-week study without permanently discontinuing study drug or suspending study drug for greater than 28 days. Tolerability of inosine vs. placebo will be based on the proportion determined tolerant at week 20 and compared by Fisher's exact test from the AT sample. Serum urate elevation via oral inosine as titrated in this study will be judged tolerable if the lower one-sided 95% confidence bound on the proportion tolerant is greater than 50%.

10.2.2.3 Efficacy and target engagement

Effects of treatment on ALSFRS-R, will be analyzed in shared-baseline generalized least squares models with among-person effects of visit and the interaction of treatment group x post-baseline visit and within-person unstructured covariance among repeated measurements. Shared-baseline random-slopes linear mixed models will be used to analyze high-frequency smartphone metrics with fixed effects of time and treatment x post-baseline time and random participant-specific intercepts and slopes with unstructured covariance. Baseline characteristics will be compared by masked treatment groups prior to finalizing analysis of efficacy outcomes to select covariates to include the models to avoid chance confounding. Two-tailed tests at alpha = 0.05 will be used.

10.2.2. Operational endpoints

Accuracy of serum urate titration will be evaluated relative to the average levels from the week 12 visit onward and relative to the proportion of samples that are within the target range. Inosine dosage associated with accurate titration will be summarized. Urine pH trajectories and the frequency of alkalinization will be summarized.

10.2.2.5 Power Calculations

Assuming that 50%, 20%, and 10% of participants in the placebo group are expected to experience an AE or a given type of AE, we will have 80% power to declare a significant treatment-dependent increase in the proportion experiencing an AE if at least 95%, 71%, and 59% of participants in the inosine group are expected to experience an AE, respectively. We will have 80% power to observing at least one instance of any inosine-associated AE with a true probability of occurrence of at least 8%. For tolerability, we will have 80% power to declare inosine tolerable if the true proportion tolerant is at least 71%. Variance component estimates for calculating power for effects of treatment on ALSFRS-R are from a single arm pilot study of 25 participants on inosine for 20 weeks. Based on the follow-up schedule for this trial and assuming a random-slopes model, the estimated effective SD for ALSFRS-R slope per month is 1.4. Given this estimate, we will have 80% power to detect a difference in ALSFRS-R slopes between the inosine and placebo groups of 1.05 units per month. We will have 12% power for a difference in slopes of 0.3 units per month.

11 DATA COLLECTION

11.1 Role of Data Management

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

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

11.1.1 Data Entry and Checks

The site personnel are instructed to enter information into the Electronic Data Capture (EDC) System within 5 days of a visit. Please Note: Serious Adverse Events (SAEs) must be reported to the Coordination Center within 24 hours of the site learning of the SAE. Data collection is the responsibility of the staff at the site under the supervision of the Site Investigator (SI). During the study, the SI must maintain complete and accurate documentation for the study.

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

11.1.2 Data Lock Process

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

11.1.3 Quality Assurance

Protocol procedures are reviewed with the Site Investigator (SI) and associated personnel prior to the study to ensure the accuracy and reliability of data. Each SI must adhere to the protocol detailed in

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

11.1.3 Data handling and record keeping

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

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

11.2 Confidentiality

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

Data generated by this study must be available for inspection by representatives of the US FDA, by the Office for Human Research Protections (OHRP), the Sponsor, all pertinent national and local health and regulatory authorities, the Coordination Center or their representative, and the sites local IRB.

11.3 Study Discontinuation

The study can be terminated at any time by the Sponsor, DSMB, or FDA. Reasons for terminating the study may include the following:

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

11.4 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 Investigator (SI) for two years after marketing application approval. If no application is filed, these records must be kept for two years after the investigation is discontinued and the US FDA and the applicable national and local health authorities are notified. The Coordination Center or their representative will notify the Site Investigators of these events. The Site Investigators should retain all study documents and records until they are notified in writing by the Sponsor or their representative.

11.5 Publications

The Principal Investigator, Sabrina Paganoni, MD, will be responsible for publications of results from this trial. 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.

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13 APPENDICES

13.1 APPENDIX I: EL ESCORIAL WORLD FEDERATION OF NEUROLOGY CRITERIA FOR THE DIAGNOSIS OF ALS

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

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

(A:1) evidence of lower motor neuron (LMN) degeneration by clinical, 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

13.2 APPENDIX II: ALS FUNCTIONAL RATING SCALE – REVISED (ALSFRS-R)

ALSFRS-R

QUESTIONS: SCORE:

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

☐

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

☐

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

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

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

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

6. Dressing and Hygiene ☐
4 = Normal function
3 = Independent, can complete self-care with effort or decreased efficiency
2 = Intermittent assistance or substitute methods
1 = Needs attendant for self-care
0 = Total dependence

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

☐

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

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

R-1. Dyspnea ☐

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

R-2 Orthopnea ☐

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

R-3 Respiratory Insufficiency ☐

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

Evaluator's Initials: _____ _____ _____

13.3 APPENDIX III: COLUMBIA-SUICIDE SEVERITY RATING SCALE - *BASELINE*

COLUMBIA-SUICIDE SEVERITY RATING SCALE (C-SSRS)

Baseline

Version 1/14/09

Posner, K.; Brent, D.; Lucas, C.; Gould, M.; Stanley, B.; Brown, G.; Fisher, P.; Zelazny, J.; Burke, A.; Oquendo, M.; Mann, J.

Disclaimer:

This scale is intended to be used by individuals who have received training in its administration. The questions contained in the Columbia-Suicide Severity Rating Scale are suggested probes. Ultimately, the determination of the presence of suicidal ideation or behavior depends on the judgment of the individual administering the scale.

Definitions of behavioral suicidal events in this scale are based on those used in The Columbia Suicide History Form, developed by John Mann, MD and Maria Oquendo, MD, Conte Center for the Neuroscience of Mental Disorders (CCNMD), New York State Psychiatric Institute, 1051 Riverside Drive, New York, NY, 10032. (Oquendo M. A., Halberstam B. & Mann J. J., Risk factors for suicidal behavior: utility and limitations of research instruments. In M.B. First [Ed.] Standardized Evaluation in Clinical Practice*, pp. 103 -130, 2003.)*

For reprints of the C-SSRS contact Kelly Posner, Ph.D., New York State Psychiatric Institute, 1051 Riverside Drive, New York, New York, 10032; inquiries and training requirements contact posnerk@nyspi.columbia.edu

SUICIDAL IDEATION

Ask questions 1 and 2. If both are negative, proceed to "Suicidal Behavior" section. If the answer to question 2 is "yes", ask questions 3, 4 and 5. If the answer to question 1 and/or 2 is "yes", complete "Intensity of Ideation" section below.

Lifetime: Time He/She Felt Most Suicidal

1. Wish to be Dead
Subject endorses thoughts about a wish to be dead or not alive anymore, or wish to fall asleep and not wake up.
Have you wished you were dead or wished you could go to sleep and not wake up?
If yes, describe:

Yes ☐ No ☐

2. Non-Specific Active Suicidal Thoughts
General, non-specific thoughts of wanting to end one's life/commit suicide (e.g., *"I've thought about killing myself"*) without thoughts of ways to kill oneself/associated methods, intent, or plan.
Have you actually had any thoughts of killing yourself?
If yes, describe:

Yes ☐ No ☐

3. Active Suicidal Ideation with Any Methods (Not Plan) without Intent to Act
Subject endorses thoughts of suicide and has thought of at least one method during the assessment period. This is different than a specific plan with time, place or method details worked out (e.g., thought of method to kill self but not a specific plan). Includes person who would say, *"I thought about taking an overdose but I never made a specific plan as to when, where or how I would actually do it…and I would never go through with it."*
Have you been thinking about how you might do this?
If yes, describe:

Yes ☐ No ☐

4. Active Suicidal Ideation with Some Intent to Act, without Specific Plan
Active suicidal thoughts of killing oneself and subject reports having some intent to act on such thoughts, as opposed to *"I have the thoughts but I definitely will not do anything about them."*
Have you had these thoughts and had some intention of acting on them?
If yes, describe:

Yes ☐ No ☐

5. Active Suicidal Ideation with Specific Plan and Intent
Thoughts of killing oneself with details of plan fully or partially worked out and subject has some intent to carry it out.
Have you started to work out or worked out the details of how to kill yourself? Do you intend to carry out this plan?

If yes, describe:

Yes ☐ No ☐

INTENSITY OF IDEATION

The following features should be rated with respect to the most severe type of ideation (i.e., 1-5 from above, with 1 being the least severe and 5 being the most severe). Ask about time he/she was feeling the most suicidal.

Most Severe

Most Severe Ideation: _____ _____

Type # (1-5) *Description of Ideation*

Frequency
How many times have you had these thoughts?
(1) Less than once a week (2) Once a week (3) 2-5 times in week (4) Daily or almost daily (5) Many times each day

____

Duration
When you have the thoughts, how long do they last?
(1) Fleeting - few seconds or minutes
(2) Less than 1 hour/some of the time
(3) 1-4 hours/a lot of time
(4) 4-8 hours/most of day
(5) More than 8 hours/persistent or continuous

____

Controllability
Could/can you stop thinking about killing yourself or wanting to die if you want to?
(1) Easily able to control thoughts
(2) Can control thoughts with little difficulty
(3) Can control thoughts with some difficulty
(4) Can control thoughts with a lot of difficulty
(5) Unable to control thoughts
(0) Does not attempt to control thoughts

____

Deterrents
Are there things - anyone or anything (e.g., family, religion, pain of death) - that stopped you from wanting to die or acting on thoughts of committing suicide?
(1) Deterrents definitely stopped you from attempting suicide
(2) Deterrents probably stopped you
(3) Uncertain that deterrents stopped you
(4) Deterrents most likely did not stop you
(5) Deterrents definitely did not stop you
(0) Does not apply

____

Reasons for Ideation
What sort of reasons did you have for thinking about wanting to die or killing yourself? Was it to end the pain or stop the way you were feeling (in other words you couldn't go on living with this pain or how you were feeling) or was it to get attention, revenge or a reaction from others? Or both?
(1) Completely to get attention, revenge or a reaction from others
(2) Mostly to get attention, revenge or a reaction from others
(3) Equally to get attention, revenge or a reaction from others and to end/stop the pain.
(4) Mostly to end or stop the pain (you couldn't go on living with the pain or how you were feeling)
(5) Completely to end or stop the pain (you couldn't go on living with the pain or how you were feeling)
(0) Does not apply

____

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

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

 C-SSRS—Baseline (Version 1/14/09)

13.4 APPENDIX IV: COLUMBIA-SUICIDE SEVERITY RATING SCALE – *SINCE LAST VISIT*

COLUMBIA-SUICIDE SEVERITY RATING SCALE (C-SSRS)

Since Last Visit

Version 1/14/09

Posner, K.; Brent, D.; Lucas, C.; Gould, M.; Stanley, B.; Brown, G.; Fisher, P.; Zelazny, J.; Burke, A.; Oquendo, M.; Mann, J.

Disclaimer:

This scale is intended to be used by individuals who have received training in its administration. The questions contained in the Columbia-Suicide Severity Rating Scale are suggested probes. Ultimately, the determination of the presence of suicidal ideation or behavior depends on the judgment of the individual administering the scale.

Definitions of behavioral suicidal events in this scale are based on those used in The Columbia Suicide History Form, developed by John Mann, MD and Maria Oquendo, MD, Conte Center for the Neuroscience of Mental Disorders (CCNMD), New York State Psychiatric Institute, 1051 Riverside Drive, New York, NY, 10032. (Oquendo M. A., Halberstam B. & Mann J. J., Risk factors for suicidal behavior: utility and limitations of research instruments. In M.B. First [Ed.] Standardized Evaluation in Clinical Practice*, pp. 103 -130, 2003.)*

For reprints of the C-SSRS contact Kelly Posner, Ph.D., New York State Psychiatric Institute, 1051 Riverside Drive, New York, New York, 10032; inquiries and training requirements contact posnerk@nyspi.columbia.edu

SUICIDAL IDEATION

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

INTENSITY OF IDEATION

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

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

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

 C-SSRS—Since Last Visit (Version 1/14/09)

13.5 APPENDIX X: SMARTPHONE USAGE QUESTIONNAIRE

1. How do you use your phone?
   - ❍Independently
   - ❍With help for some functions
   - ❍Someone else uses my phone on my behalf
   - ❍I don't use a phone. I am participating with a tablet.

2. How often is your phone with you?
   - ❍Almost all the time when I am awake.
   - ❍Most of the time
   - ❍Some of the time
   - ❍Very little

3. How do you carry your phone most of the time?
   - ❍In your pocket
   - ❍Purse/Bag
   - ❍On a wheelchair
   - ❍Other
   - ❍Someone else carries my phone.

4. What do you do with your phone when you are at home?
   - ❍I carry my phone with me most of the time.
   - ❍I leave it on a surface most of the time.

5. When do you first use your phone in the morning?
   - ❍Immediately after I wake up
   - ❍Within one hour after I wake up
   - ❍2+ hours after I wake up

6. When do you last use your phone at night?
   - ❍Just before going to bed
   - ❍About an hour before going to bed
   - ❍More than 2 hours before going to bed

7. What do you do with your phone when you sleep?
   - ❍I keep the phone with me in bed.
   - ❍I leave it on a surface in my room.
   - ❍I leave it on a surface in another room.

8. Do you use your phone as an alarm clock?
   - ❍Yes
   - ❍Sometimes
   - ❍No

13.6 APPENDIX XI EXIT QUESTIONNAIRE: STUDY PARTICIPANT

Status: ❍ Done ❍ Not Done

1. Which treatment do you believe you were assigned to during this study?

❍ Active Treatment
❍ Placebo

2. How sure are you of this answer?

❍ Just guessing, not sure at all
❍ Somewhat sure
❍ Very sure

3. If somewhat sure or very sure, provide the primary reason for your treatment guess:

❍ Improvement in symptoms of disease under study
❍ Lack of improvement in symptoms of disease under study
❍ Adverse effects of study medication
❍ Lack of adverse effects of study medication
❍ Appearance, taste or odor or other physical characteristics of the study medication
❍ Other reasons

4. If somewhat sure or very sure, provide the secondary reason for your treatment guess:

❍ Improvement in symptoms of disease under study
❍ Lack of improvement in symptoms of disease under study
❍ Adverse effects of study medication
❍ Lack of adverse effects of study medication
❍ Appearance, taste or odor or other physical characteristics of the study medication
❍ Other reasons

5. How did you take the study drug?

❍ By mouth ❍ By feeding tube ❍ Both

How easy/challenging did you find administration of the study drug? Any comments on the study drug's consistency? Anything you would like to see changed or different?

6. Do you have any general comments or suggestions that you would like to share with the study team?

13.7 APPENDIX XII EXIT QUESTIONNAIRE: SITE INVESTIGATOR

Status: ❍ Done ❍ Not Done

1. Which treatment do you believe the subject was assigned to during this study?
❍ Active Treatment
❍ Placebo

2. How sure are you of this answer?
❍ Just guessing, not sure at all
❍ Somewhat sure
❍ Very sure

3. If somewhat sure or very sure, provide the primary reason for your treatment guess:
❍ Improvement in symptoms of disease under study
❍ Lack of improvement in symptoms of disease under study
❍ Adverse effects of study medication
❍ Lack of adverse effects of study medication
❍ Appearance, taste or odor or other physical characteristics of the study medication
❍ Other reasons

4. If somewhat sure or very sure, provide the secondary reason for your treatment guess:
❍ Improvement in symptoms of disease under study
❍ Lack of improvement in symptoms of disease under study
❍ Adverse effects of study medication
❍ Lack of adverse effects of study medication
❍ Appearance, taste or odor or other physical characteristics of the study medication
❍ Other reasons