Protocol

This trial protocol has been provided by the authors to give readers additional information about their work.

Protocol for: Paganoni S, Macklin EA, Hendrix S, et al. Trial of sodium phenylbutyrate–taurursodiol for amyotrophic lateral sclerosis. N Engl J Med 2020;383:919-30. DOI: 10.1056/NEJMoa1916945

Evaluation of the Safety, Tolerability, Efficacy and Activity of AMX0035, a Fixed Combination of Phenylbutyrate (PB) and Tauroursodeoxycholic Acid (TUDCA), for Treatment of Amyotrophic Lateral Sclerosis (ALS)

This supplement contains the following items:

1. Original Clinical Trial Protocol – PDF pages: 2-94

   Final Clinical Trial Protocol – PDF pages: 95-195

   Clinical Trial Protocol Amendment Summary of Changes – PDF pages: 196-205

2. Statistical Analysis Plan Outline – PDF pages: 206-221

   Amendment to Statistical Analysis Plan Outline – PDF pages: 222-224

   Final Statistical Analysis Plan – PDF pages: 225-278

   Statistical Analysis Plan Note to File – PDF pages: 279-281

Evaluation of the Safety, Tolerability, Efficacy and Activity of AMX0035, a Fixed Combination of Phenylbutyrate (PB) and Tauroursodeoxycholic Acid (TUDCA), for Treatment of Amyotrophic Lateral Sclerosis (ALS)

Regulatory Sponsor: Amylyx Pharmaceuticals Inc.

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STATEMENT OF COMPLIANCE

This study will be conducted in compliance with the protocol, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), Good Clinical Practice (GCP), and the applicable regulatory requirements, United States Code of Federal Regulations (CFR) Title 45 CFR Part 46 and Title 21 CFR Parts 50, 56, and 312.

SIGNATURE PAGE

I have read the attached protocol entitled, **Evaluation of the Safety, Tolerability, Efficacy and Activity of AMX0035, a Fixed Combination of Phenylbutyrate (PB) and Tauroursodeoxycholic Acid (TUDCA), for Treatment of Amyotrophic Lateral Sclerosis (ALS)** dated **November 18, 2016 (Version 1.0)** and agree to abide by all described protocol procedures. I agree to comply with the International Conference on Harmonisation Tripartite Guideline on Good Clinical Practice, applicable FDA regulations and guidelines identified in 21 CFR Parts 11, 50, 54, and 312, Institutional Review Board (IRB) and local institutional guidelines and policies, and the Health Insurance Portability and Accountability Act (HIPAA).

Site Investigator:

Signed: _____ Date: _____

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LIST OF ABBREVIATIONS

ADR	Adverse Drug Reaction
AE	Adverse Event/Adverse Experience
b.i.d	Twice a Day
CFR	Code of Federal Regulations
CIB	Clinical Investigator's Brochure
cIRB	Central Institutional Review Board
CRF	Case Report Form
DSMB	Data and Safety Monitoring Board
eCRF	Electronic Case Report Form
ER	Endoplasmic Reticulum
FDA	Food and Drug Administration
FWA	Federal-wide Assurance
g	Gram
GCP	Good Clinical Practice
GUID	Globally Unique Identifier
HIPAA	Health Insurance Portability and Accountability Act
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IDE	Investigational Device Exemption
IND	Investigational New Drug Application
IRB	Institutional Review Board
ITT	Modified Intent to Treat
MOP	Manual of Procedures
MPRAGE	Magnetization Prepared Rapid Gradient Echo
N	Number (typically refers to subjects)
NDA	New Drug Application
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
OHSR	Office of Human Subjects Research
PAA	Phenylacetate (metabolite of PB)
PB	Sodium Phenylbutyrate
PET	Positron Emission Tomography
PCP	Primary Care Provider
PHI	Protected Health Information
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
ROI	Region of Interest
SAE	Serious Adverse Event/Serious Adverse Experience

SAP	Statistical Analysis Plan
SI	Site Investigator
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
t.i.d	Three Times a Day
UDCA	Ursodeoxycholic Acid (ursodiol)
TUDCA	Tauroursodeoxycholic Acid
US	United States

PROTOCOL SUMMARY

Study Title
Evaluation of the Safety, Tolerability, Efficacy and Activity of AMX0035, a Fixed Combination of Phenylbutyrate (PB) and Tauroursodeoxycholic Acid (TUDCA), for Treatment of Amyotrophic Lateral Sclerosis (ALS)

Version Number
1.0

Study Indication
Amyotrophic Lateral Sclerosis (ALS)

Phase of Development
II

Rationale for the Study
The objective of this study is to determine the safety and efficacy of AMX0035 in subjects with Amyotrophic Lateral Sclerosis (ALS). ALS is a progressive neurodegenerative disease for which there is no cure. There is just one medicine approved specifically for treating ALS, Rilutek (riluzole), and it only provides a modest benefit for subjects. ALS also exacts a significant economic burden.

AMX0035 has demonstrated efficacy in models of neurodegeneration, classical activation of neuroinflammation, and bioenergetics deficits. The individual components of AMX0035, PB and TUDCA have demonstrated efficacy in *in vivo* models of ALS, Parkinson's, Alzheimer's, ischemia, and many others. Each individual component has also been tested in small clinical trials of ALS subjects and was found to be safe and well-tolerated, and hit primary endpoints of efficacy.

The first trial under this IND will be a randomized double-blind placebo-controlled Phase II trial to evaluate the safety and efficacy of AMX0035 for treatment of ALS. The program is designed to demonstrate that treatment is safe, can slow the decline in function, muscle strength, and vital capacity, and to assess the impact of AMX0035 therapy on biomarkers of ALS including blood levels of phosphorylated axonal neurofilament H subunit and 18 kDa translocator protein PET tracer uptake. This Phase II trial would also serve as the basis for the design of a pivotal trial in this subject population.

Study Design
This is a multicenter, randomized, double-blind, placebo-controlled 28-week study evaluating the safety, tolerability, efficacy, pharmacokinetics and biological activity of AMX0035.

Study Objectives and Endpoints

The primary objective of the study is to assess safety, tolerability, and efficacy of oral (or feeding tube) administration of AMX0035 via sachet (3g PB and 1g TUDCA) twice daily vs. matched placebo administered via sachet twice daily.

The primary outcome measures will be:

1. To confirm the safety and tolerability of AMX0035 in subjects with ALS over a 24-week period
2. To measure the impact of treatment on disease progression using the slope of the revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R)

The secondary objectives of the study will be:

1. To assess the impact of AMX0035 on the rate of decline of isometric muscle strength, as measured by the Accurate Test of Limb Isometric Strength (ATLIS)
2. To assess the impact of AMX0035 on disease progression as measured by Slow Vital Capacity (SVC) decline, time to tracheostomy, and survival
3. To assess the impact of AMX0035 on biomarkers including blood levels of phosphorylated axonal neurofilament H subunit (pNF-H) and 18 kDa translocator protein (TSPO) PET tracer uptake
4. To determine the population pharmacokinetics parameters of PB and TUDCA at steady state during treatment with AMX0035
5. To measure the impact of the treatment on survival.

Study Locations

Up to 25 Northeast ALS Consortium (NEALS) centers in the United States will participate in the study.

Number of Planned Subjects

Approximately 132 subjects will be randomized in the study.

Study Population

This study will be conducted in subjects who have sporadic or familial ALS diagnosed as definite as defined by revised El Escorial criteria (Appendix 1). Subjects must provide written informed consent prior to screening. At screening, eligible subjects must be at least 18 years old and less than 80 years old, and have a VC $\geq$ 60% of predicted capacity for age, height and gender. Subjects must have had onset of ALS symptoms less than or equal to 18 months prior to the screening visit, defined as first onset of weakness. 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 Groups

Subjects will be randomly assigned in a 2:1 ratio to oral (or feeding tube) AMX0035 treatment (1 sachet= 3g PB and 1g TUDCA plus excipients) or matching placebo. For the first three weeks of dosing, subjects will take one sachet daily (i.e. half-dose) and if tolerated will increase to two sachets daily.

Duration of Treatment and Follow-up

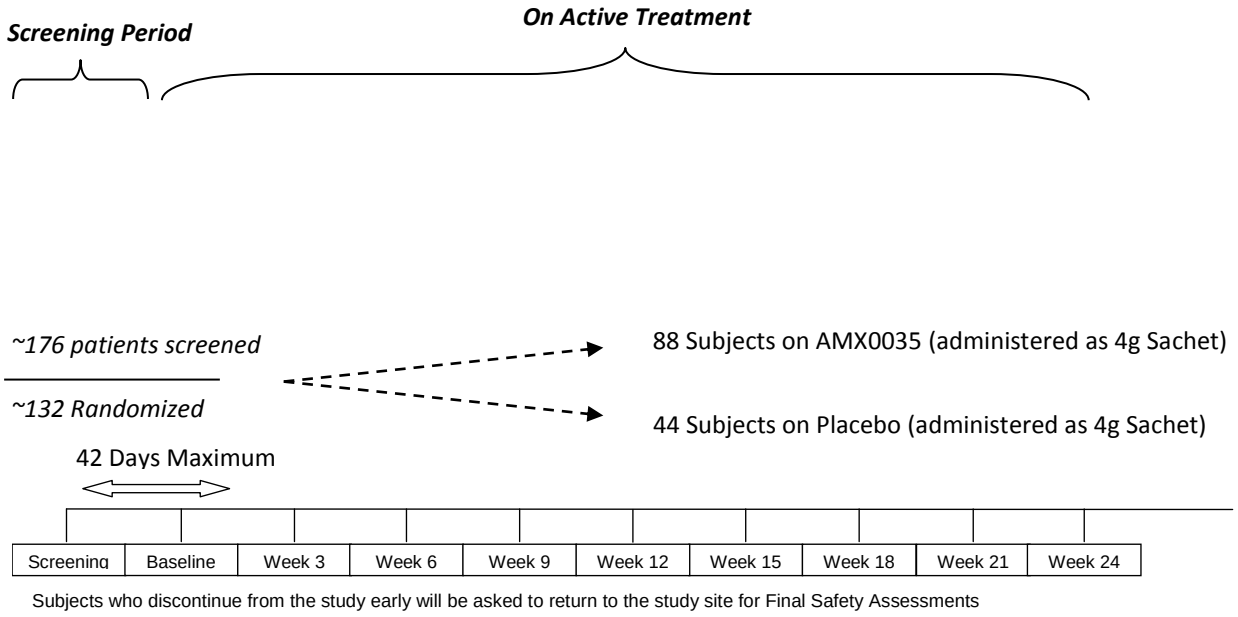
Subjects will remain on treatment until the Week 24 visit. Each randomized subject will also have a Final Telephone Interview 28 days (+ 5 days) after last dose of study drug to assess for adverse events (AEs), changes in concomitant medications and to administer the ALSFRS-R.

SCHEDULE OF ACTIVITIES

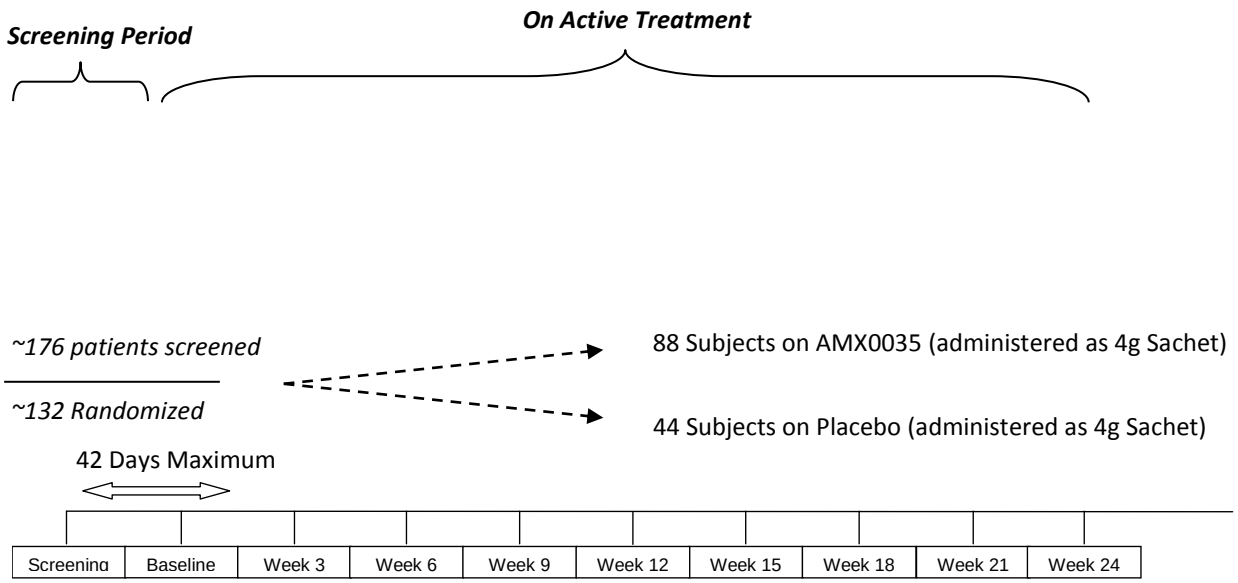
ACTIVITY	Screening Visit	Study Drug Administration (weeks)									Final Follow-up Telephone Call[2]	MR-PET Sub-Study Subjects Only
		Baseline Visit[1]	Week 3	Week 6	Week 9	Week 12	Week 15	Week 18	Week 21	Week 24 OR Early Discontinuation/ Final Safety Visit		
	Clinic	Clinic	Clinic	Clinic	Phone	Clinic	Phone	Clinic	Phone	Clinic	Phone	At MGH
	-42 Days	Day 0	Day 21 ±5	Day 42 ±5	Day 63 ±5	Day 84 ±5	Day 105 ±5	Day 126 ±5	Day 147 ±5	Day 168 ±5	28 +5 days	
Written Informed Consent	X											X
Inclusion/Exclusion Review	X	X										X
Medical History History/Demographics	X											
ALS Diagnosis/ALS History	X											
Vital Signs[3]	X	X	X	X		X		X		X		
Neurological Exam[4]	X					X				X		X[4]
Physical Exam[5]	X					X				X		
Blood Draw for Safety Labs[6]	X	X	X	X		X		X		X		
Blood Draw for Serum Pregnancy Test for WOCB[6]	X											
Urine Sample for Urinalysis[6]	X	X	X	X		X		X		X		
12-Lead ECG	X					X				X		
ALSFRS-R	X	X	X	X	X	X	X	X	X	X	X	
Slow Vital Capacity	X	X		X		X		X		X		
ATLIS Testing	X	X		X		X		X		X		
Columbia-Suicide Severity Scale[7]		X[7]	X	X		X		X		X		
Exit Questionnaire										X		
MR-PET Scan[8]		X				X						X[8]
Blood draw for Biomarker Testing[9]		X		X		X		X		X		
Blood draw for PK Analysis[10]		X				X				X[11]		
Adverse Events[12]	X	X	X	X	X	X	X	X	X	X	X	X
Blood draw for TSPO affinity testing[13]	X											
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X
Randomization[14]		X										
Dispense Study Drug[15]		X		X		X		X				
Drug Accountability/ Compliance			X[16]	X	X	X	X	X	X	X		

[1]The Baseline Visit should occur no more than 42 days after the Screening Visit.
[2]A Final Safety Telephone Call will be conducted 28 (+5 days) after the subject takes their last dose of study drug (whether or not the subject has discontinued from the study) to assess for adverse events (AEs), changes in concomitant medications and to administer the ALSFRS-R.
[3]Vital signs include systolic and diastolic pressure in mmHg, respiratory rate/minute, heart rate/minute and temperature.
[4] The standard Neurological Exam will be used for all patients. The Upper Motor Neuron Burden Scale (UMN-B) will be included for the MR-PET Sub-Study only and administered at the time of the scan.
[5]Physical Exam will include height and weight. Height will be collected at Screening Visit ONLY.
[6]Safety labs include Hematology (CBC with differential), Complete Chemistry Panel, Liver Function Tests and Urinalysis. Serum pregnancy testing will occur in women of child bearing potential (WOCBP) at the Screening Visit and as necessary during the course of the study.
[7]C-SSRS Baseline version to be completed at Baseline Visit only. C-SSRS Since Last Visit version to be completed at all other visits.
[8]Approximately 20 subjects will receive MR-PET (Magnetic Resonance-Positron Emission Tomography) scanning completed at selected sites. First scan will occur PRIOR to the Baseline Visit (pre-dose) and the second scan will occur between the Week 12 and Week 21 study visits. MR-PET subjects will also provide blood samples for peripheral blood mononuclear cell (PBMC) extraction prior to each MR-PET scan.
[9]Subjects will provide a blood sample for biomarker testing and storage in a biorepository.
[10]All subjects will provide a blood sample for pharmacokinetic (PK) testing at the Baseline Visit (pre-dose). Subjects will also provide a blood sample either 1 hour or 4 hours post-dose (±10 minute window per time point) at the Week 12 and Week 24 Visits. PK times will be randomized such that every subject has a 1-hour draw at one visit and a 4-hour draw at the other.
[11]PK should not be drawn for early termination subjects
[12]Adverse events that occur AFTER signing the consent form will be recorded.
[13]For MR-PET Sub-Study subjects only, blood will be drawn for TSPO testing at the subject's site during the Screening Visit.
[14]Randomization should occur at the Baseline Visit. Randomization will entail entering a subject's kit number into the data capture system.
[15]First dose of study drug will be administered in clinic after ALL Baseline Visit procedures are completed.
[16]Notify subjects of increase from one sachet per day to two sachets per day

STUDY WORKFLOW



Subjects who discontinue from the study early will be asked to return to the study site for final safety assessments at a Final Safety Visit, and will be asked to have a final Follow-Up Telephone Call 28 days (+5 days) after taking their last dose of study drug.



Subjects who discontinue from the study early will be asked to return to the study site for final safety assessments at a Final Safety Visit, 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. Subjects 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 ALS Overview

ALS is the most prevalent, adult-onset, progressive motor neuron disease, affecting more than 20,000 subjects in the US and an estimated 450,000 people worldwide, according to the ALS Association. ALS causes the progressive degeneration of motor neurons, resulting in rapidly progressing muscle weakness and atrophy that eventually leads to partial or total paralysis; on average, the disease is fatal within just 3-5 years. There is only one FDA-approved medication for ALS, riluzole, and it only extends survival modestly. ALS also exacts a significant economic burden.

Although the precise cause of ALS is unknown, ALS and other neurodegenerative diseases such as Alzheimer's are strongly characterized by nerve cell death and inflammation. Together these processes form a toxic cycle that is a key driver of progressive neurological decline. Recent research has highlighted mitochondrial stress and endoplasmic reticulum (ER) stress as key mediators of both the nerve cell death and neuroinflammatory processes[1]. The mitochondrion is the energy production center of the cell, while the ER is the quality control center. These two organelles are in constant communication, and are in fact physically connected by a membrane, and their health is vital to cell survival. When either of these cellular processes goes awry, the resulting stress can either kill the cell and/or create inflammation. The brain is extremely sensitive to both mitochondrial stress and ER stress, and both of these pathways have been strongly implicated in causing neurodegenerative disease. We believe that only therapeutically targeting both organelles simultaneously will enact a significant and lasting benefit.

2.1.2 AMX0035 Rationale

AMX0035 is a proprietary combination of two small molecules, phenylbutyrate (PB) and tauroursodeoxycholic acid (TUDCA), designed to block neuronal death and neurotoxic inflammation through simultaneous inhibition of endoplasmic reticulum (ER) stress and mitochondrial stress.

Both PB and TUDCA have been evaluated individually in many disease-specific models of ALS and other neurodegenerative diseases, and in many nonspecific models of ER Stress and bioenergetic stress, respectively.

PB is a pan-HDAC inhibitor and ameliorates ER stress through upregulation of the master chaperone regulator DJ-1 and through recruitment of other chaperone proteins[2,3]. The large increase in chaperone production reduces activation of canonical ER stress pathways, folds misfolded proteins, and has been shown to increase survival in many in vivo models including

the G93A SOD1 mouse model of ALS[4]. Phenylbutyrate has also been effective in additional in vivo models of Huntington's Disease, Alzheimer's, and Parkinson's[5,6,7].

TUDCA recovers mitochondrial bioenergetic deficits through incorporating into the mitochondrial membrane, reducing Bax translocation to the mitochondrial membrane, reducing mitochondrial permeability, and increasing the apoptotic threshold of the cell[8]. TUDCA has exhibited efficacy in many in vivo oxidative insult models, including mouse models of stroke, retinal disease, cardiac disease, brain lipopolysaccharide insult, the MPTP mouse model of Parkinson's, and ALS in vitro models of poly(GA)-induced toxicity[9,10,11].

Either ER stress or bioenergetic stress can result in neuronal death and a cytotoxic immune response. We therefore combined PB and TUDCA and have since demonstrated that they have synergistic efficacy when dosed in particular ratios. The combination of agents demonstrated a mathematically synergistic increase in neuronal viability in a strong oxidative insult model (H2O2-mediated toxicity) by linear modeling.

Cytotoxic neuroinflammation has been found to be a major part of neurodegeneration[12,13,14]. Different ratios of AMX0035 reduced classical activation of cytotoxic cytokines and increased phagocytic cytokines in an LPS-insult, glial model of inflammation.

2.1.3 Prior Clinical Use of PB and TUDCA in Subjects with ALS

Both PB and TUDCA have been evaluated in subjects with ALS and were found to be safe, well-tolerated, and exhibited preliminary signs of efficacy. PB was evaluated in a 20-week safety and biomarker study in ALS subjects[15]. This was a Phase I dose escalation trial and each subject was scheduled to receive PB at increasing dose from 9 to 21 g/day. A total of 40 subjects were recruited at 8 sites in the US. Twenty-six subjects completed the 20-week treatment phase. Histone acetylation was decreased by approximately 50% in blood buffy-coat specimens at screening and was significantly increased after PB administration. Blood levels of PB and the primary metabolite, phenylacetate, increased with dosage (Figure 1) with a plateau between the 3 and 6 gram t.i.d. regimen. While the majority of subjects tolerated higher dosages of PB, the lowest dose (9 g/day), was the most effective at increasing histone acetylation levels in blood (Figure 2). Treatment with PB did not alter blood riluzole levels. Adverse events in subjects taking riluzole and NaPB together did not occur more frequently, compared to those on PB alone.

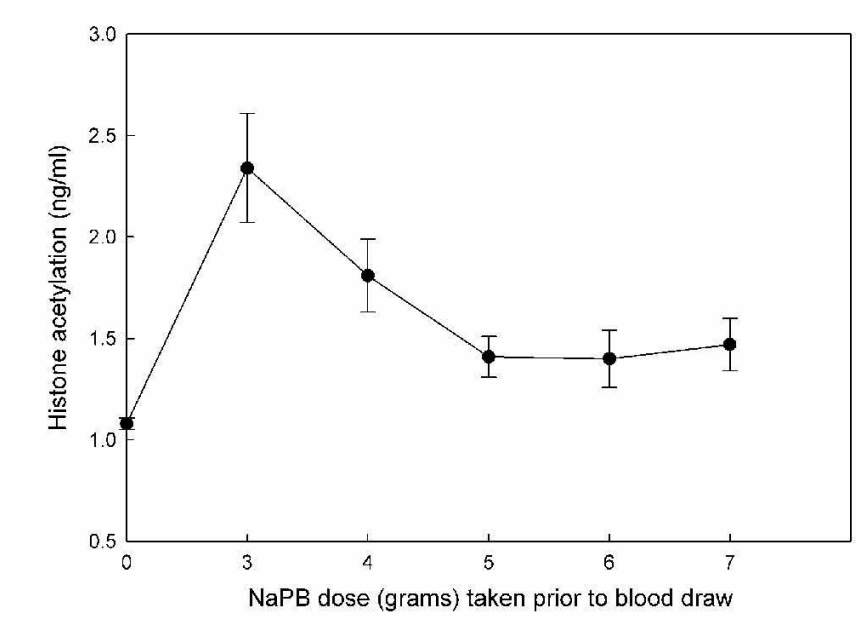


Figure 1: Histone acetylation levels with PB dose. Blood histone acetylation levels are shown compared with dose taken prior to blood draw. The error bars represent standard error. (Doses are repeated t.i.d in this study)

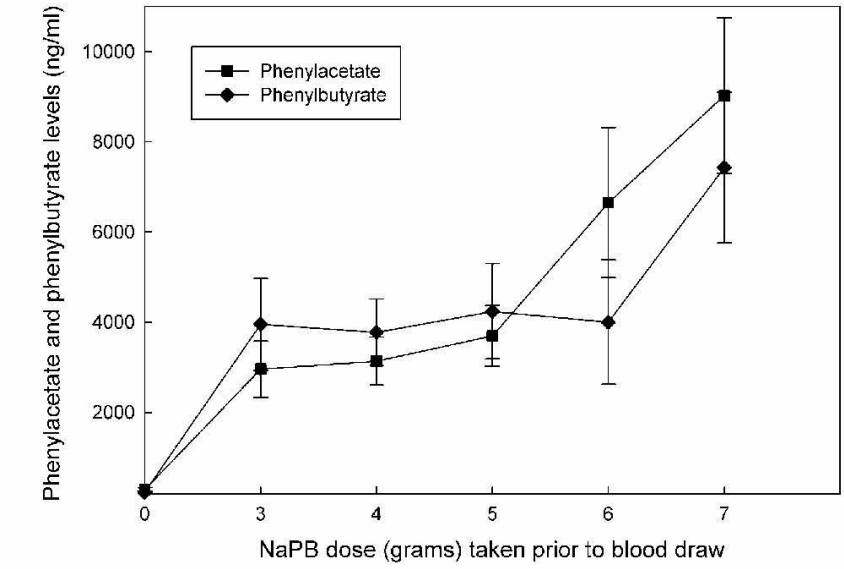


Figure 2: Phenylbutyrate and phenylacetate levels. Blood phenylbutyrate and phenylacetate levels are shown compared with dose taken prior to blood draw. The error bars represent standard error (doses are repeated t.i.d in this study).

It is not clear why acetylation levels were highest at 9 g/day. However, the author noted that in a study of PB in Huntington's disease, the effects of PB on mRNA expression levels of a 12-gene biomarker set were greatest at lowest dosages (4 g t.i.d.) with an inverse dose response[16].

For the planned Phase II trial of PB in combination with TUDCA, we therefore selected a dose of 3 grams of PB twice a day (6 grams per day) as a target dose with the desired pharmacologic effect.

Recently, TUDCA at 1g b.i.d. demonstrated a statistically significant slowing of ALSFRS-R progression rate in a year-long, multi-site, placebo-controlled clinical trial of ALS[17]. In this proof-of-principle trial, 34 ALS subjects under treatment with riluzole were randomized to placebo or TUDCA (1 gram b.i.d.) for 54 weeks. The proportion of responders (defined as subjects with >15% improvement in ALSFRS-R slope) was higher under TUDCA (87%) than under placebo (P = 0.021; 43%). At study end, baseline-adjusted ALSFRS-R was significantly higher (P = 0.007) in TUDCA than in placebo groups. Comparison of the slopes of regression analysis showed slower progression in the TUDCA than in the placebo group (P < 0.01) (Figure 3).

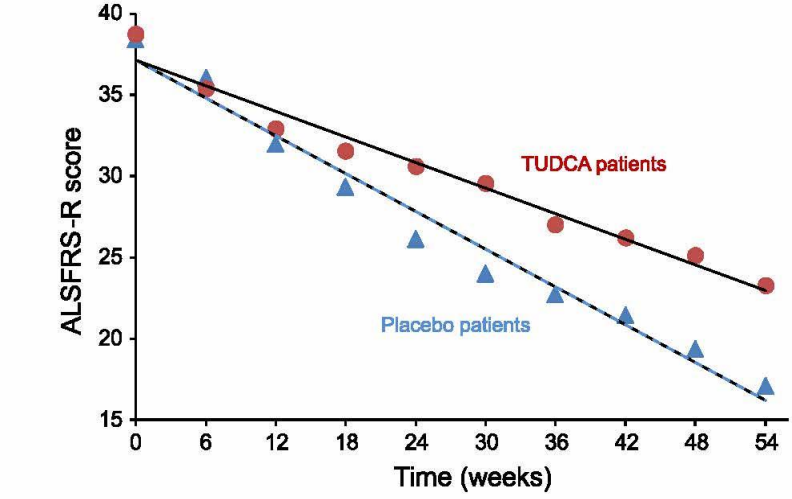


Figure 3: Linear regression analysis of ALSFRS-R mean scores over time for the TUDCA (circles, slope -0.388) and placebo groups (triangles, slope -0.262).

For the planned Phase II trial of PB in combination with TUDCA, we therefore selected a dose of 1 gram of TUDCA twice a day (2 grams per day) as a target dose.

Ursodiol (UDCA), the non-taurine conjugated form of TUDCA, was also found to be safe and well-tolerated in a crossover study subjects with ALS[18]. Subjects who received UDCA treatment also showed significant benefit as measured by the Appel ALS rating scale.

Subjects randomized to active therapy in the Phase II trial will receive 3g PB and 1g TUDCA twice a day orally (or by feeding tube). AMX0035 will be presented as a 4 gram sachet to be suspended in water and taken with a glass of water before a meal. Single agent TUDCA or PB treatment in subjects with ALS was very well tolerated.

In the TUDCA study from Elia et al., the AE profile and laboratory anomalies were not different between the TUDCA and placebo cohort. In the small group of 15 subjects treated with TUDCA, the adverse events were limited to diarrhea.

In the PB study from Cudkowicz et al., tolerability was similar to that reported in other trials of PB in other indications. There were no changes in safety laboratory tests, EKG or vital signs. The most common AEs were those previously reported with PB, including falls, dizziness, diarrhea, edema, dry mouth, headache, nausea and rash. A single subject interrupted treatment with PB at the 9 gram per day dose (i.e. a dose higher than that planned in the proposed Phase II) for the occurrence of edema on the foot and under the eye.

2.1.4 Additional Previous Clinical Experience with Phenylbutyrate

Sodium phenylbutyrate (PB) is generally well tolerated. It is FDA approved for subjects with urea cycle disorders including deficiencies of carbamylphosphate synthetase, ornithine transcarbamylase, or argininosuccinic acid synthetase. It is indicated in patients with either neonatal-onset deficiency or late-onset disease. The usual total daily dose is 450-600 mg/kg/day in patients weighing less than 20kg, or 9.9-13.0 g/m^2/day in larger patients. Detailed information can be found on the package insert for PB[19].

Sodium phenylbutyrate is also under development as an anticancer agent. In a dose escalation study in subjects with refractory solid tumor malignancies doses of up to 45g/day were administered[20]. Due to dose-limiting toxicities, the study concluded that 27g/day was the maximally tolerated dose. Nausea, vomiting, hypocalcemia and fatigue occurred at the 36g/day and 45g/day doses. Gastrointestinal upset (nausea, dyspepsia and vomiting) occurred at the lowest dose of 9g/day and was seen within 30 minutes of drug ingestion. However, 82% of subjects completed the study despite these side effects. Other frequently reported side effects include a "sweat"-like odor, usually noticeable only to the caregiver. Mild neurotoxicity (confusion, lethargy) has been noted at higher doses of close to 30g/day, but resolved with dose reduction.

A dose-escalation study of intravenous PB in subjects with myelodysplastic syndromes and acute myelogenous leukemia found a maximally tolerated dose at 375 mg/kg/day (26.3g/day for a 70kg individual) with no serious toxicities detected in subjects receiving doses between 125 and 375 mg/kg/day (8.8 and 26.3g/day for a 70kg individual) [0]. Dose-limiting toxicities (lethargy, confusion, slurred speech) were detected at 440 and 500 mg/kg/day PB (30.8 and 35g/day respectively, for a 70kg individual). Reports of edema have been blamed on the high sodium

load associated with the drug. Phase I/II studies in subjects with sickle cell anemia (see table 1 below) and beta thalassemia report similar side effects.

Another phase I study in subjects with refractory solid tumors tested IV PB doses between 150 to 515 mg/kg/day (up to 36g/day for a 70kg individual) with dose-limiting toxicities (excessive somnolence, confusion) and electrolyte abnormalities resulting at a dose of 515 mg/kg/day (36.0 g/day for a 70kg individual). The maximally tolerated dose of PB was determined to be 410 mg/kg/day (28.7 grams/day for a 70kg individual) as there were no dose-limiting toxicities at this dose and no subjects required dose reductions or escalations (see table 1).

The most common side effects of PB include: menstrual irregularities, decreased appetite, sweat-like body odor, and bad taste. Less common side effects include: nausea, vomiting, stomach upset, stomach pain, gastritis, headache, and skin rash. Rarely, cases of peptic ulcers, rectal bleeding, constipation, pancreatitis and renal tubular acidosis have been reported. Hypoalbuminemia, metabolic acidosis, alkalosis, hyperchloremia, hyperuricemia, hypokalemia, hypophosphatemia, hyperphosphatemia and hypernatremia have been observed. At higher doses, some subjects experienced confusion and fatigue, both of which resolved with dose reductions. Rarely, the following may occur, but have not been directly linked to sodium phenylbutyrate therapy: anemia, leukopenia, leukocytosis, thrombocytopenia, thrombocytosis, arrhythmia, syncope and depression.

Table 1: Prior Clinical Experience with Phenylbutyrate

Dose	Duration	Patient Population	# of patients	AE summary	Location	Status	Reference	NCT # (if ref unavailable)
9-21g/day	5 months	Amyotrophic Lateral Sclerosis	40	Well tolerated at 9 g	US	Completed	Amyotrophic Lateral Sclerosis. 2009; 10: 99106	
12-18g/day	28 days per dose level	Huntington's	24	Table included, Nausea, Headache, gain instability, were most common. Most side effects uncommon at 12g/day	US	Completed	Hogarth et al. Sodium phenylbutyrate in Huntington's disease: a dose-finding study. Mov. Disord. 2007.	
15g/day	12 months	SCA3	20	NA	Ex-US	Withdrawn	NA	NCT01096095
500mg/kg/day	14 days	Maple Syrup Urine Disease	40	NA	US	Complete	Brunetti-Pieri, et al.	NCT01529060

Dose	Duration	Patient Population	# of patients	AE summary	Location	Status	Reference	NCT # (if ref unavailable)
							Phenylbutyrate therapy for maple syrup urine disease. Hum. Mol. Gen. 2010.	
20g/day	4 days	Urea Cycle Disorders	9	NA	US	Active	NA	NCT02111200
IV Phenylbutyrate	7 years	Advanced Colorectal Cancer	46	NA	US	Cancelled	NA	NCT00002796
12.4g/day (mean dose, 198mg/kg-476mg/kg range)	12 months	Urea Cycle Disorders	11	One case of vomiting, see horizon package insert	US	Completed	Lichter-Konecki, U. et al. Mol Genet Metab. 2011 Aug;103(4)	
<20g/day	10 weeks	Urea Cycle Disorders	14	See Horizon package insert	US	Completed	See Horizon Package Insert	
1g/day	16 weeks	HIV	279	NA	Ex-US	Completed	NA	NCT01702974
9-36g/day	28 days	recurrent malignant glioma	23	No AE's at 9g/day, 1 headache, 1 lightheadedness at 18g/day, 1 fatigue at 27g/day, 2 fatigue at 36g/day	US	Completed	Neuro-oncol. 2005 Apr	
1g/day	16 weeks	Tuberculosis	390	NA	Ex-US	Completed	BMC Pulmonary MedicineBMC series 2013	
Effective dose for UCD	28 days	UCD	46	1 patient experienced Hyperammonaemia	US	Completed	NA	NCT00992459
450-600mg/kg/day	18-24 months	SMA	14 infants	NA	US	Completed	NA	NCT00528268
19g p.o./day divided into three doses	1 week	F(del)508 CF	18	Minimal and comparable side effects	US	Completed	Am J Respir Crit Care Med. 1998	

Dose	Duration	Patient Population	# of patients	AE summary	Location	Status	Reference	NCT # (if ref unavailable)
500mg/kg/day	12 weeks	SMA1	5	Terminated for slow enrollment	US	Cancelled	NA	NCT00439218
500mg/kg/day	12 weeks	SMA2/SMA3	9	Terminated for poor compliance	US	Cancelled	NA	NCT00439569
500mg/kg/day	1 week	Argininosuccinic Aciduria	12	NA	US	Completed	NA	NCT00345605
200mg/kg IV	5 days	Acute Myeloid Leukemia	10	Well tolerated, fatigue observed	US	Completed	Leukemia (2006) 20, 212–217	
7.5g/day	2 weeks	BMI>27	10	NA	Canada	Completed	NA	NCT00533559
IV Phenylbutyrate	NA	Multiple Cancers	20	NA	US	Completed	NA	NCT00006019
IV Phenylbutyrate	up to 4yrs	AML	9 to 24	NA	US	Completed	NA	NCT00006240
IV/Oral Phenylbutyrate Escalating top dose: 45g/day	4 weeks	Refractory Solid Tumor malignancies	28	Generally well tolerated <27g/day. Nausea, Hypocalemia observed	US	Completed	Clin Cancer Res August 2001 7;2292	
7.5g, 15g/day	14 day	Protinuric Nephropathy	26	NA	Ex-US	Completed	NA	NCT02343094
IV Phenylbutyrate	Ascending Dose	Hematologic Cancer	3 to 24	NA	US	Completed	NA	NCT00006239
20g/day	41 to 460 days	Thalessemia Major	11	Weight gain and/or edema caused by increase salt load in 2/12, transient epigastric discomfort in 7/12, and abnormal body odor in 3/12 subjects	US	Completed	AF Collins et al., 1995; Blood: 85 (1)	

Dose	Duration	Patient Population	# of patients	AE summary	Location	Status	Reference	NCT # (if ref unavailable)
20g/day	41 to 460 days	Thalessemia Major	11	Weight gain and/or edema caused by increase salt load in 2/12, transient epigastric discomfort in 7/12, and abnormal body odor in 3/12 subjects	US	Completed	January 1, 1995; Blood: 85 (1)	
20g/day	4 weeks	Healthy, BMI 30-45	101	Not yet posted	US	Completed	NA	NCT00771901
30-40g/day	10 days	ATT deficiency	12	NA	US	Completed	NA	NCT00067756

2.1.5 Additional Previous Clinical Experience with TUDCA

Tauroursodeoxycholic acid is currently marketed in Italy under the brand name Tudcabil (Bruschettini S.R.L.). It is exported to China and Turkey under the brand name Taurolite. It is used for the treatment of cholesterol gallstones. It has been used for the treatment of cholestatic liver diseases including primary cirrhosis, pediatric familial intrahepatic cholestasis and primary sclerosing cholangitis and cholestasis due to cystic fibrosis. To our knowledge there are no other off label uses of tauroursodeoxycholic acid.

Ursodeoxycholic acid (UDCA), which is widely used in the United States for treating gallstones, is produced and secreted endogenously by the liver as a taurine (TUDCA) or glycine (GUDCA) conjugate. Taurine conjugation increases the solubility of UDCA by making it more hydrophilic. TUDCA is taken up in the distal ileum under active transport and therefore likely has a slightly a longer dwell time within the intestine than UDCA which is taken up more proximally in the ileum (IND 118,844).

TUDCA is widely used for the dissolution of cholesterol gallstones. This generally requires long periods of treatment often 1 to 2 years to obtain complete dissolution (IND 118,844).

Between 1997 and 2007, 898,000 Tudcabil tablets were sold in Italy (taken from product profile contained in referenced IND 118,844). There were no reported cases of toxicity related to Tudcabil capsules. There were no reports of overdose or drug abuse during this period. There were no reports related to the use of pregnancy (all pregnant subjects, and those planning to become pregnant, are excluded from this trial). Common adverse events include mild abdominal pain and diarrhea. There are some cases of pruritus and a very limited number of cases of elevated liver enzymes. It should be noted that most of the studies are conducted in subjects with chronic liver disease.

TUDCA is contraindicated in subjects with biliary tree infections, frequent biliary colic, or in subjects who have trouble absorbing bile acids (e.g. ileal disease or resection). The only known or theoretical drug interactions are with substances that inhibit the absorption of bile acids such as cholestyramine and with drugs that increase the elimination of cholesterol in the bile (TUDCA reduces biliary cholesterol content). Based on similar physicochemical characteristics, it is likely that drug toxicity and interactions are very similar to those of ursodeoxycholic acid which are summarized below.

TUDCA has been and is being evaluated in multiple other studies as well. A study at Columbia of 20 subjects with new onset type 1 diabetes in which subjects are administered 1.75g TUDCA for 12 months is ongoing (see table 2). A study at Washington University assessing the effect of TUDCA on lipid markers and ER stress has been completed in 101 subjects at 1.75g daily for 4 weeks; an additional study arm in this study assessed PB at 20g/day (see table 2). We have included in the IND package a signed right to reference to the IND for a study at Washington University assessing subjects with HIV receiving 1.75g daily TUDCA for 30 days.

Table 2: Prior Clinical Experience with TUDCA

TUDCA Dose	Duration	Patient Population	# of patients	AE summary	Location	Status	Reference	NCT #
1g b.i.d.	1 year	Amyotrophic Lateral Sclerosis	29	Mild diarrhea occurred in two patients treated with TUDCA and in two treated with placebo; anorexia was reported in a placebo-treated patient.	ex-US	Completed	Elia et al. European J. Neurology	NCT00877604
1.75g/day	1 year	Type 1 Diabetes	20	NA	US	Ongoing	NA	NCT02218619
1.75g/day	4 weeks	Healthy, BMI 30-45	101	Not yet posted	US	Completed	NA	NCT00771901
750mg/day	24 weeks	Chronic Cholestatic Liver Disease	199	NA	Ex-US	Completed	NA	NCT01829698
750mg/day	18 months	Transthyretin Amyloid Cardiomyopathy	40	NA	US	Active	NA	NCT01855360
1.75g once	30 days	Protease-	48	NA	US	Recruiting	NA	NCT01877551

TUDCA Dose	Duration	Patient Population	# of patients	AE summary	Location	Status	Reference	NCT #
daily		inhibitor Associated Insulin Resistance						
750mg/day	12 months	PBC	216	NA	Ex-US	Completed	NA	NCT01857284
750mg/day	1 year	Transthyretin Amyloidosis	40	NA	Ex-US	Completed	NA	NCT01171859
UNK	3 months	Hepatobiliary Disease in Cystic Fibrosis	39	NA	US	Completed	NA	NCT00004441
500mg/day	60-80 days	Biliary Dyspepsia	30	Safe and well tolerated	Ex-US	Completed	Rivista di Patologia e Clinica, 1985, 34:3370-380	
750mg/day	254 days mean	Cholelithiasis	93	Minor side effects were observed in 4 patients treated with TUDCA (2 g.i. and 2 unspecified skin cases) none of which required suspension of treatment.	Ex-US	Completed	Acta Toxicologica et Therapeutica, Vol. 5, Oct-Dec. 1986, Vaccari ed., Parma	
1.0 g/day	10 days	Patients with Gallstones	7	NA	US	Completed	Batta, et al. Hepatology, 1982, 2(6):811-816	
.5g, 1g, 1.5g/day	6 months	Primary Biliary Cirrhosis	24	Diahrrea only observed AE	Ex-US	Completed	Crosignani, et al. Digestive Diseases and Sciences. 1996, 41(4):809-815	
.5g, 1g, 1.5g/day	6 months	Primary Biliary Cirrhosis	24	NA	Ex-US	Completed	Setchell et al. GUT, 1996; 38:439-446	
3.5-16.6mg/kg/day	4-6 weeks	Gallstones	33	NA	Ex-US	Completed	Muraca et al. International J. Clin. Pharmacol. Therapeuticcs, 1995; 33(7):391-393,	

TUDCA Dose	Duration	Patient Population	# of patients	AE summary	Location	Status	Reference	NCT #
3.5-16.6mg/kg/day	4-6 weeks	Biliary Lipid Composition	33	NA	Ex-US	Completed	Muraca et al. Ital J. Gastroenterol 1995, 27:439-440	
10mg/kg/day	1 month	Gallstones	29	NA	Ex-US	Completed	Portincasa, et al. Ital. J. Gastroenterol. 1996, 28:111-113	
10-13mg/kg/day	3 months	chronic hepatitis	5	NA	Ex-US	Completed	Panella, et al. Ital. J. Gastroenterology 1995; 27:256-258;	
10mg/kg/day	6 months	Gallstones	12	No side effects observed	Ex-US	Completed	La Clinica Terapeutica, 1986; 117:475-479	
10mg/kg/day	6 months	Gallstones	31	NA	Ex-US	Completed	The American Journal of Gastroenterology 1995; 90(6):978-981	
500mg/day	3 months	Biliary Dyspepsia	133	NA	Ex-US	Completed	Advances in Therapy - 1994; 11 (1):34-41,	
500mg/day	3 months	chronic active hepatitis	53	No side effects observed	Ex-US	Completed	Portincasa et al. Current Therapeutic Research. 1993; 53(5):521-531	
500mg/day	3 months	Patients post cholecystectomy	203	1 patient vomited, and 1 had abdominal pain in active, 1 abdominal pain, 1 rash cutaneous, 1 lipotinemia in placebo	Ex-US	Completed	Annali Italiani di Chirurgia, 1993, 64(5):533-537	
.5g, 1g, 1.5g/day	6 months	asymptomatic/mildly symptomatic PBC	24	NA	Ex-US	Completed	Hepatology, 1994, 130A.	

TUDCA Dose	Duration	Patient Population	# of patients	AE summary	Location	Status	Reference	NCT #
.5g, 1g, 1.5g/day	6 months	asymptomatic/mildly symptomatic PBC	24	TUDCA was well tolerated (rarely diarrhoea dose dependent reversible).	Ex-US	Completed	Hepatology, 1993, 10; 176A	
500mg/day	6 months	PBC	23	Well tolerated and no patient complained of side effects.	Ex-US	Completed	Aliment. Pharmacol. Ther. 1997; 11:409-414	
750mg/day	2 month with crossover	PBC	12 females	NA	US	Completed	Hepatology. 1999; 29:320-327	
675mg/day	2 months	PBC	15	Two patients experienced burning discomfort in the epigastrium during the TUDCA treatment period.	Ex-US	Completed	Clin. Res. 1986, 34(1):181	
13mg/kg/day	3 months	Chronic liver disease hystologically determined	69	NA	Ex-US	Completed	J of Hepatology. 1993; 18 (Suppl. 1) S157	
500mg/day	3 months	chronic hepatitis c	134	2.2% cases of diarrhoea solved promptly without suspension of therapy	Ex-US	Completed	Advances in therapy. 1994, 11(5):262-268	
500mg/day	3 months	patients with biopsy proved CAH due to HCV or HBV infections	162	1 patient developed abdominal discomfort, 1 patient had mild pruritus, 3 patients developed mild diarrhoea without wirthdrawal	Ex-US	Completed	Current Therapeutic Research 1995; 56(6):626-634,	
500mg/day	6 months	compensated liver cirrhosis associated with hepatitis B or C of Child's group A or B (histological tests)	30	No side effects and no treatment withdrawals occurred	Ex-US	Completed	Current Therapeutic Research 1994; 55 (11):1355-1362,	

TUDCA Dose	Duration	Patient Population	# of patients	AE summary	Location	Status	Reference	NCT #
Phase 1: TUDCA 10 mg/kg/day + lymphoblastoid IFNα 3MU/m2 ter in week; Phase 2: TUDCA idem + IFNα tapering dose down to the minimum effective	6 month phase 1, 6 months phase 2	Patients with CHC	120	NA	Ex-US	Completed	Gastroenterology 1996; 110(4) A1296.	
.25, .5, 1 g/day	6 months	Chronic Hepatitis	155	Two patients were withdrawn for minor side effects (one for diarrhea and one for dyspepsia).	Ex-US	Completed	Hepatology. 1995, 23(4):120A - 53	
500mg/day	12 months	Liver transplant	33	Safe and well tolerated	Ex-US	Completed	Ital J. Gastro and Hepatology 1999; P/C 13/37:154	

2.1.6 Previous Clinical Experience with Ursodiol (UDCA)

Ursodiol therapy has not been associated with liver damage. Lithocholic acid, a naturally occurring bile acid, is known to be a liver-toxic metabolite. This bile acid is formed in the gut from ursodiol less efficiently and in smaller amounts than that seen from chenodiol. Lithocholic acid is detoxified in the liver by sulfation and, although man appears to be an efficient sulfater, it is possible that some subjects may have a congenital or acquired deficiency in sulfation, thereby predisposing them to lithocholate-induced liver damage (IND 118,844).

Abnormalities in liver enzymes have not been associated with Actigall® (Ursodiol USP capsules) therapy and, in fact, Actigall® has been shown to decrease liver enzyme levels in liver disease. However, subjects given Actigall® should have SGOT (AST) and SGPT (ALT)

measured at the initiation of therapy and thereafter as indicated by the particular clinical circumstances.

Bile acid sequestering agents such as cholestyramine and colestipol may interfere with the action of ursodiol by reducing its absorption. Aluminum-based antacids have been shown to adsorb bile acids in vitro and may be expected to interfere with ursodiol in the same manner as the bile acid sequestering agents (IND 118,844). Estrogens, oral contraceptives, and clofibrate (and perhaps other lipid-lowering drugs) increase hepatic cholesterol secretion, and encourage cholesterol gallstone formation and hence may counteract the effectiveness of ursodiol.

Ursodeoxycholic acid was tested in 2-year oral carcinogenicity studies in CD-1 mice and Sprague-Dawley rats at daily doses of 50, 250, and 1000 mg/kg/day. It was not tumorigenic in mice. In the rat study, it produced statistically significant dose-related increased incidences of pheochromocytomas of adrenal medulla in males (p=0.014, Peto trend test) and females (p=0.004, Peto trend test). A 78-week rat study employing intrarectal instillation of lithocholic acid and tauro-deoxycholic acid, metabolites of ursodiol and chenodiol, has been conducted. These bile acids alone did not produce any tumors. A tumor-promoting effect of both metabolites was observed when they were co-administered with a carcinogenic agent. Ursodiol is not mutagenic in the Ames test (IND 118,844).

Reproduction studies have been performed in rats and rabbits with ursodiol doses up to 200-fold the therapeutic dose and have revealed no evidence of impaired fertility or harm to the fetus at doses of 20- to 100-fold the human dose in rats and at 5-fold the human dose (highest dose tested) in rabbits. Studies employing 100- to 200-fold the human dose in rats have shown some reduction in fertility rate and litter size. (IND 118,844) There have been no adequate and well-controlled studies of the use of ursodiol in pregnant women, but inadvertent exposure of 4 women to therapeutic doses of the drug in the first trimester of pregnancy during the Actigall trials led to no evidence of effects on the fetus or newborn baby. Although it seems unlikely, the possibility that ursodiol can cause fetal harm cannot be ruled out; hence, the drug is not recommended for use during pregnancy.

2.2 Potential Risks and Benefits

2.2.1 Potential Risks

The safety profile with PB administration is in large part derived from studies of subjects with urea cycle disorders. Refer to the phenylbutyrate tablet label (Buphenyl®).

In female subjects, the most common clinical adverse event reported was amenorrhea/menstrual dysfunction (irregular menstrual cycles), which occurred in 23% of the menstruating subjects. Decreased appetite occurred in 4% of all subjects. Body odor (probably caused by the metabolite, phenylacetate [PAA]) and bad taste or taste aversion were each reported in 3% of subjects.

Other adverse events reported in 2% or fewer subjects were:

- Gastrointestinal: abdominal pain, gastritis, nausea and vomiting; constipation, rectal bleeding, peptic ulcer disease, and pancreatitis each occurred in one subject.
- Hematologic: aplastic anemia and ecchymoses each occurred in one subject.
- Cardiovascular: arrhythmia and edema each occurred in one subject.
- Renal: renal tubular acidosis
- Psychiatric: depression
- Skin: rash
- Miscellaneous: headache, syncope, and weight gain

Phenylbutyrate has been evaluated in a dose-escalating study in ALS subjects over the course of 20-weeks and was found to be generally safe and tolerable[15]. Specifically, the most common adverse events included falls or other accidental injury, dizziness, diarrhea, edema, dry mouth, headache, nausea, and rash. With the exception of headache, these adverse events occurred at a higher rate compared to the comparison placebo cohort. These events are expected side effects from PB. There were no clinically significant changes in laboratory values, EKGs or vital signs. No deaths or unexpected and related serious adverse events occurred. Significant adverse events did not occur more frequently with subjects who were taking riluzole in addition to NPB, compared to subjects taking PB alone. Importantly, this study evaluated daily dosages of phenylbutyrate between 9 and 21 grams while our study will be limited to 6 grams daily.

Neurotoxicity was reported in cancer subjects receiving intravenous phenylacetate, 250–300 mg/kg/day for 14 days, repeated at 4-week intervals. Manifestations were predominately somnolence, fatigue, and lightheadedness; with less frequent headache, dysgeusia, hypoacusis, disorientation, impaired memory, and exacerbation of a pre-existing neuropathy.

These adverse events were mainly mild in severity. The acute onset and reversibility when the phenylacetate infusion was discontinued suggest a drug effect.

The most common adverse reactions reported with the use of TUDCA (≥1%) are: abdominal discomfort, abdominal pain, diarrhea, nausea, pruritus, and rash.

TUDCA is generally well tolerated. A derivative, UDCA or ursodiol, is approved for subjects with primary biliary cirrhosis. Common adverse events with TUDCA include mild abdominal pain and diarrhea. There are some cases of pruritus and a very limited number of cases of elevated liver enzymes.

TUDCA has been evaluated over a year-long placebo controlled study in ALS subjects at 1g b.i.d[17]. The population for safety analysis consisted of 15 subjects who took TUDCA and 14 subjects who took placebo. The treatment was well tolerated in all subjects. Laboratory parameters did not change in either treatment group during the course of the study. Except for

the expected complications related to ALS, no changes in vital signs and laboratory values that could possibly be attributed to the study drug or placebo were recorded. Overall, five adverse events were considered by the Investigators to be study related based on the subjects' descriptions. Two events were reported in the 15 TUDCA-treated subjects (13.3%); three events occurred in the 14 placebo-treated subjects (21.4%). The events were as follows: mild diarrhea occurred in two subjects treated with TUDCA and in two treated with placebo; anorexia was reported in a placebo-treated subject. Four subjects died during the study period, one in the TUDCA group and three in the placebo group. The one death in the treated group was not considered drug related—TUDCA trended towards a survival benefit.

The risks and side effects of muscle strength testing include fatigue and/or muscle cramping.

2.2.2 Known Potential Benefits

This study is designed to assess the safety, tolerability and biological activity of AMX0035 therapy. TUDCA and PB have both been tested individually in ALS clinical trials and met their primary endpoints of safety and tolerability. TUDCA also met its efficacy endpoint of slowing ALSFRS-R decline, and PB was therapeutically efficient in improving histone acetylation levels. If successful, this trial will allow further clinical development of this therapy to potentially slow ALS progression. The trial is also assessing multiple biomarkers in concert with clinical endpoints, which will allow both a more detailed understanding of drug activity as well as serve as a data set for the field as a whole to help understand how these biomarkers might track ALS progression.

3 OBJECTIVES

3.1 Study Objectives

This Phase II protocol is intended as a proof of concept of AMX0035 as a safe and effective treatment of adult subjects with ALS. The main strategic objectives of this protocol are below.

The primary outcome measures will be:

1. To confirm the safety and tolerability of a fixed-dose combination of PB and TUDCA in subjects with ALS over a 6-month period;
2. To measure the impact of the treatment using the slope of progression with the revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R);

The secondary objectives of the study will be:

1. To assess the impact of AMX0035 on the rate of decline of isometric muscle strength, as measured by the Accurate Test of Limb Isometric Strength (ATLIS);
2. To assess the impact of AMX0035 on disease progression as measured by Slow Vital Capacity (SVC) decline, time to tracheostomy and survival;
3. To assess the impact of AMX0035 on biomarkers including phosphorylated axonal neurofilament H subunit (pNF-H) levels and 18 kDa translocator protein (TSPO) uptake;
4. To develop concentration-response models of TUDCA and phenylbutyrate at steady-state after administration of AMX0035 sachet twice-daily.
5. To measure the impact of AMX0035 on survival.

3.2 Study Outcome Measures

3.2.1 Primary Outcome Measures

The primary outcome measures for the study will include:

- Safety and tolerability as defined as the proportion of subjects able to remain on study drug until planned discontinuation.
- The rate of decline (slope of decline) in the ALS functional rating scale (ALSFRS-R).

Safety and tolerability will be assessed by the procedures outlined in Section 9.

The revised version of the ALSFRS was created to add assessments of respiratory dysfunction, including dyspnea, orthopnea, and the need for ventilatory support. The revised ALSFRS (ALSFRS-R) has been demonstrated to retain the properties of the original scale and show strong internal consistency and construct validity.

Survival endpoint will be defined as death, tracheostomy or permanent assisted ventilation (>22 hours a day).

3.2.2 Secondary Outcome Measures

The secondary outcome measures include:

- Assessing the impact of AMX0035 on the rate of decline of isometric muscle strength, as measured by the Accurate Test of Limb Isometric Strength (ATLIS);
- Assessing the impact of AMX0035 on disease progression as measured by Slow Vital Capacity (SVC) decline;
- Assessing the impact of AMX0035 on survival, hospitalization and tracheostomies;
- Assessing the impact of AMX0035 on biomarkers including phosphorylated axonal neurofilament H subunit (pNF-H) levels and 18 kDa translocator protein (TSPO) uptake;
- Assessing the concentration-response model of TUDCA and phenylbutyrate at steady-state after administration of AMX0035 4 grams twice daily.

4 STUDY DESIGN

4.1 Overall Study Design and Plan

During the enrollment period approximately 176 subjects will be screened from approximately 25 Northeast ALS Consortium (NEALS) centers in the US. One hundred thirty-two (132) of these subjects will be randomly assigned in a 2:1 ratio to oral (or feeding tube) twice daily sachet of active therapy or matching placebo. Treatment duration will be twenty-four (24) weeks. For the first three weeks study drug will be administered once daily. If tolerated, the dose will then be increased to twice a day. Clinic visits will occur at Screening, Baseline, Week 3 (day 21), Week 6 (day 42), Week 12 (day 84), Week 18 (Day 126), and Week 24 (Day 168). Phone calls will be conducted at Week 9, Week 15, Week 21 and Week 28 (4 weeks after completion of treatment).

All visit windows are consecutive calendar days and are calculated from the day the subject starts study treatment (Day 0, the day of the Baseline Visit). Any change from this visit window will be considered an out of window visit deviation.

4.2 Study Centers

This study will be conducted at up to 25 NEALS Centers in the US. Sites will be selected based on recruitment record from prior trials, compliance with prior study protocols and regulations, clinical research expertise and availability of necessary resources.

4.3 Study Duration

Subjects will remain on randomized, placebo-controlled, double-blind treatment until the Week 24 visit. Each randomized subject will also have a Follow-up Telephone Interview 28 days after the completion of dosing to assess for adverse events (AEs), changes in concomitant medications and to administer the ALSFRS-R. Including the Screening and Follow-up Visits, each subject will be in the study for approximately 8 months. We expect the study to take up to 18 months to meet enrollment goals.

4.4 Protocol Adherence

Each Site Investigator must adhere to the protocol detailed in this document and agree that any changes to the protocol must be approved by the NCRI Coordination Center (CC) or their Central Institutional Review Board (cIRB). Each Site Investigator (SI) will be responsible for enrolling only those study subjects who have met protocol eligibility criteria.

5 STUDY ENROLLMENT AND WITHDRAWAL

5.1 Number of Study Subjects

Approximately 132 ALS subjects will be randomized.

5.2 Inclusion and Exclusion Criteria

5.2.1 Inclusion Criteria

Inclusion Criteria

1. Male or female, aged 18-80 years of age
2. Sporadic or familial ALS diagnosed as definite as defined by the World Federation of Neurology revised El Escorial criteria
3. Less than or equal to 18 months since ALS symptom onset
4. Capable of providing informed consent and following trial procedures
5. Geographically accessible to the site
6. Slow Vital Capacity (SVC) >60% of predicted value for gender, height, and age at the Screening Visit
7. Subjects must either not take riluzole or be on a stable dose of riluzole for at least 30 days prior to the Screening Visit. Riluzole-naïve subjects are permitted in the study.
8. Women of child bearing potential (e.g. not post-menopausal for at least one year or surgically sterile) must agree to use adequate birth control for the duration of the study and 3 months after last dose of study drug
    a. Women must not be planning to become pregnant for the duration of the study and 3 months after last dose of study drug
9. Men must agree to practice contraception for the duration of the study and 3 months after last dose of study drug
    a. Men must not plan to father a child or provide sperm for donation for the duration of the study and 3 months after last dose of study drug

Acceptable birth control methods for use in this study are:

- Hormonal methods, such as birth control pills, patches, injections, vaginal ring, or implants
- Barrier methods (such as a condom or diaphragm) used with a spermicide (a foam, cream, or gel that kills sperm)
- Intrauterine device (IUD)
- Abstinence (no heterosexual sex)
- Unique partner who is surgically sterile (men) or not of child bearing potential (female)

Date of ALS Symptom Onset. For the purposes of this study, the date of symptom onset will be defined as the date the subject first had symptoms of their disease, i.e., weakness. To be eligible

for this study, the date of symptom onset must be no greater than exactly 18 months prior to the Screening Visit date.

MR-PET Sub-Study
A subset of study subjects will undergo MR-PET and will need to meet the following additional inclusion criteria:

1. Ability to safely lie flat for 90 min for MR-PET procedures in the opinion of the Site Investigator
2. High or mixed affinity to bind TSPO protein (Genotype Ala/Ala or Ala/Thr)

TSPO affinity test: Venous blood for the TSPO affinity test will be drawn from all subjects who have indicated their interest in participating in the MR-PET sub-study. (This will be indicated via a checkbox on the consent form.) The blood will be drawn at Screening in order to have the subjects genotyped for the Ala147Thr TSPO polymorphism in the *TSPO* gene (rs6971). About 10% of humans show low binding affinity to PBR28[21].

Note: High or Mixed affinity binders (Ala/Ala or Ala/Thr) will be considered eligible, whereas the low affinity binders (Thr/Thr) will be considered ineligible for the MR-PET sub-study.

Note: A subject may be eligible for the main study but ineligible for the MR-PET sub-study. However, if a subject is found to be ineligible for the main study, he or she is automatically ineligible for the MR-PET sub-study as well.

5.2.2 Exclusion Criteria

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

Exclusion Criteria

1. Presence of tracheostomy
2. Exposure to PB, TUDCA or UDCA within 3 months prior to the Screening Visit or planning to use these medications during the course of the study
3. History of known allergy to PB or bile salts
4. Abnormal liver function defined as AST and/or ALT > 3 times the upper limit of the normal
5. Renal insufficiency as defined by eGFR < 60 mL/min/1.73m^2.
6. Poorly controlled arterial hypertension (SBP>160mmHg or DBP>100mmHg) at the Screening Visit
7. Pregnant women or women currently breastfeeding
8. History of cholecystectomy

9. Biliary disease which impedes biliary flow including active cholecystitis, primary biliary cirrhosis, sclerosing cholangitis, gallbladder cancer, gallbladder polyps, gangrene of the gallbladder, abscess of the gallbladder.
10. History of Class III/IV heart failure (per New York Heart Association – NYHA)
11. Severe pancreatic or intestinal disorders that may alter the enterohepatic circulation and absorption of TUDCA including biliary infections, pancreatitis and ileal resection
12. The presence of unstable psychiatric disease, cognitive impairment, dementia or substance abuse that would impair ability of the subject to provide informed consent, according to Site Investigator judgment
13. Clinically significant unstable medical condition (other than ALS) that would pose a risk to the subject if they were to participate in the study
14. Active participation in an ALS clinical trial evaluating a small molecule within 30 days of the Screening Visit
15. Exposure at any time to any biologic under investigation for the treatment of subjects with ALS (off-label use or investigational) including cell therapies, gene therapies, and monoclonal antibodies.
16. Implantation of Diaphragm Pacing System (DPS)
17. 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
18. Exposure to any disallowed medications listed below

MR-PET Sub-Study

A subset of study subjects will undergo MR-PET. The following additional exclusion criteria apply to this subset:

1. Exposure to immunomodulatory medications within 30 days of the Screening Visit
2. Any contraindication to undergo MRI studies such as:
    a. History of a cardiac pacemaker or pacemaker wires
    b. Metallic particles in the body
    c. Vascular clips in the head
    d. Prosthetic heart valves
    e. Severe claustrophobia impeding ability to participate in an imaging study
3. Low affinity binders (Thr/Thr) on the TSPO Affinity Test
4. Radiation exposure that exceeds the site's current guidelines

Note: A subject may be eligible for the main study but ineligible for the MR-PET sub-study. However, if a subject is found to be ineligible for the main study, he or she is automatically ineligible for the MR-PET sub-study as well.

Note on Benzodiazepines for MR-PET Sub-Study Subjects: If an MR-PET subject is taking a benzodiazepine, he or she should not take the benzodiazepine for at least 1 day before his or her scans with the exception of lorazepam and clonazepam that do not need to be discontinued.

Disallowed medications for all subjects include

- HDAC Inhibitors including:
    - Valproate
    - Vorinostat (Zolinza)
    - Romidepsin
    - Chidamide
    - Panobinostat
    - Lithium
    - Butyrate
    - Suramin

- Probenecid

- Bile Acid Sequestrants including:
    - Cholestyramine and Cholestyramine Light
    - Questran and Questran Light
    - Welchol
    - Colestid and Colestid Flavored
    - Prevalite

Note on Antacids Within Two Hours of AMX0035 Administration:
Antacids containing Aluminum hydroxide or smectite (aluminum oxide) may not be taken **within two hours of administration of AMX0035** as they inhibit absorption of TUDCA. These include:

- Alamag
- Alumina and Magnesia
- Antacid, Antacid M and Antacid Suspension
- Gen-Alox
- Kudrox
- M.A.H.
- Maalox HRF and Maalox TC
- Magnalox
- Madroxal
- Mylanta and Mylanta Ultimate
- Ri-Mox
- Rulox

Cautionary Note on Mexiletine
Subjects who participated in the Mexiletine trial within the last 30 days will be excluded from the trial. However, subjects who are using Mexiletine at a dosage less than or equal to 300mg/day for cramps and fasciculations will not be excluded.

There is a potential for an interaction between AMX0035 and Mexiletine; at 20 times the intended clinical concentration (C_{max}), the principal metabolite of Phenylbutyrate, Phenylacetylacetate has been shown to be inhibitory to CYP 1A2 and CYP 2D6 which are the major enzymes responsible for the breakdown of Mexiletine. Therefore, it is possible the co-administration of Phenylbutyrate and Mexiletine will increase the subject's exposure to Mexiletine.

Subjects who are co-administered AMX0035 and Mexiletine should therefore be monitored for Mexiletine-associated adverse events, and if these events present, the Site Investigator should consider stopping or reducing the dosage of Mexiletine. Adverse events associated with Mexiletine include but are not limited to cardiac arrhythmias, liver injury, and blood dyscrasias.

5.3 Treatment Assignment Procedures

Each subject who meets all eligibility criteria will be randomized to receive either therapy by twice daily sachet of AMX0035 (3g PB and 1g TUDCA) or matching placebo for 24 weeks of treatment. For the first three weeks of the study subjects will only take a single sachet daily and will be instructed to increase to 2 sachets daily at the Week 3 Visit.

5.3.1 Randomization Procedures

The randomization scheme will be independently developed and will indicate the treatment assignment and the subject numbers to be used by each site. The randomization scheme will be managed by the manufacturer.

5.4. Reasons for Withdrawal

A study subject will be discontinued from participation in the study if:

- Any clinical adverse event (AE), laboratory abnormality, requirement for a concomitant medication, concurrent illness, or other medical condition or situation occurs such that, in the opinion of the Investigator, continued participation in the study would not be in the best interest of the subject.
- The subject is non-compliant or is lost-to-follow-up.

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

5.4.1 Handling of Withdrawals

A subject may choose to discontinue participation in the study at any time. However, the Site Investigator (SI) or designee will encourage subjects to continue with follow-up, regardless of their compliance with the study drug. If the SI or designee is concerned about the use of a prohibited medication or other safety issues, then the study drug may have to be reduced to

single dose or discontinued. If a subject 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. 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 the study drug and should not begin use of the prohibited medication before an appropriate wash-out period of at least 30 days occurs. Subjects who must permanently discontinue study drug may continue in the ITT portion of the study, per protocol.

Subjects who permanently discontinue study drug and will not continue monitoring per the study schedule should complete early study drug termination procedures per protocol. Subjects who discontinue treatment should not be unblinded unless there is a specific reason to do so.

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 as soon as possible after stopping study drug, if possible within 28 days of asking to withdraw consent. The subject will also be asked to have a Follow-Up Telephone Call no sooner than 28 days (+5 days) after taking their last dose of study drug to monitor their safety and to permit review of their medical records at the end of the study to document their vital status.

Subjects who withdraw from the study due to adverse events will be followed for outcome measures under the ITT protocol as noted above. The DSMB will review these events promptly and make recommendations about potential changes to the study, including possible changes to protocol, updates to the informed consent form, or even ending the study early.

In the event a subject wishes to no longer have their personal health information used for the analysis of this study, he or she will notify the site through an authorized letter and future data will not be included in analysis; however, all data up to this letter will still be included.

5.5 Termination of Study

This study may be prematurely terminated if, in the opinion of the DSMB or sponsor, there is sufficient reasonable cause. Written notification, documenting the reason for study termination, will be provided to the Principal Investigator or Sponsor by the terminating party.

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

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

- Plans to modify, suspend or discontinue the development of the study drug.

If the study is prematurely terminated or suspended, the Sponsor will promptly inform the Site Investigators/institutions, and the regulatory authority(ies) of the termination or suspension and the reason(s) for the termination or suspension. The Central IRB (cIRB) will also be informed promptly and provided the reason(s) for the termination or suspension by the Sponsor or by the Site Investigator/institution, as specified by the applicable regulatory requirement(s).

6 TREATMENTS ADMINISTERED

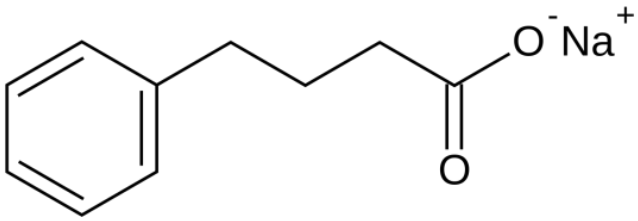
6.1 Treatments

6.1.1 Study Product Description

AMX0035 is a combination therapy comprised of two active pharmaceutical ingredients, sodium phenylbutyrate (PB and tauroursodeoxycholic acid (TUDCA).

Phenylbutyrate is an approved compound in the United States for urea cycle disorders and is marketed in the US as Buphenyl®. There is an existing USP monograph for this material.
The chemical structure for PB is provided below.

Chemical Structure PB



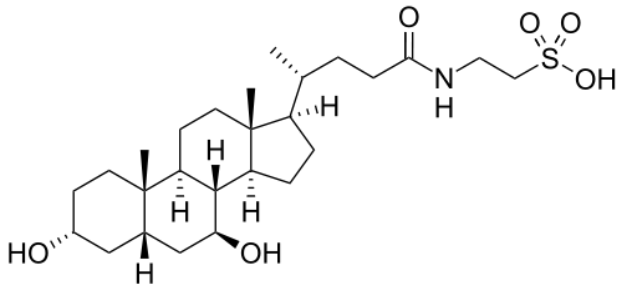
The drug substance PB is produced by Sri Krishna Pharmaceuticals, Ltd. under cGMP conditions. The manufacture and controls for PBA are described in Drug Master File No. 019569.

The specifications for PB are identical to those of the Ph.Eur.

The drug substance TUDCA is currently marketed in Italy under the brand name Tudcabil. It is exported to China and Turkey under the brand name Taurolite. It is used for the indications of treatment of cholesterol gallstones. It has been used for the treatment of cholestatic liver diseases including primary cirrhosis, pediatric familial intrahepatic cholestasis and primary sclerosing cholangitis and cholestasis due to cystic fibrosis. To our knowledge, there are no other uses of tauroursodeoxycholic acid. It is marketed by some companies in the United States on websites such as Amazon as a dietary supplement to "promote liver health".

The chemical structure for TUDCA is provided below.

Chemical Structure TUDCA



The drug substance TUDCA is produced by Prodotti Chimici E Alimentaria S.p.A.

The specifications for TUDCA are identical to those used by the supplier.

A powder filled sachet will be used as the AMX0035 drug product. The drug product will be filled under cGMP conditions in an aluminum foil lined sachet.

The sachet containing active ingredients will include:

- Active Ingredients:
  - 1g TUDCA
  - 3g PB

- Excipients
  - Sodium Phosphate Dibasic, Anhydrous
  - Dextrates, Hydrates
  - Sorbitol
  - Syloid 63FP (colloidal silica)
  - Sucralose
  - Sodium Stearyl Fumarate
  - Weber Mixed Berry Flavoring
  - Kleptose Linecaps (maltodextrin)

6.1.2 Placebo

A matched placebo will be used to maintain the dosage-blind. The placebo sachets for this study will match the corresponding AMX0035 sachets in size, color, and presentation.

The placebo sachets contain:

- Excipients
  - Sodium Phosphate Dibasic, Anhydrous
  - Dextrates, Hydrates

- Sorbitol
- Syloid 63FP (colloidal silica)
- Sucralose
- Sodium Stearyl Fumarate
- Weber Mixed Berry Flavoring
- Kleptose Linecaps (maltodextrin)
- Denatonium Benzoate Granules

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

6.2 Acquisition

Upon receipt of the study treatment supplies, an inventory must be performed and a drug receipt log filled out and signed by the person accepting the shipment. It is important that the designated study staff counts and verifies that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable study drug in a given shipment will be documented in the study files. The Site Investigator must notify the study Sponsor or their designee of any damaged or unusable study treatments that were supplied to the Site Investigator's site.

6.2.1 Formulation, Packaging, and Labeling

The study drug is prepackaged in kits containing 98 sachets and ready for oral (or feeding tube) administration. The Site Investigator (SI) has the responsibility to ensure that the integrity of packaged study drug is not jeopardized prior to dispensing. Each individual subject kit must be dispensed as provided with no further repackaging or labeling done at the investigational site, unless required by the institution per institutional polices.

6.2.2 Product Storage and Stability

The SI must ensure that all investigational drug supplies are kept in a locked, safe area at ambient temperature 15-25°C with access limited to authorized study staff. Investigational drug supplies should not be repackaged in any way.

Once subjects have access to kits containing the sachets, they will be asked to store them away from moisture at room temperature. Stability has been assessed both at ICH standard and accelerated conditions for each of the individual active ingredients and they were found to be stable over five years. Drug product will receive regular stability testing over the course of the study to ensure product does not degrade. At least one month stability will be verified prior to initiation of the proposed trial. Subjects should contact the SI or their designee in the case of damaged goods; the SI or designee will coordinate with the Sponsor or their designee to determine the most appropriate remediation.

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

It is recommended that the study drug be taken prior to a meal. Subjects should rip open the sachet of study drug and add it to a cup or other container and add approximately 8 oz. (1 cup) of room temperature water and stir vigorously. The study drug mixture should be consumed completely and within one hour of combining the contents of the sachet with water.

Subjects may resume normal eating and drinking after taking the study drug.

Note on Antacids Within Two Hours of Study Drug Administration

Antacids containing Aluminum hydroxide or smectite (aluminum oxide) may not be taken **within two hours of administration of the study drug** as they inhibit absorption of TUDCA.

These include:

- Alamag
- Alumina and Magnesia
- Antacid, Antacid M and Antacid Suspension
- Gen-Alox
- Kudrox
- M.A.H.
- Maalox HRF and Maalox TC
- Magnalox
- Madroxal
- Mylanta and Mylanta Ultimate
- Ri-Mox
- Rulox

6.3.1 Feeding Tube Study Drug Administration

For subjects with a gastrostomy or nasogastric (feeding) tube, the study drug may be dissolved in water as per the procedures outlined above in Section 6.3 and the study drug may be administered via the feeding tube.

6.4 Modification of Study Intervention/Investigational Product For A Subject

Any dosage adjustment, including the reason for and dates of adjustment, will be documented in the CRF for each subject requiring this manipulation. The SI or designated 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 reasons during the trial (the reason for, and dates of suspension or dose reduction must be documented). All dose modifications need to be discussed with the study Medical Monitor. If the AE is mild or moderate, the dosage may be reduced until the event improves. The SI or designated licensed

physician Sub-Investigator 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 within the study for 28 days (+ 5 days) after a subject's last dose of study drug.

6.4.1. Dosage Discontinuation

Reasons for discontinuation of study drug may include an AE, Medical Monitor or Site Investigator recommendation, Sponsor termination, protocol deviation, lost-to-follow-up, subject request, or death. All serious adverse events (SAEs) that occur in a subject who has discontinued early must be recorded and reported within 24-hours of awareness.

Study subjects who discontinue study drug prematurely (early termination from study) and decide to not remain in the modified intent-to-treat (ITT) portion of the study will be encouraged to return for a Final Safety/Early Termination Visit and participate in a Follow-Up Telephone Call 28 days (+ 5 days) after the last dose of study drug.

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

SAEs will be followed for resolution for 28 days (+5 days) after a subject's last dose of study drug, regardless of whether they prematurely discontinued study drug or completed 24 weeks of treatment.

6.5 Study Drug Accountability Procedures

At the completion of the study, there will be a final reconciliation of study drug shipped, drug consumed, and drug remaining. This reconciliation will be logged on the study 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.

6.6 Assessment of Subject Compliance

Subjects will be instructed to return empty and unused study drug containers at each clinic Visit (Weeks 6, 12, 18, and 24) or the Final Safety Visit (whichever occurs first). Site staff will count returned and unused sachets to determine compliance.

Non-compliance will be otherwise defined as taking less than 80% or more than 125% of study drug as determined by sachet counts. If a study subject is non-compliant with study drug, the Site Investigator (SI) or designee should re-educate and train the subject in administration of study drug. Data indicating non-compliance will be used in the end of study analysis.

6.7 Prior and Concomitant Therapy

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

Any investigational small molecule therapy being used or evaluated for the treatment of ALS is prohibited beginning 30 days prior to the Screening Visit and throughout the study. This includes, but is not limited to, the following:

- Pioglitazone
- Arimoclomol
- Olanzapine
- Tamoxifen
- NP001
- Mexiletine
- Rasagiline
- Masitinib
- Dexpramipexole
- Tirasemtiv
- Ibudilast
- TW001
- Inosine
- RNS60

Use of any biologic therapy prior to this study excludes subjects from enrollment. This includes any cell or gene therapy under evaluation for the treatment of ALS and includes but is not limited to, the following:

- ISIS 333611
- Ionis SOD1R
- NurOwn
- Q-Cells
- NSI-566
- GM604
- GSK 1223249
- Treg cell therapies

6.7.1 Prohibited Medications and Contraindications

Prohibited Medications

Throughout the course of the trial, study subjects should not be treated with the following medications. If a Site Investigator learns that a subject has begun therapy with any of these medications, this should be reported to the Medical Monitor and Coordination Center immediately and the SI should make the determination whether to stop the study drug or the prohibited medication immediately, taking into account the health, safety and preference of the study subject.

Agents which might impair bile acid processing or renal function are contraindicated with AMX0035. Prohibited medications include but are not limited to:

- HDAC Inhibitors including:
    - Valproate
    - Vorinostat (Zolinza)
    - Romidepsin
    - Chidamide
    - Panobinostat
    - Lithium
    - Butyrate
    - Suramin

- Probenecid for potential kidney interaction

- Antacids containing aluminum hydroxide or smectite (aluminum oxide) **within two hours of administration of AMX0035.** These inhibit absorption of TUDCA. These include:
    - Alamag
    - Alumina and Magnesia
    - Antacid, Antacid M and Antacid Suspension
    - Gen-Alox
    - Kudrox
    - M.A.H.
    - Maalox HRF and Maalox TC
    - Magnalox
    - Madroxal
    - Mylanta and Mylanta Ultimate
    - Ri-Mox
    - Rulox

- Bile Acid Sequestrants including:

- Cholestyramine and Cholestyramine Light
- Questran and Questran Light
- Welchol
- Colestid and Colestid Flavored
- Prevalite

Pregnancy & Nursing Mothers

There are no adequate and well-controlled studies in pregnant women. Female subjects or female 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. If a female subject becomes pregnant during the course of the study, the Medical Monitor and Coordination Center should be contacted immediately.

It is not known whether AMX0035 is excreted in human milk. Caution should be exercised; therefore, no subject should nurse an 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. It is recommended that the ALSFRS-R be completed first at every visit. After the ALSFRS-R it is recommended that SVC and ATLIS measurements are performed so as not to fatigue the subject with other testing. Blood samples are recommended to be taken at the end of the study visits. The order of testing, however, will be at the discretion of each Site Investigator (SI).

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

Subjects who withdraw consent or early terminate from the study (i.e., discontinue study drug) will be asked to come in for a Final Safety Visit and have a Final Follow-up Telephone Call 28 days (+5 days) after stopping study drug.

7.1 MR-PET Scheduling Call

Only those subjects from selected sites will be considered to participate in the MR-PET Sub-Study. The MR-PET Sub-Study procedures will be conducted at Massachusetts General Hospital (MGH) in Boston, MA. However, blood will be drawn for TSPO testing at the subject's site during the Screening Visit.

Subjects participating in the MR-PET Sub-Study may be consented over the phone by a medically licensed professional MGH study staff member to determine subject eligibility and to ensure the subject is safe to undergo the MR-PET scan. These procedures include:

- Obtain verbal pre-screening informed consent from subject
- Assess MR-PET inclusion and exclusion criteria
- Complete MR-PET safety questionnaire

During this call, MR-PET Sub-Study procedures will be discussed in detail and the subject should be given the opportunity to ask questions about the MR-PET Sub-Study. The MGH study staff will write a consent note to document the consenting process over the phone. The written informed consent will be signed by the subject and the MGH Study Investigator at the MR-PET in-person visit.

7.2 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
- Create Globally Unique Identifier (GUID)
- Assess inclusion and exclusion criteria
- Obtain medical history and demographics
- Review and document concomitant medications and therapies

- Obtain ALS diagnosis history
- Administer ALSFRS-R questionnaire
- Perform pulmonary function testing including slow vital capacity (SVC)
- Measure isometric strength using ATLIS machine
- Assess and document adverse events (AEs) that occur after subject signs informed consent form (ICF)
- Measure vital signs (blood pressure, heart and breathing rates, temperature)
- Perform neurological examination
- Perform comprehensive physical examination including height and weight
- Perform 12-lead ECG (Electrocardiogram)
- [After other tests] Collect blood samples for clinical laboratory assessments including Hematology (CBC with differential), Complete Chemistry Panel, Liver Function Tests, and serum pregnancy test (for women of child-bearing potential [WOCBP])
- MR-PET SCAN SUBJECTS ONLY: TSPO Affinity Testing
- Collect urine sample for urinalysis
- Schedule the Baseline Visit

MR-PET Scan: For those subjects that consent to participate in the MR-PET scan sub-study, the scan will be scheduled/performed ***before*** the Baseline Visit at the MGH in Boston, MA. At that time, blood will also be collected for peripheral blood mononuclear cell (PBMC) storage and analysis.

7.2.1 Screen Failures

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

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

7.3 MR-PET Visit 1 (Only for patients in MR-PET substudy)

The following procedures will be performed at an office visit to determine the subject's eligibility for the MR-PET sub-study.

- Obtain written informed consent
- Assess MR-PET inclusion and exclusion criteria
- Complete MR-PET safety questionnaire
- Perform the MR-PET Scan
- Perform the Upper Motor Neuron-Burden (UMN-B) Scale
- Measure vital signs (blood pressure, heart and breathing rates, temperature), height, and weight
- Collect blood for
    - Biomarker (PBMC) testing

    - Pregnancy testing (for women of child bearing potential)
- Review and document concomitant medications and therapies
- Assess and document adverse events (AEs) that occur after subject signs informed consent form (ICF)

MR-PET Follow-Up Call

This visit will take place 24-48 hours after the MR-PET Visit 1. The following procedures will be performed.

- Assess and document AEs directly related to the MR-PET procedures

7.4 Baseline Visit

This visit will take place a maximum of 42 days after the Screening Visit. The 42-day window allows those subjects participating in the MR-PET portion of the study to have their scans scheduled. Site staff are advised to schedule the baseline visit as soon as possible after determining eligibility. The following procedures will be performed.

- Confirm eligibility criteria are still met
- Randomize subject using kit number from the study drug
- Administer the C-SSRS baseline questionnaire
- Administer ALSFRS-R questionnaire
- Perform pulmonary function testing, including slow vital capacity (SVC)
- Measure isometric strength using ATLIS machine
- Review and document concomitant medications and therapies
- Review and document Adverse Events since last visit and following study drug administration
- Measure vital signs
- [After other tests] Collect blood samples for clinical laboratory assessments including Hematology (CBC with differential), Complete Chemistry Panel, Liver Function Tests.
- Collect blood sample for biomarkers
- Collect pre-dose blood sample for pharmacokinetic analysis
- Collect urine sample for urinalysis

After all other visit activities are completed:

- Dispense 6 weeks of study drug
- Administer first dose of study drug. The healthcare staff member will advise the subject on appropriate administration. The subject will be observed at the site for a minimum of 60 minutes by an appropriate healthcare staff member according to the site's institutional/state regulations to assess medical status and any immediate reaction to the study drug.
- Review and document any Adverse Events after first dose of study drug

7.5 Week 3 Clinic Visit

This visit will take place 21±5 days after the Baseline Visit. The following procedures will be performed.

- Administer ALSFRS-R questionnaire
- Review and document concomitant medications and therapies
- Review and assess Adverse Events
- Measure vital signs
- Administer the C-SSRS questionnaire
- Collect blood samples for clinical laboratory assessments including Hematology (CBC with differential), Complete Chemistry Panel, Liver Function Tests
- Collect urine sample for urinalysis
- Perform study drug accountability
- Unless drug is not tolerated, advise subject to increase dosage level from one sachet to two sachets daily.
- Schedule next study visit

7.6 Week 6 Clinic Visit

This visit will take place 42±5 days after the Baseline Visit. The following procedures will be performed.

- Administer ALSFRS-R questionnaire
- Perform pulmonary function testing, including slow vital capacity (SVC)
- Measure isometric strength using ATLIS machine
- Review and document concomitant medications and therapies
- Review and assess Adverse Events
- Measure vital signs
- Administer the C-SSRS questionnaire
- [After other tests] Collect blood samples for clinical laboratory assessments including Hematology (CBC with differential), Complete Chemistry Panel, Liver Function Tests
- Collect blood sample for biomarkers
- Collect urine sample for urinalysis
- Perform study drug accountability and collect all unused study drug and empty containers
- Dispense next 6 weeks of study drug
- Schedule next study visit

7.7 Week 9 Telephone Visit

This visit will take place 63±5 days after the Baseline Visit. The following procedures will be performed.

- Administer ALSFRS-R questionnaire
- Review and document concomitant medications and therapies
- Assess and document AEs
- Enquire about tolerance and compliance
- Schedule next study visit

- Remind subject to bring study drug to the Week 12 Visit

7.8 Week 12 Clinic Visit

This visit will take place 84±5 days after the Baseline Visit. **Subject must take study drug at the site upon beginning this visit due to the PK analysis.** It is recommended that this visit happens earlier in the day since the drug is administered in clinic. The following procedures will be performed:

- Record day/time of previous study drug dose, including if the subject missed a dose.
- Note time of last meal
- Administer study drug and record time of administration
- Collect blood sample for PK (i.e. at 1-hour or 4-hours post-dose) as indicated at the time of randomization
- Administer ALSFRS-R questionnaire
- Perform pulmonary function testing including slow vital capacity (SVC)
- Measure isometric strength using ATLIS machine
- Review and document concomitant medications and therapies
- Review and assess Adverse Events
- Measure vital signs
- Perform neurological examination
- Perform comprehensive physical examination including weight
- Perform 12-lead ECG (Electrocardiogram)
- Administer the C-SSRS questionnaire
- [After other tests] Collect blood samples for clinical laboratory assessments including Hematology (CBC with differential), Complete Chemistry Panel, Liver Function Tests
- Collect blood sample for biomarkers
- Collect urine sample for urinalysis
- Perform study drug accountability and collect all unused study drug and empty containers
- Dispense next 6 weeks of study drug
- Schedule next study visit

7.9 Week 15 Phone Visit

This visit will take place 105±5 days after the Baseline Visit. The following procedures will be performed.

- Administer ALSFRS-R questionnaire
- Review and document concomitant medications and therapies
- Assess and document AEs
- Enquire about tolerance and compliance
- Schedule next study visit

7.10 Week 18 Clinic Visit

This visit will take place 126±5 days after the Baseline Visit. The following procedures will be performed.

- Administer ALSFRS-R questionnaire
- Perform pulmonary function testing including slow vital capacity (SVC)
- Measure isometric strength using ATLIS machine
- Review and document concomitant medications and therapies
- Review and assess Adverse Events
- Measure vital signs
- Administer the C-SSRS questionnaire
- [After other tests] Collect blood samples for clinical laboratory assessments including Hematology (CBC with differential), Complete Chemistry Panel, Liver Function Tests
- Collect blood sample for biomarkers
- Collect urine sample for urinalysis
- Perform study drug accountability and collect all unused study drug and empty containers
- Dispense next 6 weeks of study drug
- Schedule next study visit

7.11 Week 21 Phone Visit

This visit will take place 147±5 days after the Baseline Visit. The following procedures will be performed.

- Administer ALSFRS-R questionnaire
- Review and document concomitant medications and therapies
- Assess and document AEs
- Enquire about tolerance and compliance
- Schedule next study visit
- Remind subject to bring study drug to clinic for the Week 24 Visit
- Schedule MR-PET scan for those subjects participating in the MR-PET Sub-Study

7.12 MR-PET Visit 2 (Only for patients in MR-PET Substudy)

This visit will take place between the Week 12 and Week 20 study visits.

- Complete MR-PET safety questionnaire
- Perform the MR-PET Scan
- Perform the Upper Motor Neuron-Burden (UMN-B) Scale
- Measure vital signs (blood pressure, heart and breathing rates, temperature), height, and weight
- Collect blood for
    - Biomarker (PBMC) testing
    - Pregnancy testing (for women of child bearing potential)
- Review and document concomitant medications and therapies
- Assess and document adverse events (AEs)

MR-PET Follow-Up Call

This visit will take place 24-48 hours after the MR-PET Visit 1. The following procedures will be performed.

- Assess and document AEs directly related to the MR-PET procedures

7.13 Final Study Visit (Week 24)

This visit will take place 168±5 days after the Baseline Visit. **Subject must take study drug upon beginning this visit due to the PK analysis.** It is recommended that this visit happens earlier in the day since the drug is administered in clinic. The following procedures will be performed:

- Record day/time of previous study drug dose, including if the subject missed a dose
- Record time of last meal
- Administer study drug and record time of administration
- Collect a single blood sample for PK (i.e. at 1 hour or 4 hours post-dose) as indicated at the time of randomization (Week 24 only, not Early Termination Subjects)
- Administer ALSFRS-R questionnaire
- Perform pulmonary function testing, including slow vital capacity (SVC)
- Measure isometric strength using ATLIS machine
- Review and document concomitant medications and therapies
- Review Adverse Events
- Measure vital signs
- Perform neurological examination
- Perform physical examination including weight
- Perform 12-lead ECG (Electrocardiogram)
- Administer the C-SSRS questionnaire
- Exit questionnaire
- [After other tests] Collect blood samples for clinical laboratory assessments including Hematology (CBC with differential), Complete Chemistry Panel, Liver Function Tests
- Collect blood sample for biomarkers
- Collect urine sample for urinalysis
- Perform study drug accountability and collect all unused study drug and empty containers

7.14 Final Follow-up Telephone Call (Week 28)

A follow-up phone call will take place 28 + 5 days (no earlier than 28 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

7.15 Withdrawal of Consent Final Safety Visit & Final Follow-up Telephone Call

Subjects who withdraw consent will be asked to come in for a Final Safety Visit as soon as possible after consent withdrawal and to have a final Follow-Up Telephone Call 28 + 5 days (no earlier than 28 days) after the last dose of study drug.

The following will be performed at the Final Safety Visit:

- Administer ALSFRS-R questionnaire
- Perform pulmonary function testing, including slow vital capacity (SVC)
- Measure isometric strength using ATLIS machine
- Review and document concomitant medications and therapies
- Review Adverse Events
- Measure vital signs
- Perform physical examination including weight
- Perform neurological examination
- Perform 12-lead ECG (Electrocardiogram)
- Administer the C-SSRS questionnaire
- [After other tests] Collect blood samples for clinical laboratory assessments including Hematology (CBC with differential), Complete Chemistry Panel, Liver Function Tests
- Collect blood sample for biomarkers
- Collect urine sample for urinalysis
- Perform study drug accountability and collect all unused study drug and empty containers

The following procedures will be performed via telephone 28 +5 days after the last administration of study drug:

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

7.16 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 sent to the central IRB and entered in the Protocol Deviations Log in the Electronic Data Capture (EDC) System.

7.16.1 Missed Visits and Procedures

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

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

Study drug compliance that is outside the limits set in the study operations manual will be reported as a protocol deviation.

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

7.17 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 and cardiac history, 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 for safety:

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

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

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

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

All subjects will have safety laboratory tests at the designated visits outlined in the protocol. These samples will be analyzed at a central laboratory. The Site Investigator (SI) may order additional testing, 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.

Specific instructions regarding the collection, processing, storage and shipment of these samples will be provided in the Site Manual of Procedures (MOP).

8.1.3 Biomarkers and Pharmacokinetic Analysis

Subjects will have blood drawn to assess AMX0035 concentrations for pharmacokinetics (PK) pre-dose at the Baseline Visit and then again at either 1 hour or 4 hours (± 10 minutes) post-dose at the Week 12 and 24 visits. Every attempt should be made to collect samples within the allotted timeframes; however, all samples should be analyzed regardless of actual collection time. The time of administration will be noted. The time of the last meal prior to administration and the time of the drug administration(s) in the previous 24 hours will also be noted.

Additionally, blood will be collected for biomarker analysis, including light and heavy neurofilament testing (NF-L and pNF-H, respectively). Neurofilaments will be used as a mechanistic measure of neuronal death. These proteins are greatly elevated in ALS subjects and promising results from multiple trials suggest this marker may be prognostic of clinical decline. NF-L and pNF-H will be tested over multiple time points with the intention of generating a longitudinal dataset correlating neurofilament levels to observed clinical outcomes. This dataset will help to validate AMX0035 therapeutic mechanism and provide a dataset for the ALS field.

All samples will be labeled with a code. The code will not include any identifiable information. Coded blood samples will be stored at a central laboratory prior to PK and biomarker analysis and other research use.

Specific instructions regarding the collection, processing, storage and shipment of these samples will be provided in the Site Manual of Procedures (MOP).

8.1.4 Blood Samples for Future Research Use

Subjects will provide an additional blood sample for storage in a biofluid biorepository at Barrow Neurological Institute. Any research performed on the samples is for research purposes only. These samples will be used for broad future research use in motor neuron diseases. All samples will be labeled with a code. The code will not include any identifiable information. Results of future research will not be provided to the subject or his/her physician.

There is no scheduled date on which the samples will be destroyed. Samples may be stored for research until they are used, damaged, decayed or otherwise unfit for analysis. If a subject no longer wishes to participate in the study and withdraws consent, it will not be possible to destroy samples that may have already been used.

Specific instructions regarding the collection, processing, storage and shipment of these samples will be provided in the Site Manual of Procedures (MOP).

8.1.5 12-Lead Electrocardiogram (ECG)

A standard 12-lead ECG will be performed. Tracings will be reviewed by a central ECG reader and a copy of the tracings will be kept on site as part of the source documents. The central ECG vendor will provide standard ECG devices for every site and provide training as necessary.

8.1.6 Physical Examination
A comprehensive physical examination will be performed and recorded.

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

8.1.8 Upper Motor Neuron-Burden (UMN-B)
The Penn Upper Motor Neuron-Burden (UMN-B) is the total number of pathological UMN signs on examination including pathologically brisk biceps, supinator, triceps, finger, knee and ankle reflexes, and extensor plantar responses assessed bilaterally and brisk facial and jaw jerks. The scale is a combination of Ashworth, Reflexes, and Pseudobulbar Affect scale (Range score: 0-32).

The UMN also includes scoring of the Center for Neurologic Study-Lability Scale (CNS-LS), a 7-item self-report scale that assesses pseudobulbar affect (PBA) by measuring the perceived frequency of PBA episodes (laughing or crying). Data is generated from the clinical exam and scored from 1-5, the lowest score indicating normal tone and the highest extreme spasticity.

8.1.9 Columbia Suicide Severity Rating Scale (C-SSRS)
The US FDA recommends the use of a suicidality assessment instrument that maps to the Columbia Classification Algorithm for Suicide Assessment (C-CASA)[22]. 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)[23]. The C-SSRS involves a series of probing questions to inquire about possible suicidal thinking and behavior.

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

At all clinic visits after the Baseline Visit, 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.

8.1.10 Exit Questionnaire
An exit questionnaire will be completed by subjects and Site Investigators at the Final Study Visit (Week 24). This will include questions regarding blindedness and overall experience with the trial.

8.1.11 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 (+ 5 days) after the last dose of study drug. Information on adverse effects of study drug and on inter-current events will be determined at each visit by direct questioning of the subjects, review of concomitant medications, and vital sign results.

8.2 Outcome Measures

8.2.1 ALSFRS-R (Amyotrophic Lateral Sclerosis Functional Rating Scale – Revised)

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 subjects, 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. The ALSFRS-R will therefore also be given to the study subject over the phone. All ALSFRS-R evaluators must be NEALS certified.

8.2.2 Pulmonary Function Testing - 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. All VC evaluators must be NEALS certified. Three VC trials are required for each testing session, however up to 5 trials may be performed if the variability between the highest and second highest VC is 10% or greater for the first 3 trials. Only the 3 best trials are recorded on the CRF. The highest VC recorded is utilized for eligibility.

8.2.3 Isometric Strength Testing (ATLIS)

Accurate Testing of Limb Isometric Strength: We are measuring isometric strength using the Accurate Testing of Limb Isometric Strength device (ATLIS) developed by Dr. Patricia Andres of Massachusetts General Hospital. The device was specifically designed to alleviate the reproducibility concerns that exist for prior strength measurements such as hand held dynamometry (HHD). ATLIS does not depend on experimenter strength, and has measurement settings to ensure that subjects are in the same position each time they are tested. All ATLIS evaluators must be trained and certified. ATLIS may detect functional decline before the ALSFRS-R, which may have a ceiling effect, and may be able to detect changes in function with greater sensitivity to ALSFRS-R. The measure does show a small training effect, so we will

include measurement at initial screening visit to allow subjects to become acquainted with the device.

8.2.4 Neuroimaging MR-PET Sub-Study

A subset of subjects will undergo MR-PET scans at the Baseline Visit and again between the Week 21 and 24 Visits. Prior to the scan, every MR-PET sub-study subject will complete the MR-PET Safety Questionnaire. Scanning procedures and subject instructions will be provided in the Site Manual of Procedures (MOP).

8.2.5 Survival Assessment

Survival endpoint will be considered as mortality, tracheostomy or permanent assisted ventilation.

8.2.6 Training and Validation

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

9 SAFETY AND ADVERSE EVENTS

The adverse event (AE) definitions and reporting procedures provided in this protocol comply with all applicable United States Food and Drug Administration (FDA) regulations and International Conference on Harmonization (ICH) guidelines. The Site 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, ALSFRS-R, and ATLIS 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).

An unexpected adverse event is any adverse event, the specificity or severity of which is not consistent with the current Investigator's Brochure. An unexpected, suspected adverse drug reaction is any unexpected adverse event for which, in the opinion of the Site Investigator or Sponsor (or their designee), there is a reasonable possibility that the investigational product caused the event.

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 in-patient 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 in-patient hospitalization, or the development of drug dependency or drug abuse.

An in-patient 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).

A serious, suspected adverse drug reaction (SUSAR) is an SAE for which, in the opinion of the Site Investigator or Sponsor, there is a reasonable possibility that the investigational product caused the event.

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 source document templates and eCRFs 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 Adverse Events in Prior Human Experience with Each Individual Component

TUDCA

- A small number of subjects (>1%) receiving TUDCA have presented with abdominal discomfort, abdominal pain, diarrhea, nausea, emesis, pruritus, and rash.

PB

- Common adverse events include: menstrual irregularities (23%), decreased appetite (4%), sweat-like body odor (3%), and bad taste (3%)
- Rare effects (<2%) have included Gastrointestinal: abdominal pain, gastritis, nausea and vomiting; constipation, rectal bleeding, peptic ulcer disease, and pancreatitis each occurred in one subject.
    - Hematologic: aplastic anemia and ecchymoses each occurred in one subject.
    - Cardiovascular: arrhythmia and edema each occurred in one subject.
    - Renal: renal tubular acidosis
    - Psychiatric: depression
    - Skin: rash
    - Miscellaneous: headache, syncope, and weight gain

- Hypoalbuminemia, metabolic acidosis, alkalosis, hyperchloremia, hyperuricemia, hypokalemia, hypophosphatemia, hyperphosphatemia and hypernatremia have been observed.

9.2.4 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.

10 DATA AND SAFETY MONITORING AND STATISTICAL ANALYSIS PLAN

10.1 Data and Safety Monitoring Board

An independent Data and Safety Monitoring Board (DSMB) will be assembled for the trial. The DSMB receives the blinded and unblinded summary reports of the frequency of all clinical adverse events and safety laboratory tests for planned periodic meetings as specified in the DSMB charter. In addition, the DSMB Chair may call ad hoc meetings. Meetings will be held via teleconference. A DSMB Charter will detail the processes of this group.

Summaries of serious adverse events and enrollment will be provided approximately monthly to the DSMB by the Study Biostatisticians. Any possibly, probably or definitely study drug related, serious adverse events (i.e. serious adverse drug reactions, or SUSARs) are considered events of interest and will be reported in real-time (within 1 business day of Coordination Center (CC) awareness) to the DSMB. All adverse events and abnormal laboratory values results will be listed and will be completely identified (using MedDRA adverse reaction codes) by subject and center. The DSMB can ask to receive the SAE reports more frequently. As necessary, the DSMB can review the frequencies of clinical and laboratory abnormalities. Recommendations for modification or termination of the trial based on safety data will be made by the DSMB to the PIs and Steering Committee. The DSMB will review safety data throughout the trial and may stop the trial for safety if they determine that there is a significant difference in the rate of a particular adverse event that would indicate a risk that is greater than the possible benefit of the study drug. A notable increase in the frequency of any adverse event should be examined by the DSMB although it may not lead to a recommendation by the DSMB.

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

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

10.2 Statistical Considerations

10.2.1 Statistical Methods

A challenge in ALS is generating robust data on treatment effects without running prohibitively large studies. Our analysis of the PROACT and ceftriaxone de-identified subject databases suggests that statistical powering can be significantly improved by enrolling subjects who are <1.5 years from symptom onset and have a definite diagnosis of ALS according to El Escorial Criteria . Mixed-effects modeling was used to account for both the variance between subjects and the deviation within subjects from their average rate of decline. We plan to recruit subjects at a rate of at least 10/month to allow for complete enrollment of the study population within 14 months.

Power for safety and tolerability was considered in three ways: incidence of adverse events (AEs), change in ALFSR-R and ATLIS, and change in biomarker such as pNF-H.

With 88 treated subjects, we will have an 80% probability of detecting any adverse event expected to occur in at least 2% of treated subjects. We will have 80% power to detect a 28 percentage point elevation in the rate of any adverse event relative to placebo based on a one-tailed test at alpha = 0.05. We will consider a dose tolerable if the proportion of treatment failures (discontinuation of study drug due to an adverse event) is less than 40% with 80% confidence, one-tailed. With 88 treated subjects this would occur if 30 or fewer subjects on AMX0035 fail to complete the 6-month study. By this criterion, we will have 80% power for declaring AMX0035 tolerable at the tested dose if the true treatment failure rate is 30%.

A shared-baseline, mixed-effects analysis will be used for primary analysis. A covariate of bulbar onset or onset elsewhere and a second covariate of age at enrollment will be included in the analysis. The mixed-effects model accounts for both the variance between subjects and the deviation within subjects from their average rate of decline. We will use the same analysis for clinical outcomes in this trial. We propose to test at an alpha of 0.05.

Further detail on primary analysis and analysis rationale for secondary endpoints will be included in the Statistical Analysis Plan (SAP).

10.2.2 Analysis for Safety

The safety data will be summarized by treatment group. Treatment AEs will be coded and graded using CTCAE grading criteria. The treatment groups will be compared with respect to occurrence of each adverse event and incidence of Grade III/IV adverse events. Total number of serious adverse events and abnormal laboratory tests will be compared between groups using Fisher's exact test. Withdrawal, abnormal laboratory tests, vital signs and use of concomitant medications will be assessed to characterize the safety profile of the combination of PB and TUDCA. Compliance data will be determined for each visit and by treatment group. The time to subject refusal will be compared between treatment groups to better determine tolerability. This will be accomplished using a method of survival analysis that allows informative censoring due to death. Descriptive statistics denoting the changes from baseline to the final assessment visit with respect to key laboratory parameters and vital signs will also be provided.

Further detail will be provided in a statistical analysis plan.

10.2.3 Analysis for Efficacy

Modified intention-to-treat analysis will be performed, including all randomized subjects receiving at least one dose of the study medication and having at least one primary efficacy assessment after randomization. Slope will be imputed from available data and time points. Homogeneity of clinical characteristics and efficacy variables at baseline between the two randomization groups (between-group baseline differences) will be assessed by analysis of variance for continuous variables and by a chi-squared test for discrete variables. All efficacy endpoints will compared between the two randomization groups at study end (between-group differences at study end) by means of analysis of covariance for continuous variables, adjusting for baseline value and for center effect, and by a chi-squared test for discrete variables. Survival time will compared between treatments by a Kaplan–Meier survival analysis.

The primary analysis strategy will use a shared-baseline, mixed-effects model of ALSFRS-R progression rate. The mixed-effects model accounts for both the variance between subjects and the deviation within subjects from their average rate of decline. We will use the same analysis for clinical outcomes in this trial. We propose to test at an alpha of 0.05. We are targeting an effect size (slowing of ALSFRS-R slope) greater than 30% based on the trial by Elia et al[17]. In the Phase I/II trial of TUDCA that analyzed a total of 29 subjects, the ALSFRS-R score declined 32.5% more slowly in the TUDCA group: the slopes of the two regression lines were significantly different (-0.262/week for the TUDCA group, -0.388/week for the placebo group; $P < 0.01$).

10.2.4 Analysis Populations

The modified intent to treat (ITT) population will include all study subjects who are randomized and receive at least one dose of study drug. The ITT population will be considered for primary analyses. For ITT analyses, subjects will be grouped based on randomized treatment, regardless of treatment actually received.

10.3 Missing Data

The trial will be modified intent to treat (ITT). Every effort will be made to obtain follow-up information for all subjects whether or not they continue on treatment.

11 DATA COLLECTION, MANAGEMENT AND MONITORING

11.1 Role of Data Management

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

All data will be managed in compliance with 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 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 Site Investigator must maintain complete and accurate documentation for the study.

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

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.

11.2 Clinical Monitoring

Study Monitors will visit each study site to review source documentation materials, informed consent forms, and confirm entered data and that data queries have been accurately completed, and again at a study close-out visit. Study Monitors will also verify that SAEs and protocol deviations have been reported appropriately, as required. The Study Monitors will also review clinical facilities, resources and procedures for evaluating study subjects and study drug dispensing. Subsequently, the Study Monitors will provide monitoring reports to the Project Manager and, if requested, will provide reports of protocol compliance to the Study Principal Investigator and the Steering Committee. Completed informed consent forms from each subject must be available in the subject's file and verified for proper documentation. A document outlining the monitoring plan is provided to each Study Monitor.

11.3 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.

11.3.1 Confidentiality

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

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

11.3.2 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.

11.3.3 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.

11.3.4 Publications

The Study Principal Investigator, Sabrina Paganoni, along with the Sponsor, Amylyx Pharmaceuticals, Inc., will be responsible for publications of results from this trial. Their responsibilities will include the following:

- Analyze and interpret data gathered in this study, and write publications from these data.
- Submit manuscripts to selected journals and address peer reviewers' comments.
- Submit abstracts to selected meetings and present data at the meetings.
- Determine authorship on the basis of the Uniform Requirements for Manuscripts.

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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, electrophysiology 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 (subjects 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 subjects 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 (C-SSRS) BASELINE VERSION

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

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

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

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

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

13.4 APPENDIX IV: COLUMBIA-SUICIDE SEVERITY RATING SCALE (C-SSRS) SINCE LAST VISIT VERSION

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

SUICIDAL IDEATION	
Ask questions 1 and 2. If both are negative, proceed to "Suicidal Behavior" section. If the answer to question 2 is "yes", ask questions 3, 4 and 5. If the answer to question 1 and/or 2 is "yes", complete "Intensity of Ideation" section below.	Since Last Visit
1. Wish to be Dead Subject endorses thoughts about a wish to be dead or not alive anymore, or wish to fall asleep and not wake up. ***Have you wished you were dead or wished you could go to sleep and not wake up?*** If yes, describe:	**Yes No** □ □
2. Non-Specific Active Suicidal Thoughts General, non-specific thoughts of wanting to end one's life/commit suicide (e.g., *"I've thought about killing myself"*) without thoughts of ways to kill oneself/associated methods, intent, or plan during the assessment period. ***Have you actually had any thoughts of killing yourself?*** If yes, describe:	**Yes No** □ □
3. Active Suicidal Ideation with Any Methods (Not Plan) without Intent to Act Subject endorses thoughts of suicide and has thought of at least one method during the assessment period. This is different than a specific plan with time, place or method details worked out (e.g., thought of method to kill self but not a specific plan). Includes person who would say, *"I thought about taking an overdose but I never made a specific plan as to when, where or how I would actually do it…and I would never go through with it."* ***Have you been thinking about how you might do this?*** If yes, describe:	**Yes No** □ □
4. Active Suicidal Ideation with Some Intent to Act, without Specific Plan Active suicidal thoughts of killing oneself and subject reports having some intent to act on such thoughts, as opposed to *"I have the thoughts but I definitely will not do anything about them."* ***Have you had these thoughts and had some intention of acting on them?*** If yes, describe:	**Yes No** □ □
5. Active Suicidal Ideation with Specific Plan and Intent Thoughts of killing oneself with details of plan fully or partially worked out and subject has some intent to carry it out. ***Have you started to work out or worked out the details of how to kill yourself? Do you intend to carry out this plan?*** If yes, describe:	**Yes No** □ □
INTENSITY OF IDEATION	
The following features should be rated with respect to the most severe type of ideation (i.e., 1-5 from above, with 1 being the least severe and 5 being the most severe). ***Most Severe Ideation:*** _____ _____ ***Type # (1-5) Description of Ideation***	Most Severe
Frequency ***How many times have you had these thoughts?*** (1) Less than once a week (2) Once a week (3) 2-5 times in week (4) Daily or almost daily (5) Many times each day	____
Duration ***When you have the thoughts, how long do they last?*** (1) Fleeting - few seconds or minutes (4) 4-8 hours/most of day (2) Less than 1 hour/some of the time (5) More than 8 hours/persistent or continuous (3) 1-4 hours/a lot of time	____
Controllability ***Could/can you stop thinking about killing yourself or wanting to die if you want to?*** (1) Easily able to control thoughts (4) Can control thoughts with a lot of difficulty (2) Can control thoughts with little difficulty (5) Unable to control thoughts (3) Can control thoughts with some difficulty (0) Does not attempt to control thoughts	____

Deterrents ***Are there things - anyone or anything (e.g., family, religion, pain of death) - that stopped you from wanting to die or acting on thoughts of committing suicide?*** (1) Deterrents definitely stopped you from attempting suicide (4) Deterrents most likely did not stop you (2) Deterrents probably stopped you (5) Deterrents definitely did not stop you (3) Uncertain that deterrents stopped you (0) Does not apply	____
Reasons for Ideation ***What sort of reasons did you have for thinking about wanting to die or killing yourself? Was it to end the pain or stop the way you were feeling (in other words you couldn't go on living with this pain or how you were feeling) or was it to get attention, revenge or a reaction from others? Or both?*** (1) Completely to get attention, revenge or a reaction from others (4) Mostly to end or stop the pain (you couldn't go on (2) Mostly to get attention, revenge or a reaction from others living with the pain or how you were feeling) (3) Equally to get attention, revenge or a reaction from others (5) Completely to end or stop the pain (you couldn't go on and to end/stop the pain living with the pain or how you were feeling) (0) Does not apply	____

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* _____

13.5 APPENDIX V: CENTER FOR NEUROLOGICAL STUDY – LABILITY SCALE

INSTRUCTIONS

The purpose of this questionnaire is to help us better understand your neurologic problems. Please read each statement, and using the scale below, determine the degree to which it has applied to you **DURING THE PAST WEEK**. Circle the appropriate answer, or if you need help in marking your responses, tell the interviewer the number of the best response. Please choose only one response for each item.

Please select the number that describes the degree to which each item has applied to you DURING THE PAST WEEK.					
	Does not Apply 1	Rarely Applies 2	Occasionally Applies 3	Frequently Applies 4	Applies Most of the Time 5
1. There are times when I feel fine 1 minute, and then I'll become tearful the next over something small or for no reason at all.	○	○	○	○	○
2. Others have told me that I seem to become amused very easily or that I seem to become amused about things that aren't funny.	○	○	○	○	○
3. I find myself crying very easily.	○	○	○	○	○
4. I find that even when I try to control my laughter, I am often unable to do so.	○	○	○	○	○
5. There are times when I won't be thinking of anything happy or funny at all, but then I'll suddenly be overcome by funny or happy thoughts.	○	○	○	○	○
6. I find that even when I try to control my crying, I am often unable to do so.	○	○	○	○	○
7. I find that I am easily overcome by laughter.	○	○	○	○	○

Evaluator's Initials: _____ _____ _____ ***Total:*** _____

Evaluation of the Safety, Tolerability, Efficacy and Activity of AMX0035, a Fixed Combination of Phenylbutyrate (PB) and Tauroursodeoxycholic Acid (TUDCA), for Treatment of Amyotrophic Lateral Sclerosis (ALS)

Regulatory Sponsor: Amylyx Pharmaceuticals Inc.

Funding Sponsor: Amylyx Pharmaceuticals Inc.

Study Product: AMX0035

Protocol Number: AMX3500

IND Number: 129563

Draft or Version Number: *6.0*

11 Jan 2019

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STATEMENT OF COMPLIANCE

This study will be conducted in compliance with the protocol, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), Good Clinical Practice (GCP), and the applicable regulatory requirements, United States Code of Federal Regulations (CFR) Title 45 CFR Part 46 and Title 21 CFR Parts 50, 56, and 312.

SIGNATURE PAGE

I have read the attached protocol entitled, **Evaluation of the Safety, Tolerability, Efficacy and Activity of AMX0035, a Fixed Combination of Phenylbutyrate (PB) and Tauroursodeoxycholic Acid (TUDCA), for Treatment of Amyotrophic Lateral Sclerosis (ALS)** dated **January 11, 2019 (Version 6.0)** and agree to abide by all described protocol procedures. I agree to comply with the International Conference on Harmonisation Tripartite Guideline on Good Clinical Practice, applicable FDA regulations and guidelines identified in 21 CFR Parts 11, 50, 54, and 312, Institutional Review Board (IRB) and local institutional guidelines and policies, and the Health Insurance Portability and Accountability Act (HIPAA).

Site Investigator:

Signed: _____ Date: _____

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LIST OF ABBREVIATIONS

ADR	Adverse Drug Reaction
AE	Adverse Event/Adverse Experience
b.i.d	Twice a Day
CFR	Code of Federal Regulations
CIB	Clinical Investigator's Brochure
cIRB	Central Institutional Review Board
CRF	Case Report Form
DSMB	Data and Safety Monitoring Board
eCRF	Electronic Case Report Form
ER	Endoplasmic Reticulum
FDA	Food and Drug Administration
FWA	Federal-wide Assurance
g	Gram
GCP	Good Clinical Practice
GUID	Globally Unique Identifier
HIPAA	Health Insurance Portability and Accountability Act
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IDE	Investigational Device Exemption
IND	Investigational New Drug Application
IRB	Institutional Review Board
ITT	Modified Intent to Treat
MOP	Manual of Procedures
MPRAGE	Magnetization Prepared Rapid Gradient Echo
N	Number (typically refers to subjects)
NDA	New Drug Application
NYGC	New York Genome Center
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
OHSR	Office of Human Subjects Research
OLE	Open Label Extension
PAA	Phenylacetate (metabolite of PB)
PB	Sodium Phenylbutyrate
PET	Positron Emission Tomography
PCP	Primary Care Provider
PHI	Protected Health Information
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control

ROI	Region of Interest
SAE	Serious Adverse Event/Serious Adverse Experience
SAP	Statistical Analysis Plan
SI	Site Investigator
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
t.i.d	Three Times a Day
UDCA	Ursodeoxycholic Acid (ursodiol)
TUDCA	Tauroursodeoxycholic Acid
US	United States

PROTOCOL SUMMARY

Study Title
Evaluation of the Safety, Tolerability, Efficacy and Activity of AMX0035, a Fixed Combination of Phenylbutyrate (PB) and Tauroursodeoxycholic Acid (TUDCA), for Treatment of Amyotrophic Lateral Sclerosis (ALS)

Version Number
4.0

Study Indication
Amyotrophic Lateral Sclerosis (ALS)

Phase of Development
II

Rationale for the Study
The objective of this study is to determine the safety and efficacy of AMX0035 in subjects with Amyotrophic Lateral Sclerosis (ALS). ALS is a progressive neurodegenerative disease for which there is no cure. There are only two medications approved specifically for treating ALS. This includes Rilutek (riluzole), which only provides a modest benefit for subjects, and Radicava (edaravone). ALS also exacts a significant economic burden.

AMX0035 has demonstrated efficacy in models of neurodegeneration, classical activation of neuroinflammation, and bioenergetics deficits. The individual components of AMX0035, PB and TUDCA have demonstrated efficacy in *in vivo* models of ALS, Parkinson's, Alzheimer's, ischemia, and many others. Each individual component has also been tested in small clinical trials of ALS subjects and was found to be safe and well-tolerated, and hit primary endpoints of efficacy.

The first trial under this IND will be a randomized double-blind placebo-controlled Phase II trial to evaluate the safety and efficacy of AMX0035 for treatment of ALS. The program is designed to demonstrate that treatment is safe, can slow the decline in function, muscle strength, and vital capacity, and to assess the impact of AMX0035 therapy on biomarkers of ALS including blood levels of phosphorylated axonal neurofilament H subunit and 18 kDa translocator protein PET tracer uptake. This Phase II trial would also serve as the basis for the design of a pivotal trial in this subject population.

Study Design
This is a multicenter, randomized, double-blind, placebo-controlled 28-week study evaluating the safety, tolerability, efficacy, pharmacokinetics and biological activity of AMX0035.

Study Objectives and Endpoints

The primary objective of the study is to assess safety, tolerability, and efficacy of oral (or feeding tube) administration of AMX0035 via sachet (3g PB and 1g TUDCA) twice daily vs. matched placebo administered via sachet twice daily.

The primary outcome measures will be:

1. To confirm the safety and tolerability of AMX0035 in subjects with ALS over a 24-week period
2. To measure the impact of treatment on disease progression using the slope of the revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R)

The secondary objectives of the study will be:

1. To assess the impact of AMX0035 on the rate of decline of isometric muscle strength, as measured by the Accurate Test of Limb Isometric Strength (ATLIS)
2. To assess the impact of AMX0035 on disease progression as measured by Slow Vital Capacity (SVC) decline, time to tracheostomy, and survival
3. To assess the impact of AMX0035 on biomarkers including blood levels of phosphorylated axonal neurofilament H subunit (pNF-H) and 18 kDa translocator protein (TSPO) PET tracer uptake
4. To determine the population pharmacokinetics parameters of PB and TUDCA at steady state during treatment with AMX0035
5. To measure the impact of the treatment on survival.

Study Locations

Up to 25 Northeast ALS Consortium (NEALS) centers in the United States will participate in the study.

Number of Planned Subjects

Approximately 132 subjects will be randomized in the study.

Study Population

This study will be conducted in subjects who have sporadic or familial ALS diagnosed as definite as defined by revised El Escorial criteria (Appendix 1). Subjects must provide written informed consent prior to screening. At screening, eligible subjects must be at least 18 years old and less than 80 years old, and have a VC $\geq$ 60% of predicted capacity for age, height and gender. Subjects must have had onset of ALS symptoms less than or equal to 18 months prior to the screening visit, defined as first onset of weakness. 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. There will be no restrictions for subjects taking Radicava (edaravone) at the time of screening, or if started while enrolled in the study. Detailed criteria are described in the body of the protocol.

Treatment Groups

Subjects will be randomly assigned in a 2:1 ratio to oral (or feeding tube) AMX0035 treatment (1 sachet= 3g PB and 1g TUDCA plus excipients) or matching placebo. For the first three weeks of dosing, subjects will take one sachet daily (i.e. half-dose) and if tolerated will increase to two sachets daily.

Duration of Treatment and Follow-up

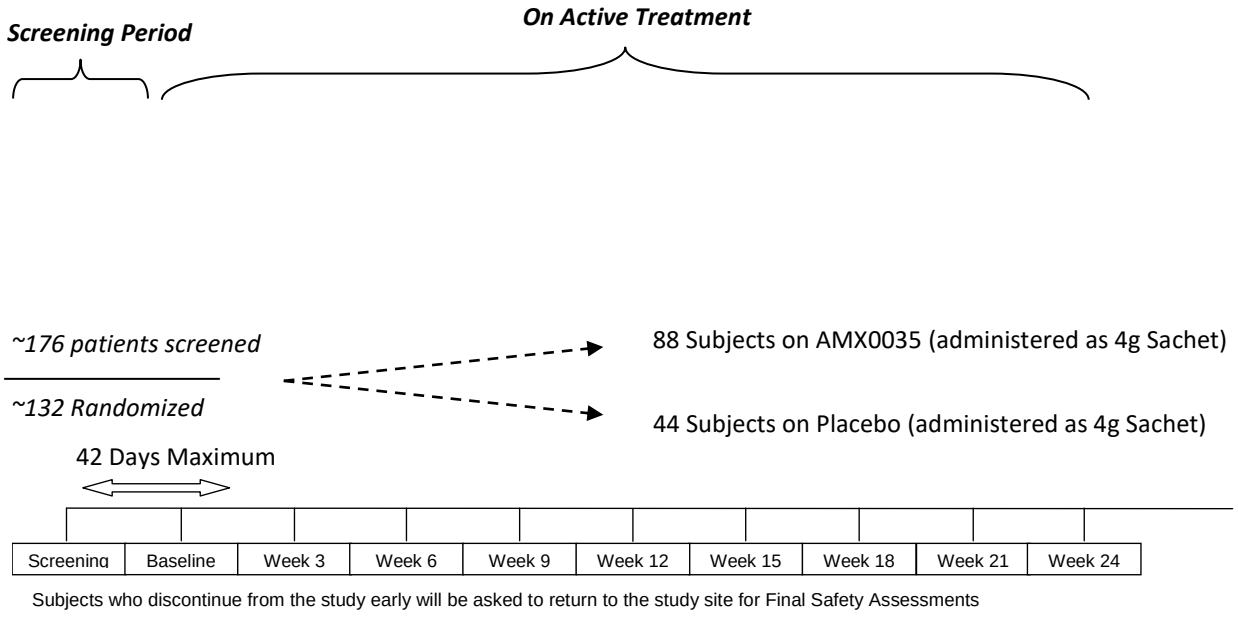
Subjects will remain on treatment until the Week 24 visit. Each randomized subject will also have a Final Telephone Interview 28 days (+ 5 days) after last dose of study drug to assess for adverse events (AEs), changes in concomitant medications and to administer the ALSFRS-R.

SCHEDULE OF ACTIVITIES

ACTIVITY	Screening Visit	Study Drug Administration (weeks)									Final Follow-up Telephone Call[2]	MR-PET Sub-Study Subjects Only
		Baseline Visit[1]	Week 3	Week 6	Week 9	Week 12	Week 15	Week 18	Week 21	Week 24 OR Early Discontinuation/ Final Safety Visit		
	Clinic	Clinic	Clinic	Clinic	Phone	Clinic	Phone	Clinic	Phone	Clinic	Phone	At MGH
	-42 Days	Day 0	Day 21 ±5	Day 42 ±5	Day 63 ±5	Day 84 ±5	Day 105 ±5	Day 126 ±5	Day 147 ±5	Day 168 ±5	28 +5 days	
Written Informed Consent	X											X
Inclusion/Exclusion Review	X	X										X
Medical History History/Demographics	X											
ALS Diagnosis/ALS History	X											
Vital Signs[3]	X	X	X	X		X		X		X		
Neurological Exam[4]	X					X				X		X[4]
Physical Exam[5]	X					X				X		
Blood Draw for Safety Labs[6]	X	X	X	X		X		X		X		
Blood Draw for Serum Pregnancy Test for WOCB[6]	X											
Urine Sample for Urinalysis[6]	X	X	X	X		X		X		X		
12-Lead ECG	X					X				X		
ALSFRS-R	X	X	X	X	X	X	X	X	X	X	X	X
Slow Vital Capacity	X	X		X		X		X		X		
ATLIS Testing	X	X		X		X		X		X		
Columbia-Suicide Severity Scale[7]		X[7]	X	X		X		X		X		
Exit Questionnaire										X		
MR-PET Scan[8]		X				X						X[8]
Blood draw for Biomarker Testing[9]		X		X		X		X		X		
Blood draw for PK Analysis[10]		X				X				X[11]		
Blood draw for optional DNA collection[12]		X	X	X		X		X		X		
Adverse Events[13]	X	X	X	X	X	X	X	X	X	X	X	X
Blood draw for TSPO affinity testing[14]	X											
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X
Randomization[15]		X										
Dispense Study Drug[16]		X		X		X		X				
Drug Accountability/ Compliance			X[17]	X	X	X	X	X	X	X		

[1]The Baseline Visit should occur no more than 42 days after the Screening Visit.
[2]A Final Safety Telephone Call will be conducted 28 (+5 days) after the subject takes their last dose of study drug (whether or not the subject has discontinued from the study) to assess for adverse events (AEs), changes in concomitant medications and to administer the ALSFRS-R. This call will only be required for subjects who do NOT enroll in the OLE.
[3]Vital signs include systolic and diastolic pressure in mmHg, respiratory rate/minute, heart rate/minute and temperature.
[4] The standard Neurological Exam will be used for all patients. The Upper Motor Neuron Burden Scale (UMN-B) will be included for the MR-PET Sub-Study only and administered at the time of the scan.
[5]Physical Exam will include height and weight. Height will be collected at Screening Visit ONLY.
[6]Safety labs include Hematology (CBC with differential), Complete Chemistry Panel, Liver Function Tests and Urinalysis. Serum pregnancy testing will occur in women of child bearing potential (WOCBP) at the Screening Visit and as necessary during the course of the study.
[7]C-SSRS Baseline version to be completed at Baseline Visit only. C-SSRS Since Last Visit version to be completed at all other visits.
[8]Approximately 20 subjects will receive MR-PET (Magnetic Resonance-Positron Emission Tomography) scanning completed at selected sites. First scan will occur PRIOR to the Baseline Visit (pre-dose) and the second scan will occur between the Week 12 and Week 21 study visits. MR-PET subjects will also provide blood samples for peripheral blood mononuclear cell (PBMC) extraction prior to each MR-PET scan.
[9]Subjects will provide a blood sample for biomarker testing and storage in a biorepository.
[10]All subjects will provide a blood sample for pharmacokinetic (PK) testing at the Baseline Visit (pre-dose). Subjects will also provide a blood sample either 1 hour or 4 hours post-dose (±10 minute window per time point) at the Week 12 and Week 24 Visits. PK times will be randomized such that every subject has a 1-hour draw at one visit and a 4-hour draw at the other.
[11]PK should not be drawn for early termination subjects
[12] If Baseline visit has already occurred or the sample was not collected, DNA should be obtained at next available visit. This is a one-time collection.
[13]Adverse events that occur AFTER signing the consent form will be recorded.
[14]For MR-PET Sub-Study subjects only, blood will be drawn for TSPO testing at the subject's site during the Screening Visit.
[15]Randomization should occur at the Baseline Visit. Randomization will entail entering a subject's kit number into the data capture system.
[16]First dose of study drug will be administered in clinic after ALL Baseline Visit procedures are completed.
[17]Notify subjects of increase from one sachet per day to two sachets per day

STUDY WORKFLOW



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. Subjects 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 ALS Overview

ALS is the most prevalent, adult-onset, progressive motor neuron disease, affecting more than 20,000 subjects in the US and an estimated 450,000 people worldwide, according to the ALS Association. ALS causes the progressive degeneration of motor neurons, resulting in rapidly progressing muscle weakness and atrophy that eventually leads to partial or total paralysis; on average, the disease is fatal within just 3-5 years. There are two FDA-approved medications for ALS, riluzole, which only extends survival modestly, and Radicava (edaravone). ALS also exacts a significant economic burden.

Although the precise cause of ALS is unknown, ALS and other neurodegenerative diseases such as Alzheimer's are strongly characterized by nerve cell death and inflammation. Together these processes form a toxic cycle that is a key driver of progressive neurological decline. Recent research has highlighted mitochondrial stress and endoplasmic reticulum (ER) stress as key mediators of both the nerve cell death and neuroinflammatory processes[1]. The mitochondrion is the energy production center of the cell, while the ER is the quality control center. These two organelles are in constant communication, and are in fact physically connected by a membrane, and their health is vital to cell survival. When either of these cellular processes goes awry, the resulting stress can either kill the cell and/or create inflammation. The brain is extremely sensitive to both mitochondrial stress and ER stress, and both of these pathways have been strongly implicated in causing neurodegenerative disease. We believe that only therapeutically targeting both organelles simultaneously will enact a significant and lasting benefit.

2.1.2 AMX0035 Rationale

AMX0035 is a proprietary combination of two small molecules, phenylbutyrate (PB) and tauroursodeoxycholic acid (TUDCA), designed to block neuronal death and neurotoxic inflammation through simultaneous inhibition of endoplasmic reticulum (ER) stress and mitochondrial stress.

Both PB and TUDCA have been evaluated individually in many disease-specific models of ALS and other neurodegenerative diseases, and in many nonspecific models of ER Stress and bioenergetic stress, respectively.

PB is a pan-HDAC inhibitor and ameliorates ER stress through upregulation of the master chaperone regulator DJ-1 and through recruitment of other chaperone proteins[2,3]. The large increase in chaperone production reduces activation of canonical ER stress pathways, folds misfolded proteins, and has been shown to increase survival in many in vivo models including

the G93A SOD1 mouse model of ALS[4]. Phenylbutyrate has also been effective in additional in vivo models of Huntington's Disease, Alzheimer's, and Parkinson's[5,6,7].

TUDCA recovers mitochondrial bioenergetic deficits through incorporating into the mitochondrial membrane, reducing Bax translocation to the mitochondrial membrane, reducing mitochondrial permeability, and increasing the apoptotic threshold of the cell[8]. TUDCA has exhibited efficacy in many in vivo oxidative insult models, including mouse models of stroke, retinal disease, cardiac disease, brain lipopolysaccharide insult, the MPTP mouse model of Parkinson's, and ALS in vitro models of poly(GA)-induced toxicity[9,10,11].

Either ER stress or bioenergetic stress can result in neuronal death and a cytotoxic immune response. We therefore combined PB and TUDCA and have since demonstrated that they have synergistic efficacy when dosed in particular ratios. The combination of agents demonstrated a mathematically synergistic increase in neuronal viability in a strong oxidative insult model (H2O2-mediated toxicity) by linear modeling.

Cytotoxic neuroinflammation has been found to be a major part of neurodegeneration[12,13,14]. Different ratios of AMX0035 reduced classical activation of cytotoxic cytokines and increased phagocytic cytokines in an LPS-insult, glial model of inflammation.

2.1.3 Prior Clinical Use of PB and TUDCA in Subjects with ALS

Both PB and TUDCA have been evaluated in subjects with ALS and were found to be safe, well-tolerated, and exhibited preliminary signs of efficacy. PB was evaluated in a 20-week safety and biomarker study in ALS subjects[15]. This was a Phase I dose escalation trial and each subject was scheduled to receive PB at increasing dose from 9 to 21 g/day. A total of 40 subjects were recruited at 8 sites in the US. Twenty-six subjects completed the 20-week treatment phase. Histone acetylation was decreased by approximately 50% in blood buffy-coat specimens at screening and was significantly increased after PB administration. Blood levels of PB and the primary metabolite, phenylacetate, increased with dosage (Figure 1) with a plateau between the 3 and 6 gram t.i.d. regimen. While the majority of subjects tolerated higher dosages of PB, the lowest dose (9 g/day), was the most effective at increasing histone acetylation levels in blood (Figure 2). Treatment with PB did not alter blood riluzole levels. Adverse events in subjects taking riluzole and NaPB together did not occur more frequently, compared to those on PB alone.

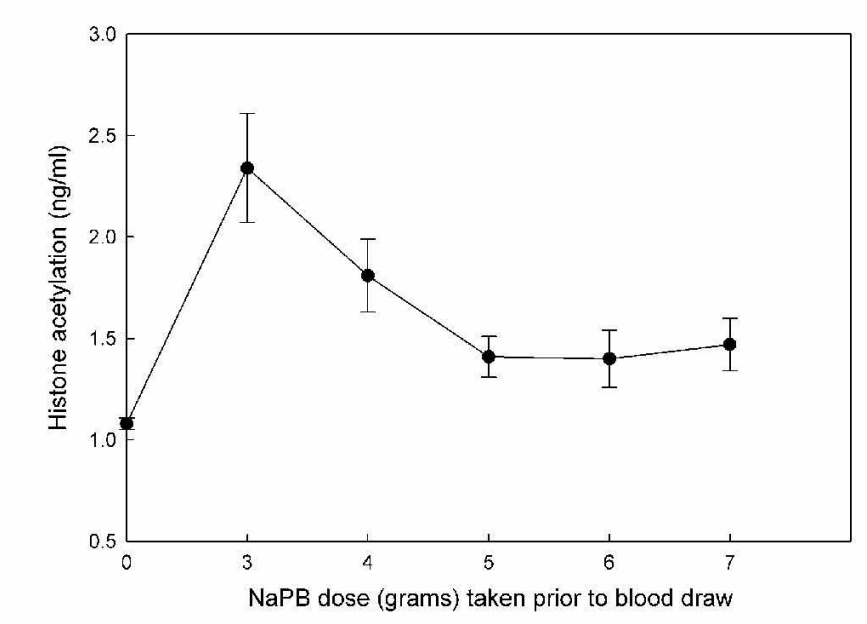


Figure 1: Histone acetylation levels with PB dose. Blood histone acetylation levels are shown compared with dose taken prior to blood draw. The error bars represent standard error. (Doses are repeated t.i.d in this study)

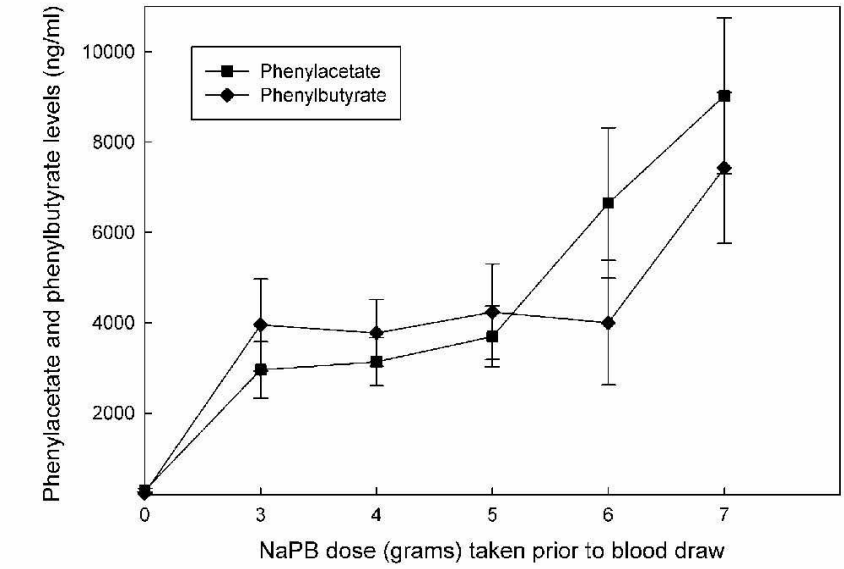


Figure 2: Phenylbutyrate and phenylacetate levels. Blood phenylbutyrate and phenylacetate levels are shown compared with dose taken prior to blood draw. The error bars represent standard error (doses are repeated t.i.d in this study).

It is not clear why acetylation levels were highest at 9 g/day. However, the author noted that in a study of PB in Huntington's disease, the effects of PB on mRNA expression levels of a 12-gene biomarker set were greatest at lowest dosages (4 g t.i.d.) with an inverse dose response[16].

For the planned Phase II trial of PB in combination with TUDCA, we therefore selected a dose of 3 grams of PB twice a day (6 grams per day) as a target dose with the desired pharmacologic effect.

Recently, TUDCA at 1g b.i.d. demonstrated a statistically significant slowing of ALSFRS-R progression rate in a year-long, multi-site, placebo-controlled clinical trial of ALS[17]. In this proof-of-principle trial, 34 ALS subjects under treatment with riluzole were randomized to placebo or TUDCA (1 gram b.i.d.) for 54 weeks. The proportion of responders (defined as subjects with >15% improvement in ALSFRS-R slope) was higher under TUDCA (87%) than under placebo (P = 0.021; 43%). At study end, baseline-adjusted ALSFRS-R was significantly higher (P = 0.007) in TUDCA than in placebo groups. Comparison of the slopes of regression analysis showed slower progression in the TUDCA than in the placebo group (P < 0.01) (Figure 3).

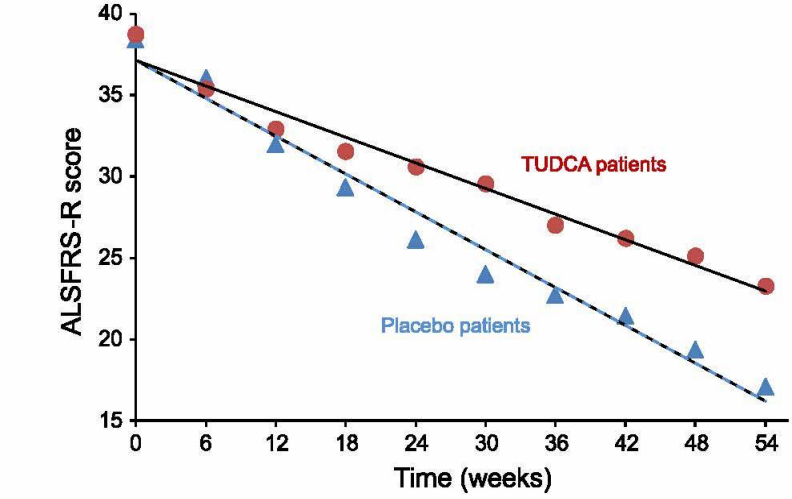


Figure 3: Linear regression analysis of ALSFRS-R mean scores over time for the TUDCA (circles, slope -0.388) and placebo groups (triangles, slope -0.262).

For the planned Phase II trial of PB in combination with TUDCA, we therefore selected a dose of 1 gram of TUDCA twice a day (2 grams per day) as a target dose.

Ursodiol (UDCA), the non-taurine conjugated form of TUDCA, was also found to be safe and well-tolerated in a crossover study subjects with ALS[18]. Subjects who received UDCA treatment also showed significant benefit as measured by the Appel ALS rating scale.

Subjects randomized to active therapy in the Phase II trial will receive 3g PB and 1g TUDCA twice a day orally (or by feeding tube). AMX0035 will be presented as a 4 gram sachet to be suspended in water and taken with a glass of water before a meal. Single agent TUDCA or PB treatment in subjects with ALS was very well tolerated.

In the TUDCA study from Elia et al., the AE profile and laboratory anomalies were not different between the TUDCA and placebo cohort. In the small group of 15 subjects treated with TUDCA, the adverse events were limited to diarrhea.

In the PB study from Cudkowicz et al., tolerability was similar to that reported in other trials of PB in other indications. There were no changes in safety laboratory tests, EKG or vital signs. The most common AEs were those previously reported with PB, including falls, dizziness, diarrhea, edema, dry mouth, headache, nausea and rash. A single subject interrupted treatment with PB at the 9 gram per day dose (i.e. a dose higher than that planned in the proposed Phase II) for the occurrence of edema on the foot and under the eye.

2.1.4 Additional Previous Clinical Experience with Phenylbutyrate

Sodium phenylbutyrate (PB) is generally well tolerated. It is FDA approved for subjects with urea cycle disorders including deficiencies of carbamylphosphate synthetase, ornithine transcarbamylase, or argininosuccinic acid synthetase. It is indicated in patients with either neonatal-onset deficiency or late-onset disease. The usual total daily dose is 450-600 mg/kg/day in patients weighing less than 20kg, or 9.9-13.0 g/m^2/day in larger patients. Detailed information can be found on the package insert for PB[19].

Sodium phenylbutyrate is also under development as an anticancer agent. In a dose escalation study in subjects with refractory solid tumor malignancies doses of up to 45g/day were administered[20]. Due to dose-limiting toxicities, the study concluded that 27g/day was the maximally tolerated dose. Nausea, vomiting, hypocalcemia and fatigue occurred at the 36g/day and 45g/day doses. Gastrointestinal upset (nausea, dyspepsia and vomiting) occurred at the lowest dose of 9g/day and was seen within 30 minutes of drug ingestion. However, 82% of subjects completed the study despite these side effects. Other frequently reported side effects include a "sweat"-like odor, usually noticeable only to the caregiver. Mild neurotoxicity (confusion, lethargy) has been noted at higher doses of close to 30g/day, but resolved with dose reduction.

A dose-escalation study of intravenous PB in subjects with myelodysplastic syndromes and acute myelogenous leukemia found a maximally tolerated dose at 375 mg/kg/day (26.3g/day for a 70kg individual) with no serious toxicities detected in subjects receiving doses between 125 and 375 mg/kg/day (8.8 and 26.3g/day for a 70kg individual) [0]. Dose-limiting toxicities (lethargy, confusion, slurred speech) were detected at 440 and 500 mg/kg/day PB (30.8 and 35g/day respectively, for a 70kg individual). Reports of edema have been blamed on the high sodium

load associated with the drug. Phase I/II studies in subjects with sickle cell anemia (see table 1 below) and beta thalassemia report similar side effects.

Another phase I study in subjects with refractory solid tumors tested IV PB doses between 150 to 515 mg/kg/day (up to 36g/day for a 70kg individual) with dose-limiting toxicities (excessive somnolence, confusion) and electrolyte abnormalities resulting at a dose of 515 mg/kg/day (36.0 g/day for a 70kg individual). The maximally tolerated dose of PB was determined to be 410 mg/kg/day (28.7 grams/day for a 70kg individual) as there were no dose-limiting toxicities at this dose and no subjects required dose reductions or escalations (see table 1).

The most common side effects of PB include: menstrual irregularities, decreased appetite, sweat-like body odor, and bad taste. Less common side effects include: nausea, vomiting, stomach upset, stomach pain, gastritis, headache, and skin rash. Rarely, cases of peptic ulcers, rectal bleeding, constipation, pancreatitis and renal tubular acidosis have been reported. Hypoalbuminemia, metabolic acidosis, alkalosis, hyperchloremia, hyperuricemia, hypokalemia, hypophosphatemia, hyperphosphatemia and hypernatremia have been observed. At higher doses, some subjects experienced confusion and fatigue, both of which resolved with dose reductions. Rarely, the following may occur, but have not been directly linked to sodium phenylbutyrate therapy: anemia, leukopenia, leukocytosis, thrombocytopenia, thrombocytosis, arrhythmia, syncope and depression.

Table 1: Prior Clinical Experience with Phenylbutyrate

Dose	Duration	Patient Population	# of patients	AE summary	Location	Status	Reference	NCT # (if ref unavailable)
9-21g/day	5 months	Amyotrophic Lateral Sclerosis	40	Well tolerated at 9 g	US	Completed	Amyotrophic Lateral Sclerosis. 2009; 10: 99106	
12-18g/day	28 days per dose level	Huntington's	24	Table included, Nausea, Headache, gain instability, were most common. Most side effects uncommon at 12g/day	US	Completed	Hogarth et al. Sodium phenylbutyrate in Huntington's disease: a dose-finding study. Mov. Disord. 2007.	
15g/day	12 months	SCA3	20	NA	Ex-US	Withdrawn	NA	NCT01096095
500mg/kg/day	14 days	Maple Syrup Urine Disease	40	NA	US	Complete	Brunetti-Pieri, et al.	NCT01529060

Dose	Duration	Patient Population	# of patients	AE summary	Location	Status	Reference	NCT # (if ref unavailable)
							Phenylbutyrate therapy for maple syrup urine disease. Hum. Mol. Gen. 2010.	
20g/day	4 days	Urea Cycle Disorders	9	NA	US	Active	NA	NCT02111200
IV Phenylbutyrate	7 years	Advanced Colorectal Cancer	46	NA	US	Cancelled	NA	NCT00002796
12.4g/day (mean dose, 198mg/kg-476mg/kg range)	12 months	Urea Cycle Disorders	11	One case of vomiting, see horizon package insert	US	Completed	Lichter-Konecki, U. et al. Mol Genet Metab. 2011 Aug;103(4)	
<20g/day	10 weeks	Urea Cycle Disorders	14	See Horizon package insert	US	Completed	See Horizon Package Insert	
1g/day	16 weeks	HIV	279	NA	Ex-US	Completed	NA	NCT01702974
9-36g/day	28 days	recurrent malignant glioma	23	No AE's at 9g/day, 1 headache, 1 lightheadedness at 18g/day, 1 fatigue at 27g/day, 2 fatigue at 36g/day	US	Completed	Neuro-oncol. 2005 Apr	
1g/day	16 weeks	Tuberculosis	390	NA	Ex-US	Completed	BMC Pulmonary MedicineBMC series 2013	
Effective dose for UCD	28 days	UCD	46	1 patient experienced Hyperammonaemia	US	Completed	NA	NCT00992459
450-600mg/kg/day	18-24 months	SMA	14 infants	NA	US	Completed	NA	NCT00528268
19g p.o./day divided into three doses	1 week	F(del)508 CF	18	Minimal and comparable side effects	US	Completed	Am J Respir Crit Care Med. 1998	

Dose	Duration	Patient Population	# of patients	AE summary	Location	Status	Reference	NCT # (if ref unavailable)
500mg/kg/day	12 weeks	SMA1	5	Terminated for slow enrollment	US	Cancelled	NA	NCT00439218
500mg/kg/day	12 weeks	SMA2/SMA3	9	Terminated for poor compliance	US	Cancelled	NA	NCT00439569
500mg/kg/day	1 week	Argininosuccinic Aciduria	12	NA	US	Completed	NA	NCT00345605
200mg/kg IV	5 days	Acute Myeloid Leukemia	10	Well tolerated, fatigue observed	US	Completed	Leukemia (2006) 20, 212–217	
7.5g/day	2 weeks	BMI>27	10	NA	Canada	Completed	NA	NCT00533559
IV Phenylbutyrate	NA	Multiple Cancers	20	NA	US	Completed	NA	NCT00006019
IV Phenylbutyrate	up to 4yrs	AML	9 to 24	NA	US	Completed	NA	NCT00006240
IV/Oral Phenylbutyrate Escalating top dose: 45g/day	4 weeks	Refractory Solid Tumor malignancies	28	Generally well tolerated <27g/day. Nausea, Hypocalemia observed	US	Completed	Clin Cancer Res August 2001 7;2292	
7.5g, 15g/day	14 day	Protinuric Nephropathy	26	NA	Ex-US	Completed	NA	NCT02343094
IV Phenylbutyrate	Ascending Dose	Hematologic Cancer	3 to 24	NA	US	Completed	NA	NCT00006239
20g/day	41 to 460 days	Thalessemia Major	11	Weight gain and/or edema caused by increase salt load in 2/12, transient epigastric discomfort in 7/12, and abnormal body odor in 3/12 subjects	US	Completed	AF Collins et al., 1995; Blood: 85 (1)	

Dose	Duration	Patient Population	# of patients	AE summary	Location	Status	Reference	NCT # (if ref unavailable)
20g/day	41 to 460 days	Thalessemia Major	11	Weight gain and/or edema caused by increase salt load in 2/12, transient epigastric discomfort in 7/12, and abnormal body odor in 3/12 subjects	US	Completed	January 1, 1995; Blood: 85 (1)	
20g/day	4 weeks	Healthy, BMI 30-45	101	Not yet posted	US	Completed	NA	NCT00771901
30-40g/day	10 days	ATT deficiency	12	NA	US	Completed	NA	NCT00067756

2.1.5 Additional Previous Clinical Experience with TUDCA

Tauroursodeoxycholic acid is currently marketed in Italy under the brand name Tudcabil (Bruschettini S.R.L.). It is exported to China and Turkey under the brand name Taurolite. It is used for the treatment of cholesterol gallstones. It has been used for the treatment of cholestatic liver diseases including primary cirrhosis, pediatric familial intrahepatic cholestasis and primary sclerosing cholangitis and cholestasis due to cystic fibrosis. To our knowledge there are no other off label uses of tauroursodeoxycholic acid.

Ursodeoxycholic acid (UDCA), which is widely used in the United States for treating gallstones, is produced and secreted endogenously by the liver as a taurine (TUDCA) or glycine (GUDCA) conjugate. Taurine conjugation increases the solubility of UDCA by making it more hydrophilic. TUDCA is taken up in the distal ileum under active transport and therefore likely has a slightly a longer dwell time within the intestine than UDCA which is taken up more proximally in the ileum (IND 118,844).

TUDCA is widely used for the dissolution of cholesterol gallstones. This generally requires long periods of treatment often 1 to 2 years to obtain complete dissolution (IND 118,844).

Between 1997 and 2007, 898,000 Tudcabil tablets were sold in Italy (taken from product profile contained in referenced IND 118,844). There were no reported cases of toxicity related to Tudcabil capsules. There were no reports of overdose or drug abuse during this period. There were no reports related to the use of pregnancy (all pregnant subjects, and those planning to become pregnant, are excluded from this trial). Common adverse events include mild abdominal pain and diarrhea. There are some cases of pruritus and a very limited number of cases of elevated liver enzymes. It should be noted that most of the studies are conducted in subjects with chronic liver disease.

TUDCA is contraindicated in subjects with biliary tree infections, frequent biliary colic, or in subjects who have trouble absorbing bile acids (e.g. ileal disease or resection). The only known or theoretical drug interactions are with substances that inhibit the absorption of bile acids such as cholestyramine and with drugs that increase the elimination of cholesterol in the bile (TUDCA reduces biliary cholesterol content). Based on similar physicochemical characteristics, it is likely that drug toxicity and interactions are very similar to those of ursodeoxycholic acid which are summarized below.

TUDCA has been and is being evaluated in multiple other studies as well. A study at Columbia of 20 subjects with new onset type 1 diabetes in which subjects are administered 1.75g TUDCA for 12 months is ongoing (see table 2). A study at Washington University assessing the effect of TUDCA on lipid markers and ER stress has been completed in 101 subjects at 1.75g daily for 4 weeks; an additional study arm in this study assessed PB at 20g/day (see table 2). We have included in the IND package a signed right to reference to the IND for a study at Washington University assessing subjects with HIV receiving 1.75g daily TUDCA for 30 days.

Table 2: Prior Clinical Experience with TUDCA

TUDCA Dose	Duration	Patient Population	# of patients	AE summary	Location	Status	Reference	NCT #
1g b.i.d.	1 year	Amyotrophic Lateral Sclerosis	29	Mild diarrhea occurred in two patients treated with TUDCA and in two treated with placebo; anorexia was reported in a placebo-treated patient.	ex-US	Completed	Elia et al. European J. Neurology	NCT00877604
1.75g/day	1 year	Type 1 Diabetes	20	NA	US	Ongoing	NA	NCT02218619
1.75g/day	4 weeks	Healthy, BMI 30-45	101	Not yet posted	US	Completed	NA	NCT00771901
750mg/day	24 weeks	Chronic Cholestatic Liver Disease	199	NA	Ex-US	Completed	NA	NCT01829698
750mg/day	18 months	Transthyretin Amyloid Cardiomyopathy	40	NA	US	Active	NA	NCT01855360
1.75g once	30 days	Protease-	48	NA	US	Recruiting	NA	NCT01877551

TUDCA Dose	Duration	Patient Population	# of patients	AE summary	Location	Status	Reference	NCT #
daily		inhibitor Associated Insulin Resistance						
750mg/day	12 months	PBC	216	NA	Ex-US	Completed	NA	NCT01857284
750mg/day	1 year	Transthyretin Amyloidosis	40	NA	Ex-US	Completed	NA	NCT01171859
UNK	3 months	Hepatobiliary Disease in Cystic Fibrosis	39	NA	US	Completed	NA	NCT00004441
500mg/day	60-80 days	Biliary Dyspepsia	30	Safe and well tolerated	Ex-US	Completed	Rivista di Patologia e Clinica, 1985, 34:3370-380	
750mg/day	254 days mean	Cholelithiasis	93	Minor side effects were observed in 4 patients treated with TUDCA (2 g.i. and 2 unspecified skin cases) none of which required suspension of treatment.	Ex-US	Completed	Acta Toxicologica et Therapeutica, Vol. 5, Oct-Dec. 1986, Vaccari ed., Parma	
1.0 g/day	10 days	Patients with Gallstones	7	NA	US	Completed	Batta, et al. Hepatology, 1982, 2(6):811-816	
.5g, 1g, 1.5g/day	6 months	Primary Biliary Cirrhosis	24	Diahrrea only observed AE	Ex-US	Completed	Crosignani, et al. Digestive Diseases and Sciences. 1996, 41(4):809-815	
.5g, 1g, 1.5g/day	6 months	Primary Biliary Cirrhosis	24	NA	Ex-US	Completed	Setchell et al. GUT, 1996; 38:439-446	
3.5-16.6mg/kg/day	4-6 weeks	Gallstones	33	NA	Ex-US	Completed	Muraca et al. International J. Clin. Pharmacol. Therapeuticcs, 1995; 33(7):391-393,	

TUDCA Dose	Duration	Patient Population	# of patients	AE summary	Location	Status	Reference	NCT #
3.5-16.6mg/kg/day	4-6 weeks	Biliary Lipid Composition	33	NA	Ex-US	Completed	Muraca et al. Ital J. Gastroenterol 1995, 27:439-440	
10mg/kg/day	1 month	Gallstones	29	NA	Ex-US	Completed	Portincasa, et al. Ital. J. Gastroenterol. 1996, 28:111-113	
10-13mg/kg/day	3 months	chronic hepatitis	5	NA	Ex-US	Completed	Panella, et al. Ital. J. Gastroenterology 1995; 27:256-258;	
10mg/kg/day	6 months	Gallstones	12	No side effects observed	Ex-US	Completed	La Clinica Terapeutica, 1986; 117:475-479	
10mg/kg/day	6 months	Gallstones	31	NA	Ex-US	Completed	The American Journal of Gastroenterology 1995; 90(6):978-981	
500mg/day	3 months	Biliary Dyspepsia	133	NA	Ex-US	Completed	Advances in Therapy - 1994; 11 (1):34-41,	
500mg/day	3 months	chronic active hepatitis	53	No side effects observed	Ex-US	Completed	Portincasa et al. Current Therapeutic Research. 1993; 53(5):521-531	
500mg/day	3 months	Patients post cholecystectomy	203	1 patient vomited, and 1 had abdominal pain in active, 1 abdominal pain, 1 rash cutaneous, 1 lipotinemia in placebo	Ex-US	Completed	Annali Italiani di Chirurgia, 1993, 64(5):533-537	
.5g, 1g, 1.5g/day	6 months	asymptomatic/mildly symptomatic PBC	24	NA	Ex-US	Completed	Hepatology, 1994, 130A.	

TUDCA Dose	Duration	Patient Population	# of patients	AE summary	Location	Status	Reference	NCT #
.5g, 1g, 1.5g/day	6 months	asymptomatic/mildly symptomatic PBC	24	TUDCA was well tolerated (rarely diarrhoea dose dependent reversible).	Ex-US	Completed	Hepatology, 1993, 10; 176A	
500mg/day	6 months	PBC	23	Well tolerated and no patient complained of side effects.	Ex-US	Completed	Aliment. Pharmacol. Ther. 1997; 11:409-414	
750mg/day	2 month with crossover	PBC	12 females	NA	US	Completed	Hepatology. 1999; 29:320-327	
675mg/day	2 months	PBC	15	Two patients experienced burning discomfort in the epigastrium during the TUDCA treatment period.	Ex-US	Completed	Clin. Res. 1986, 34(1):181	
13mg/kg/day	3 months	Chronic liver disease hystologically determined	69	NA	Ex-US	Completed	J of Hepatology. 1993; 18 (Suppl. 1) S157	
500mg/day	3 months	chronic hepatitis c	134	2.2% cases of diarrhoea solved promptly without suspension of therapy	Ex-US	Completed	Advances in therapy. 1994, 11(5):262-268	
500mg/day	3 months	patients with biopsy proved CAH due to HCV or HBV infections	162	1 patient developed abdominal discomfort, 1 patient had mild pruritus, 3 patients developed mild diarrhoea without wirthdrawal	Ex-US	Completed	Current Therapeutic Research 1995; 56(6):626-634,	
500mg/day	6 months	compensated liver cirrhosis associated with hepatitis B or C of Child's group A or B (histological tests)	30	No side effects and no treatment withdrawals occurred	Ex-US	Completed	Current Therapeutic Research 1994; 55 (11):1355-1362,	

TUDCA Dose	Duration	Patient Population	# of patients	AE summary	Location	Status	Reference	NCT #
Phase 1: TUDCA 10 mg/kg/day + lymphoblastoid IFNα 3MU/m2 ter in week; Phase 2: TUDCA idem + IFNα tapering dose down to the minimum effective	6 month phase 1, 6 months phase 2	Patients with CHC	120	NA	Ex-US	Completed	Gastroenterology 1996; 110(4) A1296.	
.25, .5, 1 g/day	6 months	Chronic Hepatitis	155	Two patients were withdrawn for minor side effects (one for diarrhea and one for dyspepsia).	Ex-US	Completed	Hepatology. 1995, 23(4):120A - 53	
500mg/day	12 months	Liver transplant	33	Safe and well tolerated	Ex-US	Completed	Ital J. Gastro and Hepatology 1999; P/C 13/37:154	

2.1.6 Previous Clinical Experience with Ursodiol (UDCA)

Ursodiol therapy has not been associated with liver damage. Lithocholic acid, a naturally occurring bile acid, is known to be a liver-toxic metabolite. This bile acid is formed in the gut from ursodiol less efficiently and in smaller amounts than that seen from chenodiol. Lithocholic acid is detoxified in the liver by sulfation and, although man appears to be an efficient sulfater, it is possible that some subjects may have a congenital or acquired deficiency in sulfation, thereby predisposing them to lithocholate-induced liver damage (IND 118,844).

Abnormalities in liver enzymes have not been associated with Actigall® (Ursodiol USP capsules) therapy and, in fact, Actigall® has been shown to decrease liver enzyme levels in liver disease. However, subjects given Actigall® should have SGOT (AST) and SGPT (ALT)

measured at the initiation of therapy and thereafter as indicated by the particular clinical circumstances.

Bile acid sequestering agents such as cholestyramine and colestipol may interfere with the action of ursodiol by reducing its absorption. Aluminum-based antacids have been shown to adsorb bile acids in vitro and may be expected to interfere with ursodiol in the same manner as the bile acid sequestering agents (IND 118,844). Estrogens, oral contraceptives, and clofibrate (and perhaps other lipid-lowering drugs) increase hepatic cholesterol secretion, and encourage cholesterol gallstone formation and hence may counteract the effectiveness of ursodiol.

Ursodeoxycholic acid was tested in 2-year oral carcinogenicity studies in CD-1 mice and Sprague-Dawley rats at daily doses of 50, 250, and 1000 mg/kg/day. It was not tumorigenic in mice. In the rat study, it produced statistically significant dose-related increased incidences of pheochromocytomas of adrenal medulla in males (p=0.014, Peto trend test) and females (p=0.004, Peto trend test). A 78-week rat study employing intrarectal instillation of lithocholic acid and tauro-deoxycholic acid, metabolites of ursodiol and chenodiol, has been conducted. These bile acids alone did not produce any tumors. A tumor-promoting effect of both metabolites was observed when they were co-administered with a carcinogenic agent. Ursodiol is not mutagenic in the Ames test (IND 118,844).

Reproduction studies have been performed in rats and rabbits with ursodiol doses up to 200-fold the therapeutic dose and have revealed no evidence of impaired fertility or harm to the fetus at doses of 20- to 100-fold the human dose in rats and at 5-fold the human dose (highest dose tested) in rabbits. Studies employing 100- to 200-fold the human dose in rats have shown some reduction in fertility rate and litter size. (IND 118,844) There have been no adequate and well-controlled studies of the use of ursodiol in pregnant women, but inadvertent exposure of 4 women to therapeutic doses of the drug in the first trimester of pregnancy during the Actigall trials led to no evidence of effects on the fetus or newborn baby. Although it seems unlikely, the possibility that ursodiol can cause fetal harm cannot be ruled out; hence, the drug is not recommended for use during pregnancy.

2.2 Potential Risks and Benefits

2.2.1 Potential Risks

The safety profile with PB administration is in large part derived from studies of subjects with urea cycle disorders. Refer to the phenylbutyrate tablet label (Buphenyl®).

In female subjects, the most common clinical adverse event reported was amenorrhea/menstrual dysfunction (irregular menstrual cycles), which occurred in 23% of the menstruating subjects. Decreased appetite occurred in 4% of all subjects. Body odor (probably caused by the metabolite, phenylacetate [PAA]) and bad taste or taste aversion were each reported in 3% of subjects.

Other adverse events reported in 2% or fewer subjects were:

- Gastrointestinal: abdominal pain, gastritis, nausea and vomiting; constipation, rectal bleeding, peptic ulcer disease, and pancreatitis each occurred in one subject.
- Hematologic: aplastic anemia and ecchymoses each occurred in one subject.
- Cardiovascular: arrhythmia and edema each occurred in one subject.
- Renal: renal tubular acidosis
- Psychiatric: depression
- Skin: rash
- Miscellaneous: headache, syncope, and weight gain

Phenylbutyrate has been evaluated in a dose-escalating study in ALS subjects over the course of 20-weeks and was found to be generally safe and tolerable[15]. Specifically, the most common adverse events included falls or other accidental injury, dizziness, diarrhea, edema, dry mouth, headache, nausea, and rash. With the exception of headache, these adverse events occurred at a higher rate compared to the comparison placebo cohort. These events are expected side effects from PB. There were no clinically significant changes in laboratory values, EKGs or vital signs. No deaths or unexpected and related serious adverse events occurred. Significant adverse events did not occur more frequently with subjects who were taking riluzole in addition to NPB, compared to subjects taking PB alone. Importantly, this study evaluated daily dosages of phenylbutyrate between 9 and 21 grams while our study will be limited to 6 grams daily.

Neurotoxicity was reported in cancer subjects receiving intravenous phenylacetate, 250–300 mg/kg/day for 14 days, repeated at 4-week intervals. Manifestations were predominately somnolence, fatigue, and lightheadedness; with less frequent headache, dysgeusia, hypoacusis, disorientation, impaired memory, and exacerbation of a pre-existing neuropathy.

These adverse events were mainly mild in severity. The acute onset and reversibility when the phenylacetate infusion was discontinued suggest a drug effect.

The most common adverse reactions reported with the use of TUDCA (≥1%) are: abdominal discomfort, abdominal pain, diarrhea, nausea, pruritus, and rash.

TUDCA is generally well tolerated. A derivative, UDCA or ursodiol, is approved for subjects with primary biliary cirrhosis. Common adverse events with TUDCA include mild abdominal pain and diarrhea. There are some cases of pruritus and a very limited number of cases of elevated liver enzymes.

TUDCA has been evaluated over a year-long placebo controlled study in ALS subjects at 1g b.i.d[17]. The population for safety analysis consisted of 15 subjects who took TUDCA and 14 subjects who took placebo. The treatment was well tolerated in all subjects. Laboratory parameters did not change in either treatment group during the course of the study. Except for

the expected complications related to ALS, no changes in vital signs and laboratory values that could possibly be attributed to the study drug or placebo were recorded. Overall, five adverse events were considered by the Investigators to be study related based on the subjects' descriptions. Two events were reported in the 15 TUDCA-treated subjects (13.3%); three events occurred in the 14 placebo-treated subjects (21.4%). The events were as follows: mild diarrhea occurred in two subjects treated with TUDCA and in two treated with placebo; anorexia was reported in a placebo-treated subject. Four subjects died during the study period, one in the TUDCA group and three in the placebo group. The one death in the treated group was not considered drug related—TUDCA trended towards a survival benefit.

The risks and side effects of muscle strength testing include fatigue and/or muscle cramping.

2.2.2 Known Potential Benefits

This study is designed to assess the safety, tolerability and biological activity of AMX0035 therapy. TUDCA and PB have both been tested individually in ALS clinical trials and met their primary endpoints of safety and tolerability. TUDCA also met its efficacy endpoint of slowing ALSFRS-R decline, and PB was therapeutically efficient in improving histone acetylation levels. If successful, this trial will allow further clinical development of this therapy to potentially slow ALS progression. The trial is also assessing multiple biomarkers in concert with clinical endpoints, which will allow both a more detailed understanding of drug activity as well as serve as a data set for the field as a whole to help understand how these biomarkers might track ALS progression.

3 OBJECTIVES

3.1 Study Objectives

This Phase II protocol is intended as a proof of concept of AMX0035 as a safe and effective treatment of adult subjects with ALS. The main strategic objectives of this protocol are below.

The primary outcome measures will be:

1. To confirm the safety and tolerability of a fixed-dose combination of PB and TUDCA in subjects with ALS over a 6-month period;
2. To measure the impact of the treatment using the slope of progression with the revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R);

The secondary objectives of the study will be:

1. To assess the impact of AMX0035 on the rate of decline of isometric muscle strength, as measured by the Accurate Test of Limb Isometric Strength (ATLIS);
2. To assess the impact of AMX0035 on disease progression as measured by Slow Vital Capacity (SVC) decline, time to tracheostomy and survival;
3. To assess the impact of AMX0035 on biomarkers including phosphorylated axonal neurofilament H subunit (pNF-H) levels and 18 kDa translocator protein (TSPO) uptake;
4. To develop concentration-response models of TUDCA and phenylbutyrate at steady-state after administration of AMX0035 sachet twice-daily.
5. To measure the impact of AMX0035 on survival.

3.2 Study Outcome Measures

3.2.1 Primary Outcome Measures

The primary outcome measures for the study will include:

- Safety and tolerability as defined as the proportion of subjects able to remain on study drug until planned discontinuation.
- The rate of decline (slope of decline) in the ALS functional rating scale (ALSFRS-R).

Safety and tolerability will be assessed by the procedures outlined in Section 9.

The revised version of the ALSFRS was created to add assessments of respiratory dysfunction, including dyspnea, orthopnea, and the need for ventilatory support. The revised ALSFRS (ALSFRS-R) has been demonstrated to retain the properties of the original scale and show strong internal consistency and construct validity.

Survival endpoint will be defined as death, tracheostomy or permanent assisted ventilation (>22 hours a day).

3.2.2 Secondary Outcome Measures

The secondary outcome measures include:

- Assessing the impact of AMX0035 on the rate of decline of isometric muscle strength, as measured by the Accurate Test of Limb Isometric Strength (ATLIS);
- Assessing the impact of AMX0035 on disease progression as measured by Slow Vital Capacity (SVC) decline;
- Assessing the impact of AMX0035 on survival, hospitalization and tracheostomies;
- Assessing the impact of AMX0035 on biomarkers including phosphorylated axonal neurofilament H subunit (pNF-H) levels and 18 kDa translocator protein (TSPO) uptake;
- Assessing the concentration-response model of TUDCA and phenylbutyrate at steady-state after administration of AMX0035 4 grams twice daily.

4 STUDY DESIGN

4.1 Overall Study Design and Plan

During the enrollment period approximately 176 subjects will be screened from approximately 25 Northeast ALS Consortium (NEALS) centers in the US. One hundred thirty-two (132) of these subjects will be randomly assigned in a 2:1 ratio to oral (or feeding tube) twice daily sachet of active therapy or matching placebo. Treatment duration will be twenty-four (24) weeks. For the first three weeks study drug will be administered once daily. If tolerated, the dose will then be increased to twice a day. Clinic visits will occur at Screening, Baseline, Week 3 (day 21), Week 6 (day 42), Week 12 (day 84), Week 18 (Day 126), and Week 24 (Day 168). Phone calls will be conducted at Week 9, Week 15, Week 21 and Week 28 (4 weeks after completion of treatment).

All visit windows are consecutive calendar days and are calculated from the day the subject starts study treatment (Day 0, the day of the Baseline Visit). Any change from this visit window will be considered an out of window visit deviation.

An one thirty-two (132) week Open Label Extension (OLE) study will be available to those subjects who complete the randomized, double-blind study. Please refer to 13.7 Appendix VII for all the details on the OLE.

4.2 Study Centers

This study will be conducted at up to 25 NEALS Centers in the US. Sites will be selected based on recruitment record from prior trials, compliance with prior study protocols and regulations, clinical research expertise and availability of necessary resources.

4.3 Study Duration

Subjects will remain on randomized, placebo-controlled, double-blind treatment until the Week 24 visit. Each randomized subject will also have a Follow-up Telephone Interview 28 days after the completion of dosing to assess for adverse events (AEs), changes in concomitant medications and to administer the ALSFRS-R. Including the Screening and Follow-up Visits, each subject will be in the study for approximately 8 months. We expect the study to take up to 18 months to meet enrollment goals.

4.4 Protocol Adherence

Each Site Investigator must adhere to the protocol detailed in this document and agree that any changes to the protocol must be approved by the NCRI Coordination Center (CC) or their Central Institutional Review Board (cIRB). Each Site Investigator (SI) will be responsible for enrolling only those study subjects who have met protocol eligibility criteria.

5 STUDY ENROLLMENT AND WITHDRAWAL

5.1 Number of Study Subjects

Approximately 132 ALS subjects will be randomized.

5.2 Inclusion and Exclusion Criteria

5.2.1 Inclusion Criteria

Inclusion Criteria

1. Male or female, aged 18-80 years of age
2. Sporadic or familial ALS diagnosed as definite as defined by the World Federation of Neurology revised El Escorial criteria
3. Less than or equal to 18 months since ALS symptom onset
4. Capable of providing informed consent and following trial procedures
5. Geographically accessible to the site
6. Slow Vital Capacity (SVC) >60% of predicted value for gender, height, and age at the Screening Visit
7. Subjects must either not take riluzole or be on a stable dose of riluzole for at least 30 days prior to the Screening Visit. Riluzole-naïve subjects are permitted in the study.
8. Women of child bearing potential (e.g. not post-menopausal for at least one year or surgically sterile) must agree to use adequate birth control for the duration of the study and 3 months after last dose of study drug
    a. Women must not be planning to become pregnant for the duration of the study and 3 months after last dose of study drug
9. Men must agree to practice contraception for the duration of the study and 3 months after last dose of study drug
    a. Men must not plan to father a child or provide sperm for donation for the duration of the study and 3 months after last dose of study drug

Acceptable birth control methods for use in this study are:

- Hormonal methods, such as birth control pills, patches, injections, vaginal ring, or implants
- Barrier methods (such as a condom or diaphragm) used with a spermicide (a foam, cream, or gel that kills sperm)
- Intrauterine device (IUD)
- Abstinence (no heterosexual sex)
- Unique partner who is surgically sterile (men) or not of child bearing potential (female)

Date of ALS Symptom Onset. For the purposes of this study, the date of symptom onset will be defined as the date the subject first had symptoms of their disease, i.e., weakness. To be eligible

for this study, the date of symptom onset must be no greater than exactly 18 months prior to the Screening Visit date.

MR-PET Sub-Study
A subset of study subjects will undergo MR-PET and will need to meet the following additional inclusion criteria:

1. Ability to safely lie flat for 90 min for MR-PET procedures in the opinion of the Site Investigator
2. High or mixed affinity to bind TSPO protein (Genotype Ala/Ala or Ala/Thr)

TSPO affinity test: Venous blood for the TSPO affinity test will be drawn from all subjects who have indicated their interest in participating in the MR-PET sub-study. (This will be indicated via a checkbox on the consent form.) The blood will be drawn at Screening in order to have the subjects genotyped for the Ala147Thr TSPO polymorphism in the *TSPO* gene (rs6971). About 10% of humans show low binding affinity to PBR28[21].

Note: High or Mixed affinity binders (Ala/Ala or Ala/Thr) will be considered eligible, whereas the low affinity binders (Thr/Thr) will be considered ineligible for the MR-PET sub-study.

Note: A subject may be eligible for the main study but ineligible for the MR-PET sub-study. However, if a subject is found to be ineligible for the main study, he or she is automatically ineligible for the MR-PET sub-study as well.

5.2.2 Exclusion Criteria

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

Exclusion Criteria

1. Presence of tracheostomy
2. Exposure to PB, TUDCA or UDCA within 3 months prior to the Screening Visit or planning to use these medications during the course of the study
3. History of known allergy to PB or bile salts
4. Abnormal liver function defined as AST and/or ALT > 3 times the upper limit of the normal
5. Renal insufficiency as defined by eGFR < 60 mL/min/1.73m^2.
6. Poorly controlled arterial hypertension (SBP>160mmHg or DBP>100mmHg) at the Screening Visit
7. Pregnant women or women currently breastfeeding
8. History of cholecystectomy

9. Biliary disease which impedes biliary flow including active cholecystitis, primary biliary cirrhosis, sclerosing cholangitis, gallbladder cancer, gallbladder polyps, gangrene of the gallbladder, abscess of the gallbladder.
10. History of Class III/IV heart failure (per New York Heart Association – NYHA)
11. Severe pancreatic or intestinal disorders that may alter the enterohepatic circulation and absorption of TUDCA including biliary infections, pancreatitis and ileal resection
12. The presence of unstable psychiatric disease, cognitive impairment, dementia or substance abuse that would impair ability of the subject to provide informed consent, according to Site Investigator judgment
13. Patients who have cancer with the exception of the following: basal cell carcinoma or successfully treated squamous cell carcinoma of the skin; cervical carcinoma in situ; prostatic carcinoma in situ; or other malignancies curatively treated and with no evidence of disease recurrence for at least 3 years.
14. Clinically significant unstable medical condition (other than ALS) that would pose a risk to the subject if they were to participate in the study
15. Active participation in an ALS clinical trial evaluating an experimental small molecule within 30 days of the Screening Visit. (*Please refer to MOP section E. Protocol Compliance for current list of experimental small molecules*).
16. Exposure at any time to any cell therapies and gene therapies under investigation for the treatment of subjects with ALS (off-label use or investigational)
17. Exposure to monoclonal antibodies under investigation for the treatment of ALS (off-label use or investigational) within 90 days from screening. If previously exposed to monoclonal antibodies under investigation for the treatment of ALS, a 90-day wash-out period will be required prior to screening.
18. Implantation of Diaphragm Pacing System (DPS)
19. 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
20. Exposure to any disallowed medications listed below

MR-PET Sub-Study

A subset of study subjects will undergo MR-PET. The following additional exclusion criteria apply to this subset:

1. Exposure to immunomodulatory medications within 30 days of the Screening Visit
2. Any contraindication to undergo MRI studies such as:
    a. History of a cardiac pacemaker or pacemaker wires
    b. Metallic particles in the body
    c. Vascular clips in the head
    d. Prosthetic heart valves
    e. Severe claustrophobia impeding ability to participate in an imaging study

3. Low affinity binders (Thr/Thr) on the TSPO Affinity Test
4. Radiation exposure that exceeds the site's current guidelines

Note: A subject may be eligible for the main study but ineligible for the MR-PET sub-study. However, if a subject is found to be ineligible for the main study, he or she is automatically ineligible for the MR-PET sub-study as well.

Note on Benzodiazepines for MR-PET Sub-Study Subjects: If an MR-PET subject is taking a benzodiazepine, he or she should not take the benzodiazepine for at least 1 day before his or her scans with the exception of lorazepam and clonazepam that do not need to be discontinued.

Disallowed medications for all subjects include

- HDAC Inhibitors including:
  - Valproate
  - Vorinostat (Zolinza)
  - Romidepsin
  - Chidamide
  - Panobinostat
  - Lithium
  - Butyrate
  - Suramin

- Probenecid

- Bile Acid Sequestrants including:
  - Cholestyramine and Cholestyramine Light
  - Questran and Questran Light
  - Welchol
  - Colestid and Colestid Flavored
  - Prevalite

Note on Antacids Within Two Hours of AMX0035 Administration:
Antacids containing Aluminum hydroxide or smectite (aluminum oxide) may not be taken **within two hours of administration of AMX0035** as they inhibit absorption of TUDCA. These include:

- Alamag
- Alumina and Magnesia
- Antacid, Antacid M and Antacid Suspension
- Gen-Alox
- Kudrox
- M.A.H.

- Maalox HRF and Maalox TC
- Magnalox
- Madroxal
- Mylanta and Mylanta Ultimate
- Ri-Mox
- Rulox

Cautionary Note on Mexiletine
Subjects who participated in the Mexiletine trial within the last 30 days will be excluded from the trial. However, subjects who are using Mexiletine at a dosage less than or equal to 300mg/day for cramps and fasciculations will not be excluded.

There is a potential for an interaction between AMX0035 and Mexiletine; at 20 times the intended clinical concentration (C_{max}), the principal metabolite of Phenylbutyrate, Phenylacetylacetate has been shown to be inhibitory to CYP 1A2 and CYP 2D6 which are the major enzymes responsible for the breakdown of Mexiletine. Therefore, it is possible the co-administration of Phenylbutyrate and Mexiletine will increase the subject's exposure to Mexiletine.

Subjects who are co-administered AMX0035 and Mexiletine should therefore be monitored for Mexiletine-associated adverse events, and if these events present, the Site Investigator should consider stopping or reducing the dosage of Mexiletine. Adverse events associated with Mexiletine include but are not limited to cardiac arrhythmias, liver injury, and blood dyscrasias.

5.3 Treatment Assignment Procedures
Each subject who meets all eligibility criteria will be randomized to receive either therapy by twice daily sachet of AMX0035 (3g PB and 1g TUDCA) or matching placebo for 24 weeks of treatment. For the first three weeks of the study subjects will only take a single sachet daily and will be instructed to increase to 2 sachets daily at the Week 3 Visit.

5.3.1 Randomization Procedures
The randomization scheme will be independently developed and will indicate the treatment assignment and the subject numbers to be used by each site. The randomization scheme will be managed by the manufacturer.

5.4. Reasons for Withdrawal
A study subject will be discontinued from participation in the study if:

- Any clinical adverse event (AE), laboratory abnormality, requirement for a concomitant medication, concurrent illness, or other medical condition or situation occurs such that, in the opinion of the Investigator, continued participation in the study would not be in the best interest of the subject.
- The subject is non-compliant or is lost-to-follow-up.

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

5.4.1 Handling of Withdrawals

A subject may choose to discontinue participation in the study at any time. However, the Site Investigator (SI) or designee will encourage subjects to continue with follow-up, regardless of their compliance with the study drug. If the SI or designee is concerned about the use of a prohibited medication or other safety issues, then the study drug may have to be reduced to single dose or discontinued. If a subject 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. 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 the study drug and should not begin use of the prohibited medication before an appropriate wash-out period of at least 30 days occurs. Subjects who must permanently discontinue study drug may continue in the ITT portion of the study, per protocol.

Subjects who permanently discontinue study drug and will not continue monitoring per the study schedule should complete early study drug termination procedures per protocol. Subjects who discontinue treatment should not be unblinded unless there is a specific reason to do so.

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 as soon as possible after stopping study drug, if possible within 28 days of asking to withdraw consent. The subject will also be asked to have a Follow-Up Telephone Call no sooner than 28 days (+5 days) after taking their last dose of study drug to monitor their safety and to permit review of their medical records at the end of the study to document their vital status.

Subjects who withdraw from the study due to adverse events will be followed for outcome measures under the ITT protocol as noted above. The DSMB will review these events promptly and make recommendations about potential changes to the study, including possible changes to protocol, updates to the informed consent form, or even ending the study early.

In the event a subject wishes to no longer have their personal health information used for the analysis of this study, he or she will notify the site through an authorized letter and future data will not be included in analysis; however, all data up to this letter will still be included.

5.5 Termination of Study

This study may be prematurely terminated if, in the opinion of the DSMB or sponsor, there is sufficient reasonable cause. Written notification, documenting the reason for study termination, will be provided to the Principal Investigator or Sponsor by the terminating party.

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

- Determination of unexpected, significant, or unacceptable risk to subjects.
- Enrollment is unsatisfactory.
- Insufficient adherence to protocol requirements.
- Data that are not sufficiently complete and/or evaluable.
- Plans to modify, suspend or discontinue the development of the study drug.

If the study is prematurely terminated or suspended, the Sponsor will promptly inform the Site Investigators/institutions, and the regulatory authority(ies) of the termination or suspension and the reason(s) for the termination or suspension. The Central IRB (cIRB) will also be informed promptly and provided the reason(s) for the termination or suspension by the Sponsor or by the Site Investigator/institution, as specified by the applicable regulatory requirement(s).

6 TREATMENTS ADMINISTERED

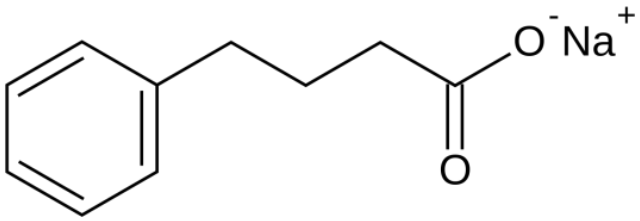
6.1 Treatments

6.1.1 Study Product Description

AMX0035 is a combination therapy comprised of two active pharmaceutical ingredients, sodium phenylbutyrate (PB and tauroursodeoxycholic acid (TUDCA).

Phenylbutyrate is an approved compound in the United States for urea cycle disorders and is marketed in the US as Buphenyl®. There is an existing USP monograph for this material.
The chemical structure for PB is provided below.

Chemical Structure PB



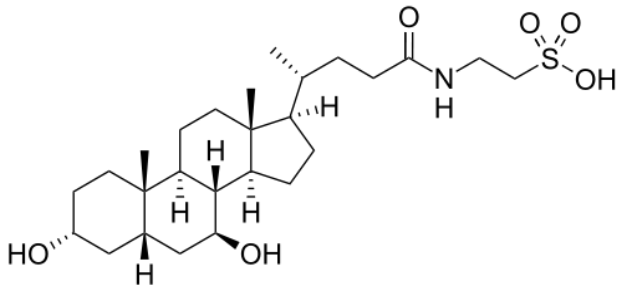
The drug substance PB is produced by Sri Krishna Pharmaceuticals, Ltd. under cGMP conditions. The manufacture and controls for PBA are described in Drug Master File No. 019569.

The specifications for PB are identical to those of the Ph.Eur.

The drug substance TUDCA is currently marketed in Italy under the brand name Tudcabil. It is exported to China and Turkey under the brand name Taurolite. It is used for the indications of treatment of cholesterol gallstones. It has been used for the treatment of cholestatic liver diseases including primary cirrhosis, pediatric familial intrahepatic cholestasis and primary sclerosing cholangitis and cholestasis due to cystic fibrosis. To our knowledge, there are no other uses of tauroursodeoxycholic acid. It is marketed by some companies in the United States on websites such as Amazon as a dietary supplement to “promote liver health”.

The chemical structure for TUDCA is provided below.

Chemical Structure TUDCA



The drug substance TUDCA is produced by Prodotti Chimici E Alimentaria S.p.A.

The specifications for TUDCA are identical to those used by the supplier.

A powder filled sachet will be used as the AMX0035 drug product. The drug product will be filled under cGMP conditions in an aluminum foil lined sachet.

The sachet containing active ingredients will include:

- Active Ingredients:
  - 1g TUDCA
  - 3g PB

- Excipients
  - Sodium Phosphate Dibasic, Anhydrous
  - Dextrates, Hydrates
  - Sorbitol
  - Syloid 63FP (colloidal silica)
  - Sucralose
  - Sodium Stearyl Fumarate
  - Weber Mixed Berry Flavoring
  - Kleptose Linecaps (maltodextrin)

6.1.2 Placebo

A matched placebo will be used to maintain the dosage-blind. The placebo sachets for this study will match the corresponding AMX0035 sachets in size, color, and presentation.

The placebo sachets contain:

- Excipients
  - Sodium Phosphate Dibasic, Anhydrous
  - Dextrates, Hydrates

- Sorbitol
- Syloid 63FP (colloidal silica)
- Sucralose
- Sodium Stearyl Fumarate
- Weber Mixed Berry Flavoring
- Kleptose Linecaps (maltodextrin)
- Denatonium Benzoate Granules

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

6.2 Acquisition

Upon receipt of the study treatment supplies, an inventory must be performed and a drug receipt log filled out and signed by the person accepting the shipment. It is important that the designated study staff counts and verifies that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable study drug in a given shipment will be documented in the study files. The Site Investigator must notify the study Sponsor or their designee of any damaged or unusable study treatments that were supplied to the Site Investigator's site.

6.2.1 Formulation, Packaging, and Labeling

The study drug is prepackaged in kits containing 98 sachets and ready for oral (or feeding tube) administration. The Site Investigator (SI) has the responsibility to ensure that the integrity of packaged study drug is not jeopardized prior to dispensing. Each individual subject kit must be dispensed as provided with no further repackaging or labeling done at the investigational site, unless required by the institution per institutional polices.

6.2.2 Product Storage and Stability

The SI must ensure that all investigational drug supplies are kept in a locked, safe area at ambient temperature 15-25°C with access limited to authorized study staff. Investigational drug supplies should not be repackaged in any way.

Once subjects have access to kits containing the sachets, they will be asked to store them away from moisture at room temperature. Stability has been assessed both at ICH standard and accelerated conditions for each of the individual active ingredients and they were found to be stable over five years. Drug product will receive regular stability testing over the course of the study to ensure product does not degrade. At least one month stability will be verified prior to initiation of the proposed trial. Subjects should contact the SI or their designee in the case of damaged goods; the SI or designee will coordinate with the Sponsor or their designee to determine the most appropriate remediation.

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

It is recommended that the study drug be taken prior to a meal. Subjects should rip open the sachet of study drug and add it to a cup or other container and add approximately 8 oz. (1 cup) of room temperature water and stir vigorously. The study drug mixture should be consumed completely and within one hour of combining the contents of the sachet with water. The site personnel will provide oral instructions to the patients and will assist the patient through the first oral administration (Appendix VI).

Subjects may resume normal eating and drinking after taking the study drug.

Note on Antacids Within Two Hours of Study Drug Administration

Antacids containing Aluminum hydroxide or smectite (aluminum oxide) may not be taken **within two hours of administration of the study drug** as they inhibit absorption of TUDCA.

These include:

- Alamag
- Alumina and Magnesia
- Antacid, Antacid M and Antacid Suspension
- Gen-Alox
- Kudrox
- M.A.H.
- Maalox HRF and Maalox TC
- Magnalox
- Madroxal
- Mylanta and Mylanta Ultimate
- Ri-Mox
- Rulox

6.3.1 Feeding Tube Study Drug Administration

For subjects with a gastrostomy or nasogastric (feeding) tube, the study drug may be dissolved in water as per the procedures outlined above in Section 6.3 and the study drug may be administered via the feeding tube.

6.4 Modification of Study Intervention/Investigational Product For A Subject

Any dosage adjustment, including the reason for and dates of adjustment, will be documented in the CRF for each subject requiring this manipulation. The SI or designated 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 reasons during the trial (the reason for, and dates of suspension or dose reduction must be documented). All dose modifications need to be discussed with the study Medical Monitor. If the AE is mild

or moderate, the dosage may be reduced until the event improves. The SI or designated licensed physician Sub-Investigator 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 within the study for 28 days (+ 5 days) after a subject's last dose of study drug.

6.4.1. Dosage Discontinuation

Reasons for discontinuation of study drug may include an AE, Medical Monitor or Site Investigator recommendation, Sponsor termination, protocol deviation, lost-to-follow-up, subject request, or death. All serious adverse events (SAEs) that occur in a subject who has discontinued early must be recorded and reported within 24-hours of awareness.

Study subjects who discontinue study drug prematurely (early termination from study) and decide to not remain in the modified intent-to-treat (ITT) portion of the study will be encouraged to return for a Final Safety/Early Termination Visit and participate in a Follow-Up Telephone Call 28 days (+ 5 days) after the last dose of study drug.

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

SAEs will be followed for resolution for 28 days (+5 days) after a subject's last dose of study drug, regardless of whether they prematurely discontinued study drug or completed 24 weeks of treatment.

6.5 Study Drug Accountability Procedures

At the completion of the study, there will be a final reconciliation of study drug shipped, drug consumed, and drug remaining. This reconciliation will be logged on the study 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.

6.6 Assessment of Subject Compliance

Subjects will be instructed to return empty and unused study drug containers at each clinic Visit (Weeks 6, 12, 18, and 24) or the Final Safety Visit (whichever occurs first). Site staff will count returned and unused sachets to determine compliance.

Non-compliance will be otherwise defined as taking less than 80% or more than 125% of study drug as determined by sachet counts. If a study subject is non-compliant with study drug, the

Site Investigator (SI) or designee should re-educate and train the subject in administration of study drug. Data indicating non-compliance will be used in the end of study analysis.

6.7 Prior and Concomitant Therapy

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

Any investigational small molecule therapy being used or evaluated for the treatment of ALS is prohibited beginning 30 days prior to the Screening Visit and throughout the study. This includes, but is not limited to, the following:

- Pioglitazone
- Arimoclomol
- Olanzapine
- Tamoxifen
- NP001
- Mexiletine
- Rasagiline
- Masitinib
- Dexpramipexole
- Tirasemtiv
- Ibudilast
- TW001
- Inosine
- RNS60
- Acetyl-L-Carnitine
- Methylcobalamine (if administered at doses equal to or greater than 25 mg per week)

Use of any biologic therapy prior to this study excludes subjects from enrollment. This includes any cell or gene therapy under evaluation for the treatment of ALS and includes but is not limited to, the following:

- ISIS 333611
- Ionis SOD1R
- NurOwn
- Q-Cells
- NSI-566
- GM604

- GSK 1223249
- Treg cell therapies

6.7.1 Prohibited Medications and Contraindications

Prohibited Medications

Throughout the course of the trial, study subjects should not be treated with the following medications. If a Site Investigator learns that a subject has begun therapy with any of these medications, this should be reported to the Medical Monitor and Coordination Center immediately and the SI should make the determination whether to stop the study drug or the prohibited medication immediately, taking into account the health, safety and preference of the study subject.

Agents which might impair bile acid processing or renal function are contraindicated with AMX0035. Prohibited medications include but are not limited to:

- HDAC Inhibitors including:
  - Valproate
  - Vorinostat (Zolinza)
  - Romidepsin
  - Chidamide
  - Panobinostat
  - Lithium
  - Butyrate
  - Suramin

- Probenecid for potential kidney interaction

- Antacids containing aluminum hydroxide or smectite (aluminum oxide) **within two hours of administration of AMX0035.** These inhibit absorption of TUDCA. These include:
  - Alamag
  - Alumina and Magnesia
  - Antacid, Antacid M and Antacid Suspension
  - Gen-Alox
  - Kudrox
  - M.A.H.
  - Maalox HRF and Maalox TC
  - Magnalox
  - Madroxal

- Mylanta and Mylanta Ultimate
- Ri-Mox
- Rulox

- Bile Acid Sequestrants including:
  - Cholestyramine and Cholestyramine Light
  - Questran and Questran Light
  - Welchol
  - Colestid and Colestid Flavored
  - Prevalite

Pregnancy & Nursing Mothers

There are no adequate and well-controlled studies in pregnant women. Female subjects or female 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. If a female subject becomes pregnant during the course of the study, the Medical Monitor and Coordination Center should be contacted immediately.

It is not known whether AMX0035 is excreted in human milk. Caution should be exercised; therefore, no subject should nurse an 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. It is recommended that the ALSFRS-R be completed first at every visit. After the ALSFRS-R it is recommended that SVC and ATLIS measurements are performed so as not to fatigue the subject with other testing. Blood samples are recommended to be taken at the end of the study visits. The order of testing, however, will be at the discretion of each Site Investigator (SI).

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

Subjects who withdraw consent or early terminate from the study (i.e., discontinue study drug) will be asked to come in for a Final Safety Visit and have a Final Follow-up Telephone Call 28 days (+5 days) after stopping study drug.

7.1 MR-PET Scheduling Call

Subjects from all sites will be considered to participate in the MR-PET Sub-Study. The MR-PET Sub-Study procedures will be conducted at Massachusetts General Hospital (MGH) in Boston, MA. However, blood will be drawn for TSPO testing at the subject's site during the Screening Visit.

Subjects participating in the MR-PET Sub-Study may be consented over the phone by a medically licensed professional MGH study staff member to determine subject eligibility and to ensure the subject is safe to undergo the MR-PET scan. These procedures include:

- Obtain verbal pre-screening informed consent from subject
- Assess MR-PET inclusion and exclusion criteria
- Complete MR-PET safety questionnaire

During this call, MR-PET Sub-Study procedures will be discussed in detail and the subject should be given the opportunity to ask questions about the MR-PET Sub-Study. The MGH study staff will write a consent note to document the consenting process over the phone. The written informed consent will be signed by the subject and the MGH Study Investigator at the MR-PET in-person visit.

7.2 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
- Create Globally Unique Identifier (GUID)
- Assess inclusion and exclusion criteria
- Obtain medical history and demographics
- Review and document concomitant medications and therapies

- Obtain ALS diagnosis history
- Administer ALSFRS-R questionnaire
- Perform pulmonary function testing including slow vital capacity (SVC)
- Measure isometric strength using ATLIS machine
- Assess and document adverse events (AEs) that occur after subject signs informed consent form (ICF)
- Measure vital signs (blood pressure, heart and breathing rates, temperature)
- Perform neurological examination
- Perform comprehensive physical examination including height and weight
- Perform 12-lead ECG (Electrocardiogram)
- [After other tests] Collect blood samples for clinical laboratory assessments including Hematology (CBC with differential), Complete Chemistry Panel, Liver Function Tests, and serum pregnancy test (for women of child-bearing potential [WOCBP])
- MR-PET SCAN SUBJECTS ONLY: TSPO Affinity Testing
- Collect urine sample for urinalysis
- Schedule the Baseline Visit

MR-PET Scan: For those subjects that consent to participate in the MR-PET scan sub-study, the scan will be scheduled/performed ***before*** the Baseline Visit at the MGH in Boston, MA. At that time, blood will also be collected for peripheral blood mononuclear cell (PBMC) storage and analysis.

7.2.1 Screen Failures

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

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

7.3 MR-PET Visit 1 (Only for patients in MR-PET substudy)

The following procedures will be performed at an office visit to determine the subject's eligibility for the MR-PET sub-study.

- Obtain written informed consent
- Assess MR-PET inclusion and exclusion criteria
- Complete MR-PET safety questionnaire
- Perform the MR-PET Scan
- Perform the Upper Motor Neuron-Burden (UMN-B) Scale
- Measure vital signs (blood pressure, heart and breathing rates, temperature), and weight
- Administer ALSFRS-R questionnaire
- Collect blood for
    - Biomarker (PBMC) testing

    - Pregnancy testing (for women of child bearing potential)
- Review and document concomitant medications and therapies
- Assess and document adverse events (AEs) that occur after subject signs informed consent form (ICF)

MR-PET Follow-Up Call

This visit will take place 24-48 hours after the MR-PET Visit 1. The following procedures will be performed.

- Assess and document AEs directly related to the MR-PET procedures

7.4 Baseline Visit

This visit will take place a maximum of 42 days after the Screening Visit. The 42-day window allows those subjects participating in the MR-PET portion of the study to have their scans scheduled. Site staff are advised to schedule the baseline visit as soon as possible after determining eligibility. The following procedures will be performed.

- Confirm eligibility criteria are still met
- Randomize subject using kit number from the study drug
- Administer the C-SSRS baseline questionnaire
- Administer ALSFRS-R questionnaire
- Perform pulmonary function testing, including slow vital capacity (SVC)
- Measure isometric strength using ATLIS machine
- Review and document concomitant medications and therapies
- Review and document Adverse Events since last visit and following study drug administration
- Measure vital signs
- [After other tests] Collect blood samples for clinical laboratory assessments including Hematology (CBC with differential), Complete Chemistry Panel, Liver Function Tests.
- Collect blood sample for biomarkers
- Collect pre-dose blood sample for pharmacokinetic analysis
- Collect blood sample for optional DNA collection (Note: if Baseline visit has passed or blood sample for DNA was not collected, the blood sample should be collected at the next available visit)
- Collect urine sample for urinalysis

After all other visit activities are completed:

- Dispense 6 weeks of study drug
- Administer first dose of study drug. The healthcare staff member will advise the subject on appropriate administration (Appendix VI). The subject will be observed at the site for a minimum of 60 minutes by an appropriate healthcare staff member according to the

site's institutional/state regulations to assess medical status and any immediate reaction to the study drug.
- Review and document any Adverse Events after first dose of study drug

7.5 Week 3 Clinic Visit

This visit will take place 21±5 days after the Baseline Visit. The following procedures will be performed.

- Administer ALSFRS-R questionnaire
- Review and document concomitant medications and therapies
- Review and assess Adverse Events
- Measure vital signs
- Administer the C-SSRS questionnaire
- Collect blood samples for clinical laboratory assessments including Hematology (CBC with differential), Complete Chemistry Panel, Liver Function Tests
- Collect urine sample for urinalysis
- Perform study drug accountability
- Unless drug is not tolerated, advise subject to increase dosage level from one sachet to two sachets daily.
- Schedule next study visit

7.6 Week 6 Clinic Visit

This visit will take place 42±5 days after the Baseline Visit. The following procedures will be performed.

- Administer ALSFRS-R questionnaire
- Perform pulmonary function testing, including slow vital capacity (SVC)
- Measure isometric strength using ATLIS machine
- Review and document concomitant medications and therapies
- Review and assess Adverse Events
- Measure vital signs
- Administer the C-SSRS questionnaire
- [After other tests] Collect blood samples for clinical laboratory assessments including Hematology (CBC with differential), Complete Chemistry Panel, Liver Function Tests
- Collect blood sample for biomarkers
- Collect urine sample for urinalysis
- Perform study drug accountability and collect all unused study drug and empty containers
- Dispense next 6 weeks of study drug
- Schedule next study visit

7.7 Week 9 Telephone Visit

This visit will take place 63±5 days after the Baseline Visit. The following procedures will be performed.

- Administer ALSFRS-R questionnaire

- Review and document concomitant medications and therapies
- Assess and document AEs
- Enquire about tolerance and compliance
- Schedule next study visit
- Remind subject to bring study drug to the Week 12 Visit

7.8 Week 12 Clinic Visit

This visit will take place 84±5 days after the Baseline Visit. **Subject must take study drug at the site upon beginning this visit due to the PK analysis.** It is recommended that this visit happens earlier in the day since the drug is administered in clinic. The following procedures will be performed:

- Record day/time of previous study drug dose, including if the subject missed a dose.
- Note time of last meal
- Administer study drug and record time of administration
- Collect blood sample for PK (i.e. at 1-hour or 4-hours post-dose) as indicated at the time of randomization
- Administer ALSFRS-R questionnaire
- Perform pulmonary function testing including slow vital capacity (SVC)
- Measure isometric strength using ATLIS machine
- Review and document concomitant medications and therapies
- Review and assess Adverse Events
- Measure vital signs
- Perform neurological examination
- Perform comprehensive physical examination including weight
- Perform 12-lead ECG (Electrocardiogram)
- Administer the C-SSRS questionnaire
- [After other tests] Collect blood samples for clinical laboratory assessments including Hematology (CBC with differential), Complete Chemistry Panel, Liver Function Tests
- Collect blood sample for biomarkers
- Collect urine sample for urinalysis
- Perform study drug accountability and collect all unused study drug and empty containers
- Dispense next 6 weeks of study drug
- Schedule next study visit

7.9 Week 15 Phone Visit

This visit will take place 105±5 days after the Baseline Visit. The following procedures will be performed.

- Administer ALSFRS-R questionnaire
- Review and document concomitant medications and therapies
- Assess and document AEs
- Enquire about tolerance and compliance
- Schedule next study visit

7.10 Week 18 Clinic Visit

This visit will take place 126±5 days after the Baseline Visit. The following procedures will be performed.

- Administer ALSFRS-R questionnaire
- Perform pulmonary function testing including slow vital capacity (SVC)
- Measure isometric strength using ATLIS machine
- Review and document concomitant medications and therapies
- Review and assess Adverse Events
- Measure vital signs
- Administer the C-SSRS questionnaire
- [After other tests] Collect blood samples for clinical laboratory assessments including Hematology (CBC with differential), Complete Chemistry Panel, Liver Function Tests
- Collect blood sample for biomarkers
- Collect urine sample for urinalysis
- Perform study drug accountability and collect all unused study drug and empty containers
- Dispense next 6 weeks of study drug
- Schedule next study visit

7.11 Week 21 Phone Visit

This visit will take place 147±5 days after the Baseline Visit. The following procedures will be performed.

- Administer ALSFRS-R questionnaire
- Review and document concomitant medications and therapies
- Assess and document AEs
- Enquire about tolerance and compliance
- Schedule next study visit
- Remind subject to bring study drug to clinic for the Week 24 Visit
- Schedule MR-PET scan for those subjects participating in the MR-PET Sub-Study

7.12 MR-PET Visit 2 (Only for patients in MR-PET Substudy)

This visit will take place between the Week 12 and Week 20 study visits.

- Complete MR-PET safety questionnaire
- Perform the MR-PET Scan
- Perform the Upper Motor Neuron-Burden (UMN-B) Scale
- Measure vital signs (blood pressure, heart and breathing rates, temperature), height, and weight
- Administer ALSFRS-R questionnaire
- Collect blood for
    - Biomarker (PBMC) testing

    - Pregnancy testing (for women of child bearing potential)
- Review and document concomitant medications and therapies
- Assess and document adverse events (AEs)

MR-PET Follow-Up Call

This visit will take place 24-48 hours after the MR-PET Visit 2. The following procedures will be performed.

- Assess and document AEs directly related to the MR-PET procedures

7.13 Final Study Visit (Week 24)

This visit will take place 168±5 days after the Baseline Visit. **Subject must take study drug upon beginning this visit due to the PK analysis.** It is recommended that this visit happens earlier in the day since the drug is administered in clinic. The following procedures will be performed:

- Record day/time of previous study drug dose, including if the subject missed a dose
- Record time of last meal
- Administer study drug and record time of administration
- Collect a single blood sample for PK (i.e. at 1 hour or 4 hours post-dose) as indicated at the time of randomization (Week 24 only, not Early Termination Subjects)
- Administer ALSFRS-R questionnaire
- Perform pulmonary function testing, including slow vital capacity (SVC)
- Measure isometric strength using ATLIS machine
- Review and document concomitant medications and therapies
- Review Adverse Events
- Measure vital signs
- Perform neurological examination
- Perform physical examination including weight
- Perform 12-lead ECG (Electrocardiogram)
- Administer the C-SSRS questionnaire
- Exit questionnaire
- [After other tests] Collect blood samples for clinical laboratory assessments including Hematology (CBC with differential), Complete Chemistry Panel, Liver Function Tests
- Collect blood sample for biomarkers
- Collect urine sample for urinalysis
- Perform study drug accountability and collect all unused study drug and empty containers

7.14 Final Follow-up Telephone Call (Week 28)

A follow-up phone call will take place 28 + 5 days (no earlier than 28 days) after the subject's last dose of study drug. Subjects who enroll in the open label extension will not be required to complete this telephone call. The following will be performed.

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

- Assess and document AEs

7.15 Withdrawal of Consent Final Safety Visit & Final Follow-up Telephone Call

Subjects who withdraw consent will be asked to come in for a Final Safety Visit as soon as possible after consent withdrawal and to have a final Follow-Up Telephone Call 28 + 5 days (no earlier than 28 days) after the last dose of study drug.

The following will be performed at the Final Safety Visit:

- Administer ALSFRS-R questionnaire
- Perform pulmonary function testing, including slow vital capacity (SVC)
- Measure isometric strength using ATLIS machine
- Review and document concomitant medications and therapies
- Review Adverse Events
- Measure vital signs
- Perform physical examination including weight
- Perform neurological examination
- Perform 12-lead ECG (Electrocardiogram)
- Administer the C-SSRS questionnaire
- [After other tests] Collect blood samples for clinical laboratory assessments including Hematology (CBC with differential), Complete Chemistry Panel, Liver Function Tests
- Collect blood sample for biomarkers
- Collect urine sample for urinalysis
- Perform study drug accountability and collect all unused study drug and empty containers

The following procedures will be performed via telephone 28 +5 days after the last administration of study drug:

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

7.16 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 sent to the central IRB and entered in the Protocol Deviations Log in the Electronic Data Capture (EDC) System.

7.16.1 Missed Visits and Procedures

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

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

Study drug compliance that is outside the limits set in the study operations manual will be reported as a protocol deviation.

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

7.17 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 and cardiac history, 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 for safety:

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

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

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

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

All subjects will have safety laboratory tests at the designated visits outlined in the protocol. These samples will be analyzed at a central laboratory. The Site Investigator (SI) may order additional testing, 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.

Specific instructions regarding the collection, processing, storage and shipment of these samples will be provided in the Site Manual of Procedures (MOP).

8.1.3 Biomarkers and Pharmacokinetic Analysis

Subjects will have blood drawn to assess AMX0035 concentrations for pharmacokinetics (PK) pre-dose at the Baseline Visit and then again at either 1 hour or 4 hours (± 10 minutes) post-dose at the Week 12 and 24 visits. Every attempt should be made to collect samples within the allotted timeframes; however, all samples should be analyzed regardless of actual collection time. The time of administration will be noted. The time of the last meal prior to administration and the time of the drug administration(s) in the previous 24 hours will also be noted.

Additionally, blood will be collected for biomarker analysis, including light and heavy neurofilament testing (NF-L and pNF-H, respectively). Neurofilaments will be used as a mechanistic measure of neuronal death. These proteins are greatly elevated in ALS subjects and promising results from multiple trials suggest this marker may be prognostic of clinical decline. NF-L and pNF-H will be tested over multiple time points with the intention of generating a longitudinal dataset correlating neurofilament levels to observed clinical outcomes. This dataset will help to validate AMX0035 therapeutic mechanism and provide a dataset for the ALS field.

All samples will be labeled with a code. The code will not include any identifiable information. Coded blood samples will be stored at a central laboratory prior to PK and biomarker analysis and other research use.

Specific instructions regarding the collection, processing, storage and shipment of these samples will be provided in the Site Manual of Procedures (MOP).

8.1.4 Blood Samples for Future Research Use

Subjects will provide an additional blood sample for storage in a biofluid biorepository at Barrow Neurological Institute. Any research performed on the samples is for research purposes only. These samples will be used for broad future research use in motor neuron diseases. All samples will be labeled with a code. The code will not include any identifiable information. Results of future research will not be provided to the subject or his/her physician.

There is no scheduled date on which the samples will be destroyed. Samples may be stored for research until they are used, damaged, decayed or otherwise unfit for analysis. If a subject no longer wishes to participate in the study and withdraws consent, it will not be possible to destroy samples that may have already been used.

Specific instructions regarding the collection, processing, storage and shipment of these samples will be provided in the Site Manual of Procedures (MOP).

Subjects will have the opportunity to provide an additional optional blood sample for deoxyribonucleic acid (DNA) extraction for genome sequencing at the Baseline Visit. Deidentified blood samples will be sent to Massachusetts General Hospital and then sent to the New York Genome Center (NYGC) in New York City, NY.

DNA may be stored, used in genome-wide association studies (GWAS), whole genome sequencing, exome sequencing, or for any other known or as yet undiscovered DNA analysis applicable to understanding or targeting disease, with a particular emphasis on ALS. The information from these genetic studies may be made available to collaborators in academia, not-for-profit settings, or industry for appropriate research. Results of DNA testing from this study will not go into the participant's medical record.

The NYGC will be conducting the sequencing of the coded samples, doing the analysis of the sequencing and sharing the results of such sequencing and analysis with researchers pursuant to this protocol, as well as uploading the data to data repositories such as the National Institutes of Health (NIH) Database of Genotypes and Phenotypes (dbGaP).

8.1.5 12-Lead Electrocardiogram (ECG)

A standard 12-lead ECG will be performed. Tracings will be reviewed by a central ECG reader and a copy of the tracings will be kept on site as part of the source documents. The central ECG vendor will provide standard ECG devices for every site and provide training as necessary.

8.1.6 Physical Examination

A comprehensive physical examination will be performed and recorded.

8.1.7 Neurological Examination

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

8.1.8 Upper Motor Neuron-Burden (UMN-B)

The Penn Upper Motor Neuron-Burden (UMN-B) is the total number of pathological UMN signs on examination including pathologically brisk biceps, supinator, triceps, finger, knee and ankle reflexes, and extensor plantar responses assessed bilaterally and brisk facial and jaw jerks. The scale is a combination of Ashworth, Reflexes, and Pseudobulbar Affect scale (Range score: 0-32).

The UMN also includes scoring of the Center for Neurologic Study-Lability Scale (CNS-LS), a 7-item self-report scale that assesses pseudobulbar affect (PBA) by measuring the perceived frequency of PBA episodes (laughing or crying). Data is generated from the clinical exam and scored from 1-5, the lowest score indicating normal tone and the highest extreme spasticity.

8.1.9 Columbia Suicide Severity Rating Scale (C-SSRS)

The US FDA recommends the use of a suicidality assessment instrument that maps to the Columbia Classification Algorithm for Suicide Assessment (C-CASA)[22]. 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)[23]. The C-SSRS involves a series of probing questions to inquire about possible suicidal thinking and behavior.

At the Baseline Visit, the C-SSRS Baseline version will be administered. This version is used to assess suicidality over the subject's lifetime .

At all clinic visits after the Baseline Visit, 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.

8.1.10 Exit Questionnaire

An exit questionnaire will be completed by subjects and Site Investigators at the Final Study Visit (Week 24). This will include questions regarding blindedness and overall experience with the trial.

8.1.11 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 (+ 5 days) after the last dose of study drug. Information on adverse effects of study drug and on inter-current events will be determined at each visit by direct questioning of the subjects, review of concomitant medications, and vital sign results.

8.2 Outcome Measures

8.2.1 ALSFRS-R (Amyotrophic Lateral Sclerosis Functional Rating Scale – Revised)

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 subjects, 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. The ALSFRS-R will therefore also be given to the study subject over the phone. All ALSFRS-R evaluators must be NEALS certified.

8.2.2 Pulmonary Function Testing - 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. All VC evaluators must be NEALS certified. Three VC trials are required for each testing session, however up to 5 trials may be performed if the variability between the highest and second highest VC is 10% or greater for the first 3 trials. Only the 3 best trials are recorded on the CRF. The highest VC recorded is utilized for eligibility.

8.2.3 Isometric Strength Testing (ATLIS)

Accurate Testing of Limb Isometric Strength: We are measuring isometric strength using the Accurate Testing of Limb Isometric Strength device (ATLIS) developed by Dr. Patricia Andres of Massachusetts General Hospital. The device was specifically designed to alleviate the reproducibility concerns that exist for prior strength measurements such as hand held dynamometry (HHD). ATLIS does not depend on experimenter strength, and has measurement settings to ensure that subjects are in the same position each time they are tested. All ATLIS evaluators must be trained and certified. ATLIS may detect functional decline before the ALSFRS-R, which may have a ceiling effect, and may be able to detect changes in function with greater sensitivity to ALSFRS-R. The measure does show a small training effect, so we will include measurement at initial screening visit to allow subjects to become acquainted with the device.

8.2.4 Neuroimaging MR-PET Sub-Study

A subset of subjects will undergo MR-PET scans at the Baseline Visit and again between the Week 12 and 21 Visits. Prior to the scan, every MR-PET sub-study subject will complete the MR-PET Safety Questionnaire. Scanning procedures and subject instructions will be provided in the Site Manual of Procedures (MOP).

8.2.5 Survival Assessment

Survival endpoint will be considered as mortality, tracheostomy or permanent assisted ventilation.

8.2.6 Training and Validation

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

9 SAFETY AND ADVERSE EVENTS

The adverse event (AE) definitions and reporting procedures provided in this protocol comply with all applicable United States Food and Drug Administration (FDA) regulations and International Conference on Harmonization (ICH) guidelines. The Site 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, ALSFRS-R, and ATLIS 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).

An unexpected adverse event is any adverse event, the specificity or severity of which is not consistent with the current Investigator's Brochure. An unexpected, suspected adverse drug reaction is any unexpected adverse event for which, in the opinion of the Site Investigator or Sponsor (or their designee), there is a reasonable possibility that the investigational product caused the event.

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 in-patient 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 in-patient hospitalization, or the development of drug dependency or drug abuse.

An in-patient 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).

A serious, suspected adverse drug reaction (SUSAR) is an SAE for which, in the opinion of the Site Investigator or Sponsor, there is a reasonable possibility that the investigational product caused the event.

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 source document templates and eCRFs 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 Adverse Events in Prior Human Experience with Each Individual Component

TUDCA

- A small number of subjects (>1%) receiving TUDCA have presented with abdominal discomfort, abdominal pain, diarrhea, nausea, emesis, pruritus, and rash.

PB

- Common adverse events include: menstrual irregularities (23%), decreased appetite (4%), sweat-like body odor (3%), and bad taste (3%)
- Rare effects (<2%) have included Gastrointestinal: abdominal pain, gastritis, nausea and vomiting; constipation, rectal bleeding, peptic ulcer disease, and pancreatitis each occurred in one subject.
    - Hematologic: aplastic anemia and ecchymoses each occurred in one subject.
    - Cardiovascular: arrhythmia and edema each occurred in one subject.
    - Renal: renal tubular acidosis
    - Psychiatric: depression
    - Skin: rash
    - Miscellaneous: headache, syncope, and weight gain

- Hypoalbuminemia, metabolic acidosis, alkalosis, hyperchloremia, hyperuricemia, hypokalemia, hypophosphatemia, hyperphosphatemia and hypernatremia have been observed.

9.2.4 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.

10 DATA AND SAFETY MONITORING AND STATISTICAL ANALYSIS PLAN

10.1 Data and Safety Monitoring Board

An independent Data and Safety Monitoring Board (DSMB) will be assembled for the trial. The DSMB receives the blinded and unblinded summary reports of the frequency of all clinical adverse events and safety laboratory tests for planned periodic meetings as specified in the DSMB charter. In addition, the DSMB Chair may call ad hoc meetings. Meetings will be held via teleconference. A DSMB Charter will detail the processes of this group.

Summaries of serious adverse events and enrollment will be provided approximately monthly to the DSMB by the Study Biostatisticians. Any possibly, probably or definitely study drug related, serious adverse events (i.e. serious adverse drug reactions, or SUSARs) are considered events of interest and will be reported in real-time (within 1 business day of Coordination Center (CC) awareness) to the DSMB. All adverse events and abnormal laboratory values results will be listed and will be completely identified (using MedDRA adverse reaction codes) by subject and center. The DSMB can ask to receive the SAE reports more frequently. As necessary, the DSMB can review the frequencies of clinical and laboratory abnormalities. Recommendations for modification or termination of the trial based on safety data will be made by the DSMB to the PIs and Steering Committee. The DSMB will review safety data throughout the trial and may stop the trial for safety if they determine that there is a significant difference in the rate of a particular adverse event that would indicate a risk that is greater than the possible benefit of the study drug. A notable increase in the frequency of any adverse event should be examined by the DSMB although it may not lead to a recommendation by the DSMB.

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

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

10.2 Statistical Considerations

10.2.1 Statistical Methods

A challenge in ALS is generating robust data on treatment effects without running prohibitively large studies. Our analysis of the PROACT and ceftriaxone de-identified subject databases suggests that statistical powering can be significantly improved by enrolling subjects who are <1.5 years from symptom onset and have a definite diagnosis of ALS according to El Escorial Criteria . Mixed-effects modeling was used to account for both the variance between subjects and the deviation within subjects from their average rate of decline. We plan to recruit subjects at a rate of at least 10/month to allow for complete enrollment of the study population within 14 months.

Power for safety and tolerability was considered in three ways: incidence of adverse events (AEs), change in ALFSR-R and ATLIS, and change in biomarker such as pNF-H.

With 88 treated subjects, we will have an 80% probability of detecting any adverse event expected to occur in at least 2% of treated subjects. We will have 80% power to detect a 28 percentage point elevation in the rate of any adverse event relative to placebo based on a one-tailed test at alpha = 0.05. We will consider a dose tolerable if the proportion of treatment failures (discontinuation of study drug due to an adverse event) is less than 40% with 80% confidence, one-tailed. With 88 treated subjects this would occur if 30 or fewer subjects on AMX0035 fail to complete the 6-month study. By this criterion, we will have 80% power for declaring AMX0035 tolerable at the tested dose if the true treatment failure rate is 30%.

A shared-baseline, mixed-effects analysis will be used for primary analysis. A covariate of bulbar onset or onset elsewhere and a second covariate of age at enrollment will be included in the analysis. The mixed-effects model accounts for both the variance between subjects and the deviation within subjects from their average rate of decline. We will use the same analysis for clinical outcomes in this trial. We propose to test at an alpha of 0.05.

Further detail on primary analysis and analysis rationale for secondary endpoints will be included in the Statistical Analysis Plan (SAP).

10.2.2 Analysis for Safety

The safety data will be summarized by treatment group. Treatment AEs will be coded and graded using MedDRA grading criteria. The treatment groups will be compared with respect to occurrence of each adverse event and incidence of Grade III/IV adverse events. Total number of serious adverse events and abnormal laboratory tests will be compared between groups using Fisher's exact test. Withdrawal, abnormal laboratory tests, vital signs and use of concomitant medications will be assessed to characterize the safety profile of the combination of PB and TUDCA. Compliance data will be determined for each visit and by treatment group. The time to subject refusal will be compared between treatment groups to better determine tolerability. This will be accomplished using a method of survival analysis that allows informative censoring due to death. Descriptive statistics denoting the changes from baseline to the final assessment visit with respect to key laboratory parameters and vital signs will also be provided.

Further detail will be provided in a statistical analysis plan.

10.2.3 Analysis for Efficacy

Modified intention-to-treat analysis will be performed, including all randomized subjects receiving at least one dose of the study medication and having at least one primary efficacy assessment after randomization. Slope will be imputed from available data and time points. Homogeneity of clinical characteristics and efficacy variables at baseline between the two randomization groups (between-group baseline differences) will be assessed by analysis of variance for continuous variables and by a chi-squared test for discrete variables. All efficacy endpoints will compared between the two randomization groups at study end (between-group differences at study end) by means of analysis of covariance for continuous variables, adjusting for baseline value and for center effect, and by a chi-squared test for discrete variables. Survival time will compared between treatments by a Kaplan–Meier survival analysis.

The primary analysis strategy will use a shared-baseline, mixed-effects model of ALSFRS-R progression rate. The mixed-effects model accounts for both the variance between subjects and the deviation within subjects from their average rate of decline. We will use the same analysis for clinical outcomes in this trial. We propose to test at an alpha of 0.05. We are targeting an effect size (slowing of ALSFRS-R slope) greater than 30% based on the trial by Elia et al[17]. In the Phase I/II trial of TUDCA that analyzed a total of 29 subjects, the ALSFRS-R score declined 32.5% more slowly in the TUDCA group: the slopes of the two regression lines were significantly different (-0.262/week for the TUDCA group, -0.388/week for the placebo group; $P < 0.01$).

10.2.4 Analysis Populations

The modified intent to treat (ITT) population will include all study subjects who are randomized and receive at least one dose of study drug. The ITT population will be considered for primary analyses. For ITT analyses, subjects will be grouped based on randomized treatment, regardless of treatment actually received.

10.3 Missing Data

The trial will be modified intent to treat (ITT). Every effort will be made to obtain follow-up information for all subjects whether or not they continue on treatment.

11 DATA COLLECTION, MANAGEMENT AND MONITORING

11.1 Role of Data Management

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

All data will be managed in compliance with 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 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 Site Investigator must maintain complete and accurate documentation for the study.

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

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.

11.2 Clinical Monitoring

Study Monitors will visit each study site to review source documentation materials, informed consent forms, and confirm entered data and that data queries have been accurately completed, and again at a study close-out visit. Study Monitors will also verify that SAEs and protocol deviations have been reported appropriately, as required. The Study Monitors will also review clinical facilities, resources and procedures for evaluating study subjects and study drug dispensing. Subsequently, the Study Monitors will provide monitoring reports to the Project Manager and, if requested, will provide reports of protocol compliance to the Study Principal Investigator and the Steering Committee. Completed informed consent forms from each subject must be available in the subject's file and verified for proper documentation. A document outlining the monitoring plan is provided to each Study Monitor.

11.3 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.

11.3.1 Confidentiality

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

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

11.3.2 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.

11.3.3 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.

11.3.4 Publications

The Study Principal Investigator, Sabrina Paganoni, along with the Sponsor, Amylyx Pharmaceuticals, Inc., will be responsible for publications of results from this trial. Their responsibilities will include the following:

- Analyze and interpret data gathered in this study, and write publications from these data.
- Submit manuscripts to selected journals and address peer reviewers' comments.
- Submit abstracts to selected meetings and present data at the meetings.
- Determine authorship on the basis of the Uniform Requirements for Manuscripts.

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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, electrophysiology 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 (subjects 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 subjects 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 (C-SSRS) BASELINE VERSION

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

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

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

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

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

13.4 APPENDIX IV: COLUMBIA-SUICIDE SEVERITY RATING SCALE (C-SSRS) SINCE LAST VISIT VERSION

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

SUICIDAL IDEATION	
Ask questions 1 and 2. If both are negative, proceed to "Suicidal Behavior" section. If the answer to question 2 is "yes", ask questions 3, 4 and 5. If the answer to question 1 and/or 2 is "yes", complete "Intensity of Ideation" section below.	Since Last Visit
1. Wish to be Dead Subject endorses thoughts about a wish to be dead or not alive anymore, or wish to fall asleep and not wake up. ***Have you wished you were dead or wished you could go to sleep and not wake up?*** If yes, describe:	**Yes No** □ □
2. Non-Specific Active Suicidal Thoughts General, non-specific thoughts of wanting to end one's life/commit suicide (e.g., *"I've thought about killing myself"*) without thoughts of ways to kill oneself/associated methods, intent, or plan during the assessment period. ***Have you actually had any thoughts of killing yourself?*** If yes, describe:	**Yes No** □ □
3. Active Suicidal Ideation with Any Methods (Not Plan) without Intent to Act Subject endorses thoughts of suicide and has thought of at least one method during the assessment period. This is different than a specific plan with time, place or method details worked out (e.g., thought of method to kill self but not a specific plan). Includes person who would say, *"I thought about taking an overdose but I never made a specific plan as to when, where or how I would actually do it…and I would never go through with it."* ***Have you been thinking about how you might do this?*** If yes, describe:	**Yes No** □ □
4. Active Suicidal Ideation with Some Intent to Act, without Specific Plan Active suicidal thoughts of killing oneself and subject reports having some intent to act on such thoughts, as opposed to *"I have the thoughts but I definitely will not do anything about them."* ***Have you had these thoughts and had some intention of acting on them?*** If yes, describe:	**Yes No** □ □
5. Active Suicidal Ideation with Specific Plan and Intent Thoughts of killing oneself with details of plan fully or partially worked out and subject has some intent to carry it out. ***Have you started to work out or worked out the details of how to kill yourself? Do you intend to carry out this plan?*** If yes, describe:	**Yes No** □ □
INTENSITY OF IDEATION	
The following features should be rated with respect to the most severe type of ideation (i.e., 1-5 from above, with 1 being the least severe and 5 being the most severe). ***Most Severe Ideation:*** _____ _____ ***Type # (1-5) Description of Ideation***	Most Severe
Frequency ***How many times have you had these thoughts?*** (1) Less than once a week (2) Once a week (3) 2-5 times in week (4) Daily or almost daily (5) Many times each day	____
Duration ***When you have the thoughts, how long do they last?*** (1) Fleeting - few seconds or minutes (4) 4-8 hours/most of day (2) Less than 1 hour/some of the time (5) More than 8 hours/persistent or continuous (3) 1-4 hours/a lot of time	____
Controllability ***Could/can you stop thinking about killing yourself or wanting to die if you want to?*** (1) Easily able to control thoughts (4) Can control thoughts with a lot of difficulty (2) Can control thoughts with little difficulty (5) Unable to control thoughts (3) Can control thoughts with some difficulty (0) Does not attempt to control thoughts	____

Deterrents ***Are there things - anyone or anything (e.g., family, religion, pain of death) - that stopped you from wanting to die or acting on thoughts of committing suicide?*** (1) Deterrents definitely stopped you from attempting suicide (4) Deterrents most likely did not stop you (2) Deterrents probably stopped you (5) Deterrents definitely did not stop you (3) Uncertain that deterrents stopped you (0) Does not apply	____
Reasons for Ideation ***What sort of reasons did you have for thinking about wanting to die or killing yourself? Was it to end the pain or stop the way you were feeling (in other words you couldn't go on living with this pain or how you were feeling) or was it to get attention, revenge or a reaction from others? Or both?*** (1) Completely to get attention, revenge or a reaction from others (4) Mostly to end or stop the pain (you couldn't go on (2) Mostly to get attention, revenge or a reaction from others living with the pain or how you were feeling) (3) Equally to get attention, revenge or a reaction from others (5) Completely to end or stop the pain (you couldn't go on and to end/stop the pain living with the pain or how you were feeling) (0) Does not apply	____

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* _____

13.5 APPENDIX V: CENTER FOR NEUROLOGICAL STUDY – LABILITY SCALE

INSTRUCTIONS

The purpose of this questionnaire is to help us better understand your neurologic problems. Please read each statement, and using the scale below, determine the degree to which it has applied to you **DURING THE PAST WEEK**. Circle the appropriate answer, or if you need help in marking your responses, tell the interviewer the number of the best response. Please choose only one response for each item.

Please select the number that describes the degree to which each item has applied to you DURING THE PAST WEEK.					
	Does not Apply 1	Rarely Applies 2	Occasionally Applies 3	Frequently Applies 4	Applies Most of the Time 5
1. There are times when I feel fine 1 minute, and then I'll become tearful the next over something small or for no reason at all.	○	○	○	○	○
2. Others have told me that I seem to become amused very easily or that I seem to become amused about things that aren't funny.	○	○	○	○	○
3. I find myself crying very easily.	○	○	○	○	○
4. I find that even when I try to control my laughter, I am often unable to do so.	○	○	○	○	○
5. There are times when I won't be thinking of anything happy or funny at all, but then I'll suddenly be overcome by funny or happy thoughts.	○	○	○	○	○
6. I find that even when I try to control my crying, I am often unable to do so.	○	○	○	○	○
7. I find that I am easily overcome by laughter.	○	○	○	○	○

Evaluator's Initials: _____ _____ _____ ***Total:*** _____

13.6 APPENDIX VI: INSTRUCTIONS TO PATIENTS

The following instructions will be provided orally to the patient at the Baseline Visit by a healthcare staff member. Please have the Listerine® products (Pocketpaks® and Pocketmist®) available for demonstration.

- Alert the patient that the study drug has a strong bitter taste, but that there are ways to make it more palatable (see below).

- Rip open the sachet of study drug and add it to a cup or other container and add approximately 8 oz. (1 cup) of room temperature water and stir vigorously. Study drug may require significant stirring or gentle crushing to dissolve.

- The treatment should be taken within one hour of mixing into water.

- Several things may be done to reduce the bad taste and make the drug more palatable:

    1) Use Listerine Pocket Packs® (strips) or a Listerine PocketMist® (spray) immediately before and/or immediately after taking the drug. Use liberally to coat the mouth. This has been found to significantly mask the bitter taste.

    2) Take a snack or a meal after taking your treatment.

    3) Follow drug immediately with milk to remove taste from the mouth.

    4) Avoid drinking fruit juice at the same time as the drug as this may make flavor worse.

- Mixing the study drug with a liquid other than water should be avoided.

13.7 APPENDIX VII: AMX-0035 IN ALS OPEN LABEL EXTENSION (OLE):

OPEN LABEL EXTENSION SCHEDULE OF ACTIVITIES

ACTIVITY	Screening/ Baseline Visit	Study Drug Administration (weeks)					Week 68*	Week 84*	Week 100	Week 116	Week 132* OR Early Discontinuation/ Final Safety Visit
		Week 6	Week 12	Week 24	Week 36	Week 52					
	Clinic	Clinic	Clinic	Clinic	Clinic	Clinic	Clinic	Clinic	Clinic	Clinic	Clinic
	Day 0[8]	Day 42 ±10	Day 84 ±10	Day 168 ±28	Day 252 ±28	Day 364 ±28	Day 476 ±28	Day 588 ±28	Day 700 ±28	Day 812 ±28	Day 924 ±28
Written Informed Consent	X										
Inclusion/Exclusion Review	X										
Vital Signs[1]	X	X	X	X	X	X	X	X	X	X	X
Blood Draw for Safety Labs[2]	X	X	X	X	X	X	X	X	X	X	X
Blood Draw for Serum Pregnancy Test for WOCB[2]	X										
Blood draw for optional DNA collection	X[3]	X[3]	X[3]	X[3]	X[3]	X[3]	X[3]	X[3]	X[3]	X[3]	X[3]
Urine Sample for Urinalysis[2]	X	X	X	X	X	X	X	X	X	X	X
12-Lead ECG[4]	X	X	X	X	X	X	X	X	X	X	X
ALSFRS-R	X	X	X	X	X	X	X	X	X	X	X
Slow Vital Capacity	X	X	X	X	X	X	X	X	X	X	X
ATLIS	X	X	X	X	X	X			X	X	X
Columbia-Suicide Severity Scale[5]	X	X	X	X	X	X	X	X	X	X	X
Adverse Events[6]	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications	X										
Dispense Study Drug[7]	X	X	X	X	X	X	X	X	X	X	
Key Study Events	X	X	X	X	X	X	X	X	X	X	X
Drug Accountability/ Compliance		X	X	X	X	X	X	X	X	X	X

[1] Vital signs include systolic and diastolic pressure in mmHg, respiratory rate/minute, heart rate/minute, temperature and weight. Vital signs only need to be taken if they were not already recorded as part of a standard of care visit that occurred within the study visit window.

[2] Safety labs include Hematology (CBC with differential), Complete Chemistry Panel, Liver Function Tests and Urinalysis. Serum pregnancy testing will occur in women of child bearing potential (WOCBP) at the Screening Visit and as necessary during the

course of the study. Blood draws and urine samples will only be taken if they were not already recorded as part of a standard of care visit that occurred within the study visit window.

[3] Optional one-time blood draw for DNA analysis can be completed during the OLE, if not completed during main study.

[4] 12-Lead ECG only needs to be completed if it was not already recorded as part of a standard of care visit that occurred within the study visit window.

[5] C-SSRS Since Last Visit version to be completed at all visits.

[6] Adverse events that are ongoing from the main study should be recorded and followed during the OLE. Any new adverse events that occur AFTER start of OLE treatment will be recorded.

[7] First dose of study drug will be administered in clinic after ALL Screening/Baseline Visit procedures are completed.

[8] Day 0 Visit of the open label extension sub-study may be the same as Week 24 Visit of the main study – so that exams and tests do not need to be duplicated if the Day 0 visit occurs within 7 days of the Week 24 visit. If the patient enrolls day 8-28 then all assessments for the Day 0 visit should be completed. Patients must enroll in the OLE within 28 days of the Week 24 visit of the main study.

[*] If subject is unable to complete all procedures, minimal procedures should be completed in the following suggested order: AE Review, Safety labs, ECG, Concomitant Medication, ALSFRS-R.

OPEN LABEL EXTENSION PLAN

Study Indication
Amyotrophic Lateral Sclerosis (ALS)

Phase of Development
II

Rationale for the Study
The objective of this study is to determine the long-term safety of AMX0035 in subjects with Amyotrophic Lateral Sclerosis (ALS). ALS is a progressive neurodegenerative disease for which there is no cure. There are only two medicines approved specifically for treating ALS, Rilutek (riluzole) and Radicava (edaravone). ALS also exacts a significant economic burden.

AMX0035 has demonstrated efficacy in models of neurodegeneration, classical activation of neuroinflammation, and bioenergetics deficits. The individual components of AMX0035, PB and TUDCA have demonstrated efficacy in in vivo models of ALS, Parkinson's, Alzheimer's, ischemia, and many others. Each individual component has also been tested in small clinical trials of ALS subjects and was found to be safe and well-tolerated, and hit primary endpoints of efficacy.

Study Design and Plan
This is a multicenter, open label extension, up to 132-week study evaluating the long-term safety of AMX0035. Up to one hundred thirty-two (132) subjects will be given oral (or feeding tube) twice daily sachet of active therapy. Treatment duration will be up to one thirty-two (132) weeks starting at the Screening/Baseline visit. Clinic visits will occur at Screening/Baseline, Week 6 (day 42), Week 12 (day 84), Week 24 (day 168), Week 36 (day 252), and Week 52 (Day 364), Week 68 (Day 476), Week 84 (Day 588), Week 100 (Day 700), Week 116 (Day 812), Week 132 (Day 924).

All visit windows are consecutive calendar days and are calculated from the day the subject starts study treatment (Day 0, the day of the Screening/Baseline Visit). The screening/Baseline visit must occur within 28 days of the Week 24 visit of the main study. If the Screening/Baseline visit occurs on the day of the Week 24 visit or within 7 days of that visit then it is not necessary to complete the assessments, labs and outcomes. If the Screening/Baseline visit occurs Day 8 – Day 28 then all assessments, labs and outcomes need to be completed. Visit windows will be +/- 10 days for the Week 6 and Week 12 visits and +/- 28 days for the Week 24, Week 36, Week 52, Week 68, Week 84, Week 100, Week 116 and Week 132 visits. Any change from this visit window will be considered an out of window visit deviation.

Study Objectives
The primary objective of the study is to assess long-term safety of oral (or feeding tube) administration of AMX0035 via sachet (3g PB and 1g TUDCA) twice daily for compassionate use.

The primary outcome measure is:

1. To confirm the long-term safety of AMX0035 in subjects with ALS over a 132-week period

Secondary outcome measures will include:

1. The rate of key study events including tracheostomy, hospitalization, and death
2. Rate of progression on the ALSFRS-R scale
3. ATLIS rate of progression
4. Rate of progression of slow vital capacity

Study Locations

Up to 25 Northeast ALS Consortium (NEALS) centers in the United States that participated in the randomized, double-blind trial will participate in this study.

Number of Planned Subjects

Up to 132 subjects that participated in the randomized, double-blind trial will be able to enroll in this study.

Study Population

This study will be conducted in subjects who have sporadic or familial ALS diagnosed as definite as defined by revised El Escorial criteria (Appendix 1). Subjects must provide written informed consent prior to screening. At screening/baseline subjects must have completed participation in the randomized, double-blind trial.

Study Enrollment

Inclusion Criteria:

1. Completion of all visits in the randomized, double blind AMX0035 study. Subjects that receive tracheostomy or PAV during the course of the main study will still be followed as ITT until the week 24 visit before enrollment in the OLE.
2. Must enroll in the OLE within 28 days of the Week 24 visit of the main study.
3. Signed informed consent to enter the open label extension phase.

Exclusion Criteria:

1. Discontinued study drug prematurely in the double-blind phase of the study for reasons other than tracheostomy or PAV.
2. Exposure to or anticipated requirement for any disallowed medication listed below.
3. Any ongoing adverse events that in the opinion of the Site Investigator are clear contraindications to the study drug.
4. Unstable cardiac or other life-threatening disease emergent during the randomized, double blind study
5. Any major medical condition that in the opinion of the Site Investigator would interfere with the study and place the subject at increased risk.

Subjects who receive tracheostomy or PAV while in the randomized, double-blind trial can elect to enroll in the OLE so long as they complete all visits in the main study.

Disallowed medications for all subjects include:

- HDAC Inhibitors including:
    - Valproate
    - Vorinostat (Zolinza)
    - Romidepsin
    - Chidamide
    - Panobinostat
    - Lithium
    - Butyrate
    - Suramin

- Probenecid

- Bile Acid Sequestrants including:
    - Cholestyramine and Cholestyramine Light
    - Questran and Questran Light
    - Welchol
    - Colestid and Colestid Flavored
    - Prevalite

Note on Antacids Within Two Hours of Study Drug Administration
Antacids containing Aluminum hydroxide or smectite (aluminum oxide) may not be taken **within two hours of administration of the study drug** as they inhibit absorption of TUDCA.

These include:

- Alamag
- Alumina and Magnesia
- Antacid, Antacid M and Antacid Suspension
- Gen-Alox
- Kudrox
- M.A.H.
- Maalox HRF and Maalox TC
- Magnalox
- Madroxal
- Mylanta and Mylanta Ultimate
- Ri-Mox
- Rulox

Please refer to section 6.7 and 6.7.1 of the main protocol for all medication information.

Study Drug and Treatment Administration
There will be a new formulation for the open label extension that has been optimized for better taste.

A powder filled sachet will be used as the AMX0035 drug product. The drug product will be filled under cGMP conditions in an aluminum foil lined sachet.

The sachet containing active ingredients will include:

- Active Ingredients:
  - 1g TUDCA
  - 3g PB (Phenylbutyrate)

- Excipients
  - Dextrates
  - Sorbitol
  - Sucralose
  - Syloid 63FP (colloidal silica)
  - Kleptose Linecaps (maltodextrin)
  - Firmenich Flavor Masking Flavorant
  - Firmenich Mixed Berry Flavorant
  - Sodium Phosphate Dibasic
  - Sodium Stearyl Fumarate

Changes from the batch used in the randomized, double blind study include a different level of sucralose, the mixed berry flavor being provided by a new company and the addition of a flavor masking agent.

Please see section 6 of the main protocol for more information on the description, packaging, storage, dosage, administration, concomitant therapies and prohibited medications of the study drug.

Study drug will be provided in clinic on the day of the screening/baseline visit and re-supplied at each subsequent visit.

Subjects should bring in unused sachets so that the site may check compliance. Any unused sachets should be re-dispensed to the subject during the visit. Please refer to the site MOP for a detailed description of study drug dispensing.

Subjects will take 2 sachets daily, 1 sachet in the morning and 1 sachet in the afternoon, throughout the study.

Duration of Treatment and Follow-up

Subjects will remain on treatment until the Week 132 or early discontinuation visit.

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. It is recommended that the ALSFRS-R be

completed first at every visit. After the ALSFRS-R it is recommended that SVC and ATLIS measurements are performed so as not to fatigue the subject with other testing. Blood samples are recommended to be taken at the end of the study visits and will be processed locally. The order of testing, however, will be at the discretion of each Site Investigator (SI).

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

Subjects who withdraw consent or early terminate from the study (i.e., discontinue study drug) will be asked to come in for a Final Safety Visit.

Screening/Baseline Clinic Visit:

Day 0 Visit of the open label extension sub-study may be the same as Week 24 Visit of the main study – so that exams and tests do not need to be duplicated if they were previously completed.
The following procedures will be performed:

- Obtain written informed consent from subject
- Assess inclusion and exclusion criteria
- Review and document concomitant medications and therapies
- Administer C-SSRS (Baseline Version)
- Administer ALSFRS-R questionnaire
- Perform pulmonary function testing including slow vital capacity (SVC)* Note height should be recorded from the main study Screening Visit.
- Measure isometric strength using ATLIS machine
- Assess and document adverse events (AEs) that occur after subject signs informed consent form (ICF)
- Measure vital signs (blood pressure, heart and breathing rates, temperature, and weight)
- Perform 12-lead ECG (Electrocardiogram)
- [After other tests] Collect blood samples for clinical laboratory assessments including Hematology (CBC with differential), Complete Chemistry Panel, Liver Function Tests, serum pregnancy test (for women of child-bearing potential [WOCBP]), optional DNA analysis if not completed during main study
- Collect urine sample for urinalysis
- Dispense 2 kits of study drug (12 weeks + 2 weeks extra)
- Capture key study events
- Schedule the Week 6 Visit

Week 6, Week 12, Week 24, Week 36, Week 52, Week 68, Week 84, Week 100, Week 116, Week 132 or Early Discontinuation/Final Safety Clinic Visit:

The Week 6 and Week 12 visits will take place +/- 10 days and the Week 24, Week 36, Week 52, Week 68, Week 84, Week 100, Week 116 and Week 132 visits will take place +/- 28 days from the time specified in the schedule of activities (table as beginning of this section).
The following procedures will be performed:

- Review and assess Adverse Events
- Measure vital signs
- Administer the C-SSRS questionnaire (Since Last Visit)
- Administer ALSFRS-R questionnaire
- Perform pulmonary function testing including slow vital capacity (SVC)
- Measure isometric strength using ATLIS machine
- Perform 12-lead ECG (Electrocardiogram)
- Collect blood samples for clinical laboratory assessments including Hematology (CBC with differential), Complete Chemistry Panel, Liver Function Tests, optional DNA analysis if not completed during main study
- Collect urine sample for urinalysis
- Perform study drug accountability
- Dispense study drug (Except at Week 132/Early Discontinuation)
- Capture key study events
- Schedule next study visit (Except Week 132/Early Discontinuation)

Please note – safety labs, urine collection for urinalysis, pregnancy test, and 12-lead ECG do not need to be repeated if they were completed as part of clinical or SOC visit within the approved study visit window.

Laboratory Testing:

The following laboratory tests will be performed for safety:

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

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

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

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

Please note: for the OLE portion of the study, local laboratories will be used.

Safety and Adverse Events

For the purposes of this study, an adverse event (AE) will be defined as any unfavorable and unintended sign, symptom, or disease that the Site Investigator deems to be definitely, probably, possibly, unlikely or not related to the conduct of the study procedures or study drug.

Please refer to section 9 of the main protocol for more information on adverse events.

Data Safety and Monitoring and Statistical Analysis Plan

Please refer to section 7.16 and 7.17 regarding protocol deviations, missed visits and deaths. Please refer to section 9 of this protocol regarding safety management. The safety management plan contains additional details regarding safety management. Please refer to section 11 regarding data capture. A separate statistical analysis plan will be prepared for this study.

Statistical analysis will mainly entail analysis of types and frequencies of adverse events. Measures of SVC, ATLIS, ALSFRS-R and study events in the open label extension study will be compared to the double-blind section of this study and to historical data. A detailed description of the statistical plan is contained in the section titled, “Open Label Extension Statistical Analysis Plan.”

While the blinded study is ongoing, the DSMB will review safety events in this study during its regular meeting, as detailed in the DSMB charter. When the main study is concluded (last patient out), the independent Medical Monitor will review ongoing safety for the remainder of the extension.

Evaluation of the Safety, Tolerability, Efficacy and Activity of AMX0035, a Fixed Combination of Phenylbutyrate (PB) and Tauroursodeoxycholic Acid (TUDCA), for Treatment of Amyotrophic Lateral Sclerosis (ALS)

Summary of Protocol Changes v1.0 to 2.0

Section	Revision	Rationale
Title Page, Footer and Signature Page	**Updated** the version number and date to 2.0 / 25 JULY 2017	Updated to current version number and date
Protocol Summary	**Updated** version number to 2.0	Updated to include the current version number
6.3 Dosage, Preparation and Administration of Study Intervention/ Investigational Product	**Added:** The site personnel will provide oral instructions to the patients and will assist the patient through the first oral administration (Appendix VI).	Clarified how patients will receive instructions on how to administer the Study Intervention/ Investigational Product
7.4 Baseline Visit	**Added:** (Appendix VI)	Clarified that appropriate administration would be advised to patients at the Baseline Visit
7.12 MR-PET Visit 2 (Only for patients in MR-PET Substudy)	**Updated** visit number to Visit 2	Clarified that MR-Pet Follow-up Call is to occur at Visit 2
8.2.4 Neuroimaging MR-PET Sub-Study	**Changed** visit number from Week 21 and Week 24 to Week 12 and Week 21.	Clarified that MR-Pet is to occur on Week 12 and Week 21
10.2.2 Analysis for Safety	**Changed** CTCAE to MedDRA	Clarified grading criteria for treatment AEs
13.6 APPENDIX VI: INSTRUCTIONS TO PATIENTS	**Added** Instructions to Patients script for drug administration	Provided instructions for study staff member to orally inform patients about how to administer drug

Evaluation of the Safety, Tolerability, Efficacy and Activity of AMX0035, a Fixed Combination of Phenylbutyrate (PB) and Tauroursodeoxycholic Acid (TUDCA), for Treatment of Amyotrophic Lateral Sclerosis (ALS)

Summary of Protocol Changes v2.0 to 3.0

Section	Revision	Rationale
Title Page, Footer and Signature Page	**Updated** the version number and date to 3.0 / 22 NOV 2017	Updated to current version number and date
Protocol Summary	**Updated** version number to 3.0	Updated to include the current version number
List of Abbreviations	**Added**: OLE Open Label Extension	Clarified abbreviation of new term in the study
Rationale for the Study	**Changed:** There are only two medications approved specifically for treating ALS. This includes Rilutek (riluzole), which only provides a modest benefit for subjects, and Radicava (edaravone).	Stated the current medications that are approved to treat ALS, and provide rationale for the objective of the study
Study Population	**Changed:** There will be no restrictions for subjects taking Radicava (edaravone) at the time of screening, or if started while enrolled in the study.	Clarified that patients taking certain medication are not restricted to volunteer for the study
Schedule of Activities	**Added** ALSFRS-R will be completed at MGH for those sub-study subjects enrolled in the MR-PET section of the study	Clarified the schedule of activities for subjects enrolled in the MR-PET sub-study
Schedule of Activities	**Added:** This call will only be required for subjects who do NOT enroll in the OLE.	Clarified the schedule of activities for Final Follow-up Telephone Call
2.1.1 ALS Overview	**Updated:** There is ~~only one~~ are two FDA-approved medications for ALS, riluzole, which ~~and it~~ only extends survival modestly, and Radicava (edaravone). ALS also exacts a significant economic burden.	Update the FDA-approved medications for ALS
2.1 Background Information	**Updated** references in this section	Updated to include additional references

4.1 Overall Study Design and Plan	**Added**: A fifty-two (52) week Open Label Extension (OLE) study will be available to those subjects who complete the randomized, double-blind study. Please refer to 13.7 Appendix VII for all the details on the OLE.	Clarified that the Open Label Extension study will be available for subjects who completed the randomized, double-blind study
5.2.2 Exclusion Criteria	**Updated:** 12. The presence of unstable psychiatric disease, cognitive impairment, dementia or substance abuse that would impair ability of the subject to provide informed consent, according to Site Investigator judgment 12.13. Patients who have cancer with the exception of the following: basal cell carcinoma or successfully treated squamous cell carcinoma of the skin; cervical carcinoma in situ; prostatic carcinoma in situ; or other malignancies curatively treated and with no evidence of disease recurrence for at least 3 years. 13.14. Clinically significant unstable medical condition (other than ALS) that would pose a risk to the subject if they were to participate in the study 14.15. Active participation in an ALS clinical trial evaluating an experimental small molecule within 30 days of the Screening Visit. (Please refer to MOP section E. Protocol Compliance for current list of prohibitedexperimental drugssmall molecules). 16. Exposure at any time to any cell therapies and gene therapies biologic under investigation for the treatment of subjects with ALS (off-label use or investigational) including cell therapies, gene therapies, and monoclonal antibodies. 15.17. Exposure to monoclonal antibodies under investigation for the treatment of ALS (off-label use or investigational) within 90 days from screening. If previously exposed to monoclonal antibodies under investigation for the treatment of ALS, a 90-day wash-out period will be required prior to screening. 16.18. Implantation of Diaphragm Pacing System (DPS) 17.19. 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 18.20. Exposure to any disallowed medications listed below	Clarified the exclusion criteria for entry into the study

5.2.2 Exclusion Criteria	**Updated:** • Probenecid • Acetyl-L-Carnitine • Methylcobalamine (if administered at doses equal to or greater than 25 mg per week)	Clarified the medication that are included in the exclusion criteria
7.3 MR-PET Visit 1 (Only for patients in MR-PET substudy)	**Added:** Administer ALSFRS-R questionnaire	Clarified the schedule of activities for the MR-PET Visit 1
7.12 MR-PET Visit 2 (Only for patients in MR-PET Substudy)	**Added:** Administer ALSFRS-R questionnaire	Clarified the schedule of activities for the MR-PET Visit 2
7.14 Final Follow-up Telephone Call (Week 28)	**Added:** Subjects who enroll in the open label extension will not be required to complete this telephone call.	Clarified the schedule of activities for Final Follow-up Telephone Call
13.7 Appendix VII	**Added:** Open Label Extension Schedule of Activities and the Open Label Extension Plan	Clarified the Schedule of Activities for the OLE portion of the study and the Open Label Extension Plan

Evaluation of the Safety, Tolerability, Efficacy and Activity of AMX0035, a Fixed Combination of Phenylbutyrate (PB) and Tauroursodeoxycholic Acid (TUDCA), for Treatment of Amyotrophic Lateral Sclerosis (ALS)

Summary of Protocol Changes v3.0 to4.0

Section	Revision	Rationale
Title Page, Footer and Signature Page	**Updated** the version number and date to 4.0 / 16 May 2018	Updated to current version number and date
List of Abbreviations	Added: New York Genome Center (NYGC)	Updated to include new term and abbreviation
Protocol Summary	• *Updated version number to 4.0*	Updated to include the current version number
Schedule of Assessments	• ***Added:*** *Blood draw to be completed for optional DNA collection*	Added an additional assessment at the MR-PET visits and clarified that the Final Safety Phone Call will not be required for any subjects that enroll in the OLE
Schedule of Assessments	• ***Added:*** *[12] If Baseline visit has already occurred or the sample was not collected, DNA should be obtained at next available visit*	Footnote added to Schedule of Activities to explain DNA collection timeframe
Study Workflow	*Removed duplicate workflow*	Added in error
Section 5.2.2 Exclusion Criteria and Section 13.7 Appendix VII: AMX-0035 in ALS Open Label Extension (OLE)	Removed: • ~~Acetyl-L-Carnitine~~ • ~~Methylcobalamine (if administered at doses equal to or greater than 25 mg per week)~~	Removed two medications from the prohibited medications list as they were added in error. These medications are disallowed because they are currently being studied in active trials so are captured under exclusion criteria 15
Section 5.2.2 Exclusion Criteria	• *Added:* ***15.*** Active participation in an ALS clinical trial evaluating an ***experimental*** small molecule within 30 days of the Screening Visit. ***(Please refer to MOP section E. Protocol Compliance for current list of experimental small molecules).*** •	Clarified that the current list of experimental trials will be include in the MOP
Section 6.7 Prior and Concomitant Therapy	Added: • Acetyl-L-Carnitine • Methylcobalamine (if administered at doses equal to or greater than 25 mg per week)	Medications added as they are currently being studied in active trials

Section 7.4 Baseline Visit	• *Collect blood sample for DNA collection (Note: if Baseline visit has passed or blood sample for DNA was not collected, the blood sample should be collected at the next available visit)*	Adding
Section 8.1.4 Blood Samples for Future Research Use	***Added:*** ***All subjects will provide blood samples for deoxyribonucleic acid (DNA) extraction for genome sequencing at the Baseline Visit. Deidentified blood samples will be stored at the New York Genome Center (NYGC) in New York City, NY.*** ***DNA may be stored, used in genome-wide association studies (GWAS), whole genome sequencing, exome sequencing, or for any other known or as yet undiscovered DNA analysis applicable to understanding or targeting disease, with a particular emphasis on ALS. The information from these genetic studies may be made available to collaborators in academia, not-for-profit settings, or industry for appropriate research. Results of DNA testing from this study will not go into the participant's medical record.*** ***The NYGC will be conducting the sequencing of the coded samples, doing the analysis of the sequencing and sharing the results of such sequencing and analysis with researchers pursuant to this protocol, as well as uploading the data to data repositories such as the National Institutes of Health (NIH) Database of Genotypes and Phenotypes (dbGaP).*** •	Added information about the DNA blood draw to be collected at Baseline and sent to the New York Genome Center for sequencing.
Section 8.1.9 Columbia Suicide Severity Rating Scale	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~~.	Clarified that the C-SSRS will assess suicidality over a subject's lifetime.
Open Label Extension- Study Schedule	**Blood samples are recommended to be taken at the end of the study visits *and will be processed locally.***	Clarified that OLE labs will be processed at the site's local lab rather than centrally.
Open Label Extension Screening/Baseline Clinic Visit	**Measure vital signs (blood pressure, heart and breathing rates, temperature, ~~height~~ and weight)**	Removed height from the list of procedures to perform.

Evaluation of the Safety, Tolerability, Efficacy and Activity of AMX0035, a Fixed Combination of Phenylbutyrate (PB) and Tauroursodeoxycholic Acid (TUDCA), for Treatment of Amyotrophic Lateral Sclerosis (ALS)

Summary of Protocol Changes v4.0 to 5.0

Section	Revision	Rationale
Title Page, Footer and Signature Page	**Updated** the version number and date to 5.0 / 06 Sep 2018	Updated to current version number and date
Protocol Summary	**Updated** version number to 5.0	Updated to include the current version number
Open Label Extension- Schedule of Activities	**Removed:** After the Screening/Baseline visit, Con Meds in the OLE will only be recorded if an SAE occurs.	Con Meds will be collected the same as the main study in the OLE
Open Label Extension- Study Schedule	**Added: Note height should be recorded from the main study Screening Visit.**	Clarified that height from the main study should be used for the SVC calculation
Open Label Extension- Study Schedule	**Added:** **Laboratory Testing:** **The following laboratory tests will be performed for safety:** o **Hematology with differential panel: complete blood count with differential (hematocrit, hemoglobin, platelet count, RBC indices, Total RBC, Total WBC, and WBC & differential)** o **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, potassium, sodium, total bilirubin and total protein** o **Urinalysis:, bilirubin, blood, clarity, color, glucose, ketones, nitrate, pH, protein, specific gravity, urobilinogen and WBC screen** o **Serum human chorionic gonadotrophin (hCG) for women of childbearing potential (WOCBP) (collected only at Screening Visit, and as necessary throughout course of study)**	Clarified the laboratory tests that should be done locally.

	Please note: for the OLE portion of the study, local laboratories will be used.	
Open Label Extension- Safety and Adverse Events	~~For the purposes of this study~~ An adverse event (AE) will be defined as any unfavorable and unintended sign, symptom, or disease that the Site Investigator deems to be definitely, probably, ~~or~~ possibly, **unlikely or not related** ~~associated with~~ **to** the conduct of the study procedures or study drug.	Modified the Adverse Event collection to include all events so it matches the main study procedure

Evaluation of the Safety, Tolerability, Efficacy and Activity of AMX0035, a Fixed Combination of Phenylbutyrate (PB) and Tauroursodeoxycholic Acid (TUDCA), for Treatment of Amyotrophic Lateral Sclerosis (ALS)

Summary of Protocol Changes v5.0 to 6.0

Section	Revision	Rationale
Title Page, Footer and Signature Page	**Updated** the version number and date to 6.0 / 11 Jan 2019	Updated to current version number and date
Protocol Summary	Updated version number to **6**.0	Updated to include the current version number
Schedule of Activities	Updated to include option for DNA blood draw at every visit	The optional DNA blood draw should be completed at the Baseline visit. However, if the subject has passed the Baseline visit, the blood draw should be completed at the next visit.
Schedule of Activities	**Footnote 12** updated **to include:** This is a one-time collection.	Footnote updated to note that the DNA collection will be a one time collection.
Section 4.1 Overall Study Design and Plan	An ~~fifty-two~~ one hundred thirty-two (132) week Open Label Extension (OLE) study will be available to those subjects who complete the randomized, double-blind study.	Updated to include the extension to the OLE timeline
Section 7.1 MR-PET Scheduling Call	~~Only those~~ Subjects from all ~~selected~~ sites will be considered to participate in the MR-PET Sub-Study	The MR-PET sub study was opened up to all sites
Section 7.4 Baseline Visit	Collect blood sample for optional DNA collection (Note: if Baseline visit has passed or blood sample for DNA was not collected, the blood sample should be collected at the next available visit)	Clarified that the DNA collection is optional
Section 8.1.4 Blood Samples for Future Research	~~All~~ Subjects will have the opportunity to provide an additional optional blood sample~~s~~ for deoxyribonucleic acid (DNA) extraction for genome sequencing at the Baseline Visit.	
Open Label Extension-Schedule of Activities	Added: Week 68, Week 84, Week 100, Week 116, Week 132 (Early Discontinuation Visit or Final Safety Visit)	

Summary of Protocol Changes v5.0 to 6.0

Open Label Extension- Schedule of Activities	Updated to include option for DNA blood draw at every visit	The optional DNA blood draw should be completed at the Baseline visit. However, if the subject has passed the Baseline visit, the blood draw should be completed at the next visit.
Open Label Extension- Study Schedule	Added: *If subject is unable to complete all procedures, minimal procedures should be completed in the following suggested order: AE Review, Safety labs, ECG, Concomitant Medication, ALSFRS-R.	Clarified the procedures that should be completed if subject is unable to complete the full study visit.
Open Label Extension- Study Design and Plan	This is a multi-center, open label extension, **up to** ~~52~~ **132** week study evaluating the long-term safety of AMX0035…Treatment duration will be **up to** ~~fifty-two~~ **one hundred thirty-two** (~~52~~ **132**) weeks starting at the Screening/Baseline visit. Clinic visits will occur at Screening/Baseline, Week 6 (Day 42), Week 12 (day 84), Week 24 (day 168), Week 36 (day 252), and Week 52 (day 364), **Week 68 (476), Week 84 (588), Week 100 (700) , Week 116 (812), Week 132 (924).**	Updated language to include extension to the study schedule
Open Label Extension- Study Objectives	The primary outcome measure is: 1. The confirm the long-term safety of AMX0035 in subjects with ALS over **132** ~~52~~ week period	Updated to extend the OLE from 52 weeks to 132 weeks
Open Label Extension- Duration of Treatment and Follow-up	Subjects will remain on treatment until the Week ~~52~~ **132** or early discontinuation visit.	Updated to extend the OLE from 52 weeks to 132 weeks
Open Label Extension- Study Schedule	Week 6, Week 12, Week 24, Week 36, Week 52, **Week 68, Week 84, Week 100, Week 116, Week 132 or** Early Discontinuation/Final Safety Clinic Visit: The Week 6 and Week 12 visits will take place +/- 10 days and the Week 24, Week 36, ~~and~~ Week 52, **Week 68, Week 84, Week 100, Week 116, and Week 132** visits will take place +/- 28 days from the time specified in the schedule of activities (table as beginning of this section).	Updated language to include extension to the study schedule

Statistical Plan

Amylyx Pharmaceuticals Inc.

Statistical Analysis Plan

Evaluation of the Safety, Tolerability, Efficacy and Activity of AMX0035, a Fixed Combination of Phenylbutyrate (PB) and Tauroursodeoxycholic Acid (TUDCA), for Treatment of Amyotrophic Lateral Sclerosis (ALS)

PIs: Sabrina Paganoni, Nazem Atassi

DATE

Version 1.0

Prepared by:

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Date

Reviewed and Approved by:

David Schoenfeld, Ph.D — Date: apr 7, 2017

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Principal Investigator

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Date

Sponsor

Statistical Analysis Plan

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1. Study Design

This is a multicenter, randomized, double-blind, placebo-controlled 28-week study evaluating the safety, tolerability, efficacy, pharmacokinetics and biological activity of AMX0035.

This study will be conducted in subjects who have sporadic or familial ALS diagnosed as definite as defined by revised El Escorial criteria. Subjects must provide written informed consent prior to screening. At screening, eligible subjects must be at least 18 years old and have a VC $\geq$ 60% of predicted capacity for age, height and gender. Subjects must have had onset of ALS symptoms less than or equal to 18 months prior to the screening visit. Subjects on a stable dose of riluzole defined as riluzole dosing for at least 30 days prior to screening visit 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.

Subjects will be randomly assigned in a 2:1 ratio to oral (or feeding tube) AMX0035 treatment (1 sachet = 3g PB and 1g TUDCA) or matching placebo. For the first three weeks of dosing, subjects will take one sachet daily (i.e. half-dose) and if tolerated increase to two sachets daily.

Subjects will remain on treatment or placebo until the Week 24 visit. Each randomized subject will have a Final Telephone Interview 28 days (+/- 5 days) after last administration of study drug or placebo to assess for adverse events (AEs), changes in concomitant medications and to administer the ALSFRS-R.

1.1 Study Title

Evaluation of the Safety, Tolerability, Efficacy and Activity of AMX0035, a Fixed Combination of Phenylbutyrate (PB) and Tauroursodeoxycholic Acid (TUDCA), for Treatment of Amyotrophic Lateral Sclerosis (ALS)

1.2 Study Objectives and Endpoints

The primary objectives of the study are to assess safety, tolerability, and efficacy of oral (or feeding tube) administration of AMX0035 sachet (3g PB and 1g TUDCA) treatment administered twice daily vs. matched placebo administered twice daily.

The primary outcome measures will be:

1. To confirm the safety and tolerability of a fixed-dose combination of PB and TUDCA in subjects with ALS over a 6-month period
2. To measure the impact of the treatment using the slope of disease progression with the revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R)

The secondary objectives of the study will be:

1. To assess the impact of AMX0035 on the rate of decline of isometric muscle strength, as measured by the Accurate Test of Limb Isometric Strength (ATLIS)
2. To assess the impact of AMX0035 on disease progression as measured by Slow Vital Capacity (SVC) decline, time to tracheostomy, and survival
3. To assess the impact of AMX0035 on biomarkers including phosphorylated axonal neurofilament H subunit (pNF-H) levels and 18 kDa translocator protein (TSPO) uptake
4. To determine the population pharmacokinetics parameters of PB and TUDCA at steady state during treatment with 4 g AMX0035

1.3 Rationale for the Study

The objectives of this study are to determine the safety and efficacy of AMX0035 in subjects with Amyotrophic Lateral Sclerosis (ALS). ALS is a progressive neurodegenerative disease for which there is no cure. There is one medicine approved specifically for treating ALS, Rilutek (riluzole), and it only provides a modest benefit for subjects. ALS also exacts a significant economic burden.

AMX0035 has demonstrated efficacy in models of neurodegeneration, classical activation of neuroinflammation, and bioenergetics deficits. The individual components of AMX0035, PB and TUDCA have demonstrated efficacy in *in vivo* models of ALS, Parkinson's, Alzheimer's, ischemia, and many others. Each individual component has been tested in small clinical trials of ALS subjects and was found to be safe and well-tolerated, and hit primary endpoints of efficacy.

The first trial under this IND will be a randomized, double-blind, placebo-controlled Phase II trial to evaluate the safety and efficacy of AMX0035 for the treatment of ALS. The program is designed to demonstrate that treatment can slow the progressive decline in function, muscle strength, and vital capacity, and to assess the impact of AMX0035 therapy on biomarkers of ALS including blood levels of phosphorylated axonal neurofilament H subunit and 18 kDa translocator protein PET tracer uptake.

1.4 Schedule of Activities

ACTIVITY	Screening Visit	Study Drug Administration (weeks)									Final Follow-up Telephone Call[2]	PET Sub-Study Subjects Only
		Baseline Visit[1]	Week 3	Week 6	Week 9	Week 12	Week 15	Week 18	Week 21	Week 24 OR Early Discontinuation/ Final Safety Visit		
	Clinic	Clinic	Clinic	Clinic	Phone	Clinic	Phone	Clinic	Phone	Clinic	Phone	At MGH
	-42 Days	Day 0	Day 21 ±5	Day 42 ±5	Day 63 ±5	Day 84 ±5	Day 105 ±5	Day 126 ±5	Day 147 ±5	Day 168 ±5	28 +5 days	
Written Informed Consent	X											X
Inclusion/Exclusion Review	X	X										X
Medical History/Cardiac History/Demographics	X											
ALS Diagnosis/ALS History	X											
Vital Signs[3]	X	X	X	X		X		X		X		
Neurological Exam[4]	X					X				X		X[4]
Physical Exam[5]	X					X				X		
Blood Draw for Safety Labs[6]	X	X	X	X		X		X		X		
Blood Draw for Serum Pregnancy Test for WOCB[6]	X											
Urine Sample for Urinalysis[6]	X	X	X	X		X		X		X		
12-Lead ECG	X					X				X		
ALSFRS-R	X	X	X	X	X	X	X	X	X	X	X	
Slow Vital Capacity	X	X		X		X		X		X		
ATLIS Testing[7]	X	X		X		X		X		X		
Columbia-Suicide Severity Scale[7]		X[7]	X	X		X		X		X		
PET Scan[8]		X								X		X[8]
Blood draw for Biomarker Testing[9]		X		X		X		X		X		
Blood draw for PK Analysis[10]		X				X				X		
Adverse Events[11]	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X
Randomization[12]		X										
Dispense Study Drug[13]		X		X		X		X				
Drug AccountabilityCompliance		X	X[14]	X	X	X	X	X	X	X		

[1]The Baseline Visit should occur no more than 42 days after the Screening Visit.
[2]A Final Safety Telephone Call will be conducted 28 (+5 days) after the subject takes their last dose of study drug (whether or not the subject has discontinued from the study) to assess for adverse events (AEs), changes in concomitant medications and to administer the ALSFRS-R.
[3]Vital signs include systolic and diastolic pressure in mmHg, respiratory rate/minute, heart rate/minute and temperature.
[4]Neurological Exam will include the Upper Motor Neuron Burden Scale (UMN-B) for the PET Sub-Study only. UMN-B will be done for subjects participating in the PET Sub-Study at the time of their scan.
[5]Physical Exam will include height and weight. Height will be collected at Screening Visit ONLY.
[6]Safety labs include Hematology (CBC with differential), Complete Chemistry Panel, Liver Function Tests and Urinalysis. Serum pregnancy testing will occur in women of child bearing potential (WOCBP) at the Screening Visit and as necessary during the course of the study.
[7]C-SSRS Baseline version to be completed at Baseline Visit only. C-SSRS Since Last Visit version to be completed at all other visits.
[8]Approximately 20 subjects will receive PET (Positron Emission Tomography) scanning completed at selected sites. First scan will occur PRIOR to the Baseline Visit (pre-dose) and the second scan will occur between the Week 21 and Week 24 study visits. PET subjects will also provide a blood sample for peripheral blood mononuclear cell (PBMC) testing and TSPO affinity testing.
[9]Subjects will provide a blood sample for biomarker testing and storage in a biorepository.
[10]All subjects will provide a blood sample for pharmacokinetic (PK) testing at the Baseline Visit (pre-dose). Subjects will also provide a blood sample either 1 hour or 4 hours post-dose (±10 minute window per time point) at the Week 12 and Week 24 Visits. PK times will be randomized such that every subject has a 1-hour draw at one visit and a 4-hour draw at the other.
[11]Adverse events that occur AFTER signing the consent form will be recorded.
[12]Randomization should occur at the Baseline Visit. Randomization will entail entering a subject's kit number into the data capture system.
[13]First dose of study drug will be administered in clinic after ALL Baseline Visit procedures are completed.
[14]Notify subjects of increase from one sachet per day to two sachets per day

2. Study Population

This study will be conducted in subjects who have sporadic or familial ALS diagnosed as definite as defined by revised El Escorial criteria. Subjects must provide written informed consent prior to screening. At screening, eligible subjects must be at least 18 years old and have a VC $\geq$ 60% of predicted capacity for age, height and gender. Subjects must have had onset of ALS symptoms less than or equal to 18 months prior to the screening visit. 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.

Approximately 132 Subjects will be randomly assigned in a 2:1 ratio to oral (or feeding tube) AMX0035 treatment (1 sachet= 3g PB and 1g TUDCA) or matching placebo. For the first three weeks of dosing, subjects will take one sachet daily (i.e. half-dose) and if tolerated will increase to two sachets daily.

Modified Intent to Treat (Primary)

Primary analysis will be based on a modified intent-to-treat criteria. The modified intent-to-treat population will include all patients who were administered at least one dose of study material. Patients will be followed for ALSFRS-R and mortality even if they stop study medication.

Per Protocol (Secondary)

Since this is a longitudinal study Per-Protocol will be defined per visit rather than per patient. All patients who were eligible for the study will be in the Per-Protocol analysis. Patients will remain in that analysis up until the time that they have a major protocol violation or have not taken study medication for one month.

Safety Population

The Safety population is defined as all randomized subjects who received at least one dose of study treatment. Since randomization occurs when the first dose is administered, the safety population will be identical to the modified ITT population. The Safety population will be used to present the safety summaries by actual treatment received.

3. Definitions, Demographics and Derived Variables

3.1 Dosage

Dosage: The treatment arms are AMX0035 and placebo. Both AMX0035 and placebo are administered as one sachet daily for the first three weeks and then escalated to 2 sachets daily for the remainder of the study.

Dose reduction, suspension, and re-challenge: If an AE occurs but is mild or moderate, the dose may be reduced as per protocol section 6.3.1. The site investigator may then choose to continue the study medication at the reduced dose or titrate it back to the initial dose. In the event of a serious or life-threatening AE, the study medication will be immediately discontinued and will not be reinstituted. In addition, the investigator may suspend study medication for any reason during the trial. If the suspension exceeds 14 day, study medication will not be re-challenged. All subjects for whom the study medication was permanently discontinued will continue to be followed till the end of the study, except for the safety laboratory tests as they will become unnecessary.

3.2 Demography and Baseline Characteristics

At screening the following measures will be taken: Age, SVC, Time since Symptom Onset, El Escorial Diagnosis, Site of Onset, ALSFRS-R score, Sex, Height and Weight, ATLIS.

Age. Age will be calculated using the Date of Birth (DOB) and the date of the Screening visit, and presented as age at last birthday as an integer.

Age = Integer part of [(Date of Screening visit – Date of Birth) / 365.25]

SVC. Slow vital capacity (SVC) is the maximum volume of air that can be exhaled slowly after slow maximum inhalation. The SVC may be measured by spirometer. Normative data for the SVC have been established. 3-5 measurements will be taken at every visit and the maximum value will be inputted for analysis.

Symptom Onset will be defined as the first onset of weakness. The time since symptom onset will be calculated using the below formula. Patients less than 548 days from symptom onset at Screening are eligible for the study.

Time since Symptom Onset = [(Date of Screening visit – Date of Symptom Onset)]

El Escorial Diagnosis. The El Escorial Diagnostic Criteria will be applied to each patient. Patients much meet definite criteria to be enrolled.

Site of Onset. Each patient will be asked the site of ALS onset. Patients may be 'limb', 'bulbar', or 'other'.

ALSFRS-R Score. The ALSFRS-R is a quickly administered (five minute) ordinal rating scale (ratings 0-4) used to determine patients' assessment of their capability and independence

in 12 functional activities. All 12 activities are relevant in ALS. The maximum score is 48. Each patient will receive an ALSFRS-R score at screening.

Sex. Each patient's biological sex will be recorded.

Height and Weight. Each patient's height and weight will be recorded.

ATLIS. ATLIS is an isometric strength measurement device. Each patient's absolute strength in twelve muscle areas will be measured at screening by ATLIS and then normalized to standard values based on Patricia Andres per predicted normal dataset (Andres, P. et al. Developing normalized strength scores for neuromuscular research. Muscle and Nerve. 2013.).

All measured muscle groups will then be averaged to produce a global composite percentage strength outcome as well as an upper limb and lower limb composite. The global composite will be considered the most meaningful of these analysis and be the primary analysis. If a patient is unable to apply any force in a particular muscle area they will be assigned "0%".

3.3 Datasets Analyzed

The final analysis will include all 6-month follow-up data of all subjects. All patients who are administered at least one dose of study drug will be included in the primary analysis.

3.4 Time Points

A schematic of these visits can be found in 1.4 *Schedule of Activities*. Below is a description of each visit.

Screening visit: The screening visit will determine eligibility for the trial as well as get initial baseline data. Patients who meet criteria will need to have their baseline visit within 42 days.

Baseline visit: The procedures under "Baseline" in the Schedule of Assessments (1.4) are all performed at this study visit. The last procedure at this visit will be administration of the study material to the patient and randomizing that patient.

Follow-up visits: Follow-up visits are calculated from the first day of the baseline visit (day 0). The visit window is ±5 days from the expected visit date. After the baseline visit, which takes place at the study site, subjects are again evaluated in person at week 3, 6, 12, 18, and 24. At weeks 9, 15, and 21 are evaluated remotely via telephone.

For details of the various study procedures performed at each visit, see Schedule of Activities (1.4).

All subjects will stop taking study medication after the week 24 visit. A final follow-up via telephone will be completed four weeks after drug discontinuation (28+5 days).

Nominal vs. actual time point: For each visit, the visit type that falls closest to the visit date will be the visit's nominal time point, while the actual time point will be the number of days between the visit and the subject's randomization/baseline visit. Graphs and tables will use

nominal time points while analyses by mixed models or survival methods will use actual time points.

4. Efficacy Parameters

4.1 Primary Efficacy Endpoint

The primary efficacy endpoint is the slope of change in the Amyotrophic Lateral Sclerosis Functional Rating Scale Revised (ALSFRS-R) over time. The placebo and active arms will be compared by a shared-baseline, mixed-effects analysis. A covariate of bulbar onset or onset elsewhere and a second covariate of age at enrollment will be included in the analysis. The mixed-effects model accounts for both the variance between subjects and the deviation within subjects from their average rate of decline. We will use the same analysis for clinical outcomes in this trial. The model used is as follows: Let Yi,t be the dependent variable observed at time t, let z be a treatment indicator which is 0 in the control group and one in the treatment group. Then the model is that $Yi,t=\mu+ui+(\beta 0+bi)t+\beta 1*z_i*t+\epsilon i,t$, where ui and bi are random effects which have an unspecified bivariate normal distribution. The covariates are not shown in this model statement.

We propose to test at a two sided alpha of 0.05.

4.2 Secondary Efficacy Endpoints

These include:

4.2.1 Accurate Testing of Limb Isometric Strength (ATLIS)

ATLIS will use the same model as ALSFRS-R with the addition of height, weight, age, and gender as covariates in the analysis.

4.2.2 Neurofilament Testing

We will be evaluating blood levels of Neurofilament light (NFL) and heavy chains (pNFH). Neurofilaments will be used as a surrogate measure of neuronal death. These proteins are greatly elevated in ALS patients and promising results from multiple trials suggest this marker may be prognostic of clinical decline. We intend to evaluate these markers at week 0, 6, 12, 18, and 24 building a longitudinal dataset for them.

There is significant data on Neurofilament heavy chain (pNF-H) in ALS.. As such, the primary analysis to be conducted with the neurofilaments will include a shared-baseline, mixed-effects analysis to compare the slope of change in pNF-H between the active arm and placebo arm over the course of the trial. A covariate of bulbar onset or onset elsewhere, a second covariate of age at enrollment, and a third covariate of baseline ALSFRS-R will be included in the analysis.

The same analysis will be conducted on NFL but this will be considered more exploratory.

Additionally, exploratory analyses will include the difference at endpoint pNF-H and NFL between the active and placebo arms.

4.2.3 TSPO Testing

To assess neuroinflammation, we will use a novel positron emission tomography (PET) radiotracer called [11C]-PBR28 that specifically binds to activated microglia and astrocytes in a subset of patients (N=15-20).

We will have two measurements one at baseline, say j=0 and j=1 and one at the end of the study, we fit a repeated measures analysis of variance using the following model. $Yi,j=\mu+\beta 0j+\beta 1*z_i*j+\epsilon i,j$ where $\epsilon i,j$ are bivariate normal. The treatment effect is measured by $\beta 1$.

4.2.4 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. All VC evaluators must be NEALS certified. Three VC trials are required for each testing session, however up to 5 trials may be performed if the variability between the highest and second highest VC is 10% or greater for the first 3 trials. Only the 3 best trials are recorded on the CRF. The highest VC recorded is utilized for analysis.

A shared-baseline, mixed-effects analysis will be used to compare the slope of change in the SVC between the active arm and placebo arm. A covariate of bulbar onset or onset elsewhere and a second covariate of age at enrollment will be included in the analysis.

4.2.5 Survival

Survival endpoint will be considered as mortality, tracheostomy or permanent assisted ventilation (defined as >22h per day assisted ventilation).

Tracheostomy-free survival will be compared between the active and placebo arms using a proportional hazards model with covariates of baseline ALSFRS-R, age at enrollment, and site of onset.

4.2.6 Hospitalizations

Hospitalizations will be considered as in-patient hospital stays due to disease progression. Hospitalizations will be compared between groups with a proportional hazards model with baseline ALSFRS-R, age, and site of onset as covariates.

4.2.7 Pharmacokinetics

Plasma concentration over time data will be recorded in tabular and graphical forms based on dose of AMX0035. The individual plasma concentration- time data will be pooled and subjected to a population PK concentration-response analysis, using currently accepted software methods. An exploratory analysis will be performed to examine the relationship between derived PK parameters and the concentrations of the 3 analytes and AMX0035 dose, as well as select efficacy and/or biomarker parameters obtained at (Baseline, Week 12 and Week 24 samples). The influence of various covariates (e.g. age, body weight, BMI, gender, race, co-medication, serum creatinine) on the PK parametersconcentrations of TUDCA or Phenylbutyrate/Phenylacetate may be examined. The population PK concentration-response analysis will be documented under a separate study report to be produced independently of the clinical study report.

4.3 Exploratory Analyses

While exploratory, we may also try to assess whether sex, baseline neurofilament level, or drug exposure correlate to differential drug effects.

5. Safety Endpoints

5.1 Primary Analysis—Safety and Tolerability

Safety will be evaluated using adverse events, laboratory values, vital signs, ECG recordings, neurological examinations, and physical examinations. The schedule of these assessments is in section 1.4.

Safety

AE's will be tabulated by the *MEDRA Preferred Term, High level Term and system organ class* and compared between treatment groups using an exact test based on the number of patients with 0,1,2… events. Using scores equal to the number of events.

The following AE summaries will be provide:

- All AEs
- All ≥Grade 3 AEs
- All vaccine related (including related and possibly related) AEs
- All SAEs
- Deaths
- AEs leading to withdrawal

Adverse events are summarized by patient incidence rates; the

Hothorn, T., Hornik, K., van de Wiel, M. A. and Zeileis, A. (2008). Implementing a class of permutation tests: the coin package. *Journal of Statistical Software* **28**(8), 1–23.http://www.jstatsoft.org/v28/i08/

Compliance data will be determined for each visit and by treatment group. The time to subject refusal will be compared between treatment groups to better determine tolerability. Descriptive statistics denoting the changes from baseline to the final assessment visit with respect to key laboratory parameters and vital signs will also be provided. Compliance over the entire course of the study will also be calculated.

Tolerability

With 88 treated subjects, we will have an 80% probability of detecting any adverse event expected to occur in at least 2% of treated subjects. We will have 80% power to detect a 28 percentage point elevation in the rate of any adverse event relative to placebo based on a one-tailed test at alpha = 0.05. We will consider a dose tolerable if the proportion of treatment failures (discontinuation of study drug due to an adverse event or reduction of treatment) is less than 40% with 80% confidence, one-tailed. If a patient completes the study on a reduced dose they will be considered to have tolerated that dose. With 88 treated subjects this would occur if

30 or fewer subjects on AMX0035 fail to complete the 6-month study. By this criterion, we will have 80% power for declaring AMX0035 tolerable at the tested dose if the true treatment failure rate is 30%.

5.2 Description of Safety Parameters

5.2.1 Adverse Events

Adverse events will be recorded both during clinic and phone contacts with the patients (see 1.4). Treatment AEs will be coded using MEDRA.

5.2.2 Laboratory Values

Laboratory values and vital signs will be tabulated by treatment and visit and compared between treatment groups using a t-test. Laboratory measures will be compared with their corresponding normal ranges and the incidence of abnormally high and abnormally low values will be calculated for each measure. If normal ranges are not available, the textbook normal will be adopted. The shift in toxicity grade from those collected at baseline to the worst value on study will be summarized using NCI CTCAE or similar toxicity grades for laboratory toxicity.The following laboratory tests will be performed for safety.

- Hematology with differential panel: complete blood count with differential (hematocrit, hemoglobin, platelet count, RBC indices, Total RBC, Total WBC, and WBC & differential)
- Blood chemistry panel/Liver function tests (LFTs): alanine aminotransferase (ALT (SGPT)), aspartate aminotransferase (AST (SGOT)), albumin, alkaline phosphatase, bicarbonate, blood urea nitrogen, calcium, chloride, creatinine, glucose, magnesium, phosphate, potassium, sodium, total bilirubin and total protein
- Urinalysis: albumin, bilirubin, blood, clarity, color, glucose, ketones, nitrate, pH, protein, specific gravity, urobilinogen and WBC screen
- Serum human chorionic gonadotrophin (hCG) for women of childbearing potential (WOCBP) (collected only at Screening Visit, and as necessary throughout course of study)

5.2.3 Vital Signs

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.

5.2.4 ECG Recordings

A standard 12-lead ECG will be performed. Tracings will be reviewed by a central ECG reader and a copy of the tracings will be kept on site as part of the source documents.

5.2.5 Neurological Examinations

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

5.2.6 Physical Examinations

A comprehensive physical examination will be performed and recorded.

Amylyx Pharmaceuticals Inc.

Statistical Analysis Plan Amendment 1

Evaluation of the Safety, Tolerability, Efficacy and Activity of AMX0035, a Fixed Combination of Phenylbutyrate (PB) and Tauroursodeoxycholic Acid (TUDCA), for Treatment of Amyotrophic Lateral Sclerosis (ALS)

PIs: Sabrina Paganoni, Nazem Atassi

August 1st 2018

Version 1.0

Statistical Analysis Plan

Reviewed and Approved by:  Aug 20, 2018

David Schoenfeld, Ph.D — Date

Sabrina Paganoni — 8/24/18

Date

Principal Investigator

 8-20-2018

Date

Sponsor

Statistical Analysis Plan

1 Analysis for Edaravone Interaction

During the course of the AMX-3500 (CENTAUR) clinical study, a new therapy was FDA approved for ALS called Edaravone (Radicava). We do not exclude edaravone use in the current trial.

As an exploratory analysis, we therefore plan will test a model which will include an edarvone term and a time*edaravone term and a time*edaravone*treatment interaction. The latter will test whether there is compelling evidence that the drug works differently in patients receiving edaravone as compared to other patients. If this interaction is significant we will use this model to estimate the treatment effect in each of the edaravone groups. The edaravone group is defined as patients who have taken edaravone anytime during the trial.

STATISTICAL ANALYSIS PLAN

Protocol
AMX3500 – Main Study

Evaluation of the Safety, Tolerability, Efficacy and Activity of AMX0035, a Fixed Combination of Phenylbutyrate (PB) and Taurursodiol (TURSO), for Treatment of Amyotrophic Lateral Sclerosis (ALS)

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Evaluation of the Safety, Tolerability, Efficacy and Activity of AMX0035, a Fixed Combination of Phenylbutyrate (PB) and Taurursodiol (TURSO), for Treatment of Amyotrophic Lateral Sclerosis (ALS)

By signing below, all parties accept that the analysis methods and data presentations are acceptable and that this document is final.

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List of Abbreviations

ΔFS	Del-FS Score
AE	Adverse Event
ALP	Alkaline phosphatase
ALS	Amyotrophic Lateral Sclerosis
ALSFRS-R	Amyotrophic Lateral Sclerosis Functional Rating Scale Revised
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATLIS	Accurate Testing of Limb Isometric Strength
BUN	Blood Urea Nitrogen
CFB	Change from Baseline
CSF	Cerebro-spinal fluid
C-CASA	Columbia Classification Algorithm for Suicide Assessment
C-SSRS	Columbia Suicidality Severity Rating Scale
eCRF	Electronic Case Report Form
ECG	Electrocardiogram
EDC	Electronic Data Capture system
ER	Endoplasmic Reticulum
hCG	Human Chorionic Gonadotropin
HDL	High-density Lipoprotein
HHD	Hand-held Dynamometry
LDL	Low-density Lipoprotein
LDH	Lactate dehydrogenase
LFT	Liver Function Test
LS	Least-squares
LSMEANS	Least-squares Means
mITT	Modified Intent-to-treat
MMRM	Mixed model with repeated measures
MAR	Missing at Random
MNAR	Missing Not at Random
MOP	Site Manual of Procedures
MRI	Magnetic resonance imaging
N	Number of subjects
NEALS	Northeast Amyotrophic Lateral Sclerosis Consortium
NFL	Neurofilament Light Chain

OLE	Open Label Extension
PAV	Permanent Assisted Ventilation
PB	Phenylbutyrate
PBMC	Peripheral Blood Mononuclear Cell
PET	Positron Emission Tomography
PK	Pharmacokinetic
PMM	Pattern Mixture Model
pNF-H	Phosphorylated Neurofilament Heavy Chain
PP	Per Protocol
PPK	Population pharmacokinetics
RBC	Red blood cell
SAE	Serious Adverse Event
SVC	Slow Vital Capacity
SD	Standard Deviation
SDTM	Study Data Tabulation Model
SI	Site Investigator
TC	Total cholesterol
TEAE	Treatment Emergent Adverse Events
TG	Triglyceride
TSH	Thyroid stimulating hormone
TSPO	18 kDa translocator protein
TURSO	Taurursodiol
WBC	White Blood Cell
WOCBP	Women of Child Bear Potential

DEFINITIONS

Safety Population	All subjects who receive at least one dose of study medication.
Modified Intent-to-Treat Population (mITT)	All subjects who receive at least one dose of study medication and have at least one post-baseline total ALSFRS-R score.
Per Protocol Population	Since this is a longitudinal study Per-Protocol will be defined per visit rather than per subject. All mITT subjects will remain in that analysis up until the time that they have a major protocol violation or have not taken study medication for one month.
Treatment	There are two treatment groups for this study: - AMX0035 via sachet (3g PB and 1g TURSO) once daily for the first three weeks and increased to two sachets daily if tolerated. - Matching placebo.

1 INTRODUCTION

The objective of the Statistical Analysis Plan is to ensure the credibility of all study findings by means of a predefined data analysis plan. This plan assumes familiarity with the study protocol and will provide further details of the summaries and analyses planned therein. This Statistical Analysis Plan (SAP) was finalized via signatory prior to the treatment unblinding.

1.1 Amyotrophic Lateral Sclerosis

ALS is a progressive neurodegenerative disease for which there is no cure. There are only two medications approved specifically for treating ALS. This includes Rilutek (riluzole), which only provides a modest benefit for subjects, and Radicava (edaravone). ALS also exacts a significant economic burden. ALS is the most prevalent, adult-onset, progressive motor neuron disease, affecting more than 20,000 subjects in the US and an estimated 450,000 people worldwide, according to the ALS Association. ALS causes the progressive degeneration of motor neurons, resulting in rapidly progressing muscle weakness and atrophy that eventually leads to partial or total paralysis; on average, the disease is fatal within 18-24 months from diagnosis. There are two FDA-approved medications for ALS, riluzole, which only extends survival modestly, and Radicava (edaravone). ALS also exacts a significant economic burden.

Although the precise cause of ALS is unknown, ALS and other neurodegenerative diseases such as Alzheimer's are characterized by nerve cell death and inflammation. Together these processes form a toxic cycle that is a key driver of progressive neurological decline. Recent research has highlighted mitochondrial stress and endoplasmic reticulum (ER) stress as key mediators of nerve cell death [Manfredi, G 2015]. The mitochondrion is the energy production center of the cell, while the ER is the quality control center. These two organelles are in constant communication, and are in fact physically connected by a membrane, and their health is vital to cell survival. When either of these cellular processes goes awry, the resulting stress can either kill the cell and/or create inflammation. The brain is extremely sensitive to both mitochondrial stress and ER stress, and both of these pathways have been strongly implicated in causing neurodegenerative disease. We believe that only therapeutically targeting both organelles simultaneously will enact a significant and lasting benefit.

1.2 AMX3500

AMX0035 is a proprietary combination of two small molecules, phenylbutyrate (PB) and Taurursodiol (TURSO), designed to block neuronal death and neurotoxic inflammation through simultaneous inhibition of endoplasmic reticulum (ER) stress and mitochondrial stress.

The individual components of AMX0035, PB and TURSO have demonstrated efficacy in *in vivo* models of ALS, Parkinson's, Alzheimer's, ischemia, and many others [Ryu 2005, Del Signore 2009, Ricobarza 2009, Wiley 2011, Ricobarza 2012, Rodrigues 2003, Castro-Caldas 2012, Zhang 2014]. Each individual component has also been tested in small clinical trials of ALS subjects and was found to be safe and well-tolerated, and achieved intended results on efficacy endpoints.

Both PB and TURSO have also been evaluated in subjects with ALS and were found to be safe, well-tolerated, and exhibited preliminary signs of efficacy. Adverse events in subjects taking riluzole and NaPB together did not occur more frequently, compared to those on PB alone.

Recently, TURSO (called "TUDCA" in the publication) at 1g b.i.d. demonstrated a statistically significant slowing of ALSFRS-R progression rate in a year-long, multi-site, placebo-controlled clinical trial of ALS [Elia et al, 2016]. In this proof-of-principle trial, 34 ALS subjects under treatment with riluzole were randomized to placebo or TURSO (1 gram b.i.d.) for 54 weeks. The proportion of responders (defined as subjects with >15% improvement in ALSFRS-R slope) was higher under TURSO (87%) than under placebo (P = 0.021; 43%). At study end, baseline-adjusted ALSFRS-R was significantly higher (P = 0.007) in TURSO than in placebo groups. Comparison of the slopes of regression analysis showed slower progression in the TURSO than in the placebo group (P < 0.01) (Figure 3).

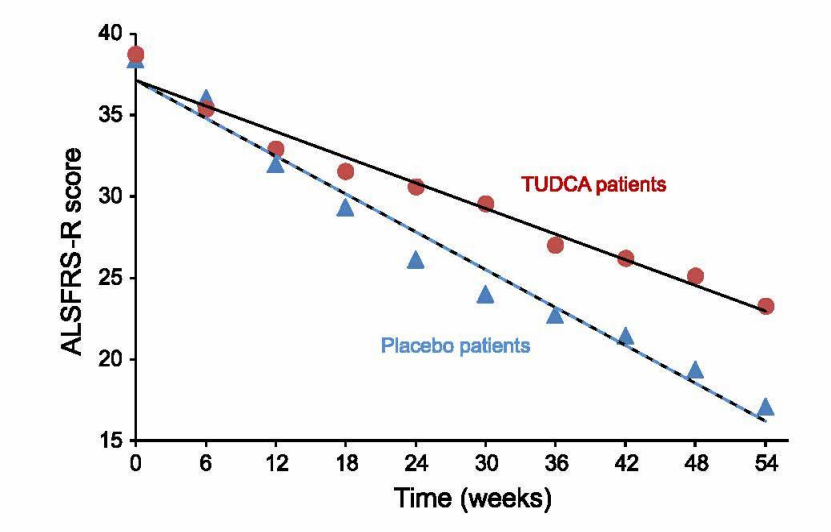


Figure 3: Linear regression analysis of ALSFRS-R mean scores over time for the TURSO (TUDCA in the publication) (circles, slope -0.388) and placebo groups (triangles, slope -0.262).

For the planned Phase II trial of PB in combination with TURSO, a dose of 1 gram of TURSO twice a day (2 grams per day) as a target dose was selected.

Sodium phenylbutyrate (PB) is generally well tolerated. It is FDA approved for subjects with urea cycle disorders including deficiencies of carbamylphosphate synthetase, ornithine transcarbamylase, or argininosuccinic acid synthetase. It is indicated in subjects with either neonatal-onset deficiency or late-onset disease. The usual total daily dose is 450-600 mg/kg/day in subjects weighing less than 20kg, or 9.9-13.0 g/m^2/day in larger subjects. Detailed information can be found on the package insert for PB [Buphenyl, Package Insert].

PB has also been studied in pre-clinical ALS models and in subjects with ALS. In mice it was shown to reduce neuronal death and increase survival through mechanisms thought to be related to its HDAC inhibition activity. In a small Phase 2a trial in subjects with ALS it was also shown to affect HDAC activity in human and to be safe and well tolerated [Cudkowicz, 2009].

Across models of oxidative stress, mitochondrial deficits, endoplasmic reticulum stress, glutamate toxicity and protein misfolding, and multiple sclerosis the combination has been shown to be effective in improving neuronal viability and function. In most of these models, the combination had significant benefit over either drug alone and furthermore in all models the combination showed efficacy whereas the individual drugs did not always show efficacy.

The program is designed to demonstrate that treatment is safe and can slow the decline in function for subjects with ALS. The study is additionally looking at muscle strength, and vital capacity, and the impact of AMX0035 therapy on biomarkers of ALS including blood levels of phosphorylated axonal neurofilament H subunit. This study is expected to provide a robust dataset which could support the efficacy and safety of AMX0035.

2 OBJECTIVES

2.1 Primary Objectives

The primary objective of the study will be to assess safety, tolerability, and efficacy of oral (or feeding tube) administration of AMX0035 via sachet (3g PB and 1g TURSO) twice daily vs. matched placebo administered via sachet twice daily.

The primary outcome measures will be:

1. To confirm the safety and tolerability of a fixed-dose combination of PB and TURSO in subjects with ALS over a 6-month period;
2. To measure the impact of treatment on disease progression using the slope of the revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R).

2.2 Secondary Objectives

The secondary objectives of the study will be:

1. To assess the impact of AMX0035 on the rate of decline of isometric muscle strength, as measured by the Accurate Test of Limb Isometric Strength (ATLIS);
2. To assess the impact of AMX0035 on phosphorylated axonal neurofilament H subunit;
3. To assess the impact of AMX0035 on disease progression as measured by Slow Vital Capacity (SVC) decline, time to tracheostomy and survival;
4. To develop concentration-response models of PB and TURSO at steady-state after administration of AMX0035 sachet twice-daily;
5. To measure the impact of AMX0035 on survival;
6. To assess the impact of AMX0035 on 18 kDa translocator protein (TSPO) uptake.

3 STUDY DESIGN

3.1 Number of Subjects

This study will be conducted in subjects who have sporadic or familial ALS diagnosed as definite as defined by revised El Escorial criteria. Subjects must provide written informed consent prior to screening. At screening, eligible subjects must be at least 18 years old and have a SVC $\geq$ 60% of predicted capacity for age, height and gender. Subjects must have had onset of ALS symptoms less than or equal to 18 months prior to the screening visit. 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 (Protocol Section 5.2).

Approximately 132 ALS subjects will be randomized in the study in up to 25 Northeast ALS Consortium (NEALS) centers in the United States. Sites were selected based on their recruitment record from prior trials, compliance with prior study protocols and regulations, clinical research expertise and availability of necessary resources. Subjects will be randomly assigned in a 2:1 ratio to oral (or feeding tube) AMX0035 treatment (1 sachet= 3g PB and 1g TURSO plus excipients) or matching placebo. For the first three weeks of dosing, subjects will take one sachet daily (i.e. half-dose) and if tolerated will increase to two sachets daily.

3.2 Sample Size Considerations

We found in the PRO-ACT database that subjects who were <540 days since symptom onset and had definite El Escorial Diagnosis progressed considerably faster than the overall population. An initial shared-baseline, mixed-effects analysis using these criteria in a different database (Ceftriaxone) with a 2:1 subject randomization between treatment and placebo indicated that approximately 131 subjects tracked over 6 months would provide 80% power to detect a 30% treatment effect when tested at a two-sided alpha of 0.1. We have added covariates which may improve the model's ability to fit this population and have higher power.

3.3 Study Design

During the enrollment period approximately 176 subjects will be screened from approximately 25 NEALS centers in the US. Approximately, one hundred thirty-two (132) of these subjects will be randomly assigned in a 2:1 ratio to oral (or feeding tube) twice daily sachet of active therapy or matching placebo. Treatment duration will be twenty-four (24) weeks. For the first three weeks study drug will be administered once daily. If tolerated, the dose will then be increased to twice a day. Clinic visits will occur at Screening, Baseline, Week 3 (day 21), Week 6 (day 42), Week 12 (day 84), Week 18 (Day 126), and Week 24 (Day 168). Phone calls will be conducted at Week 9, Week 15, Week 21 and Week 28 (4 weeks after completion of treatment).

All visit windows are consecutive calendar days and are calculated from the day the subject starts study treatment (Day 0, the day of the Baseline Visit). Any change from this visit window will be considered an out of window visit deviation.

Subjects will remain on randomized, placebo-controlled, double-blind treatment until the Week 24 visit.

A one thirty-two (132) week Open Label Extension (OLE) study will be available to those subjects who complete the randomized, double-blind study. Please refer to 13.7 Appendix VII of the protocol for all the details on the OLE. If a subject does not enroll in the extension, they will also have a Follow-up Telephone Interview 28 days (+5 days) after the completion of dosing to assess for adverse events (AEs), changes in concomitant medications and the ALSFRS-R.

Table 1: Schedule of Activities

ACTIVITY	Screening Visit	Study Drug Administration (weeks)									Final Follow-up Telephone Call[2]	MR-PET Sub-Study Subjects Only
		Baseline Visit[1]	Week 3	Week 6	Week 9	Week 12	Week 15	Week 18	Week 21	Week 24 OR Early Discontinuation/ Final Safety Visit		
	Clinic	Clinic	Clinic	Clinic	Phone	Clinic	Phone	Clinic	Phone	Clinic	Phone	At MGH
	-42 Days	Day 0	Day 21 ±5	Day 42 ±5	Day 63 ±5	Day 84 ±5	Day 105 ±5	Day 126 ±5	Day 147 ±5	Day 168 ±5	28 +5 days	
Written Informed Consent	X											X
Inclusion/Exclusion Review	X	X										X
Medical History History/Demographics	X											
ALS Diagnosis/ALS History	X											
Vital Signs[3]	X	X	X	X		X		X		X		
Neurological Exam[4]	X					X				X		X[4]
Physical Exam[5]	X					X				X		
Blood Draw for Safety Labs[6]	X	X	X	X		X		X		X		
Blood Draw for Serum Pregnancy Test for WOCB[6]	X											
Urine Sample for Urinalysis[6]	X	X	X	X		X		X		X		
12-Lead ECG	X					X				X		
ALSFRS-R	X	X	X	X	X	X	X	X	X	X	X	X
Slow Vital Capacity	X	X		X		X		X		X		
ATLIS Testing	X	X		X		X		X		X		

ACTIVITY	Screening Visit	Study Drug Administration (weeks)									Final Follow-up Telephone Call[2]	MR-PET Sub-Study Subjects Only
		Baseline Visit[1]	Week 3	Week 6	Week 9	Week 12	Week 15	Week 18	Week 21	Week 24 OR Early Discontinuation/ Final Safety Visit		
	Clinic	Clinic	Clinic	Clinic	Phone	Clinic	Phone	Clinic	Phone	Clinic	Phone	At MGH
	-42 Days	Day 0	Day 21 ±5	Day 42 ±5	Day 63 ±5	Day 84 ±5	Day 105 ±5	Day 126 ±5	Day 147 ±5	Day 168 ±5	28 +5 days	
Columbia-Suicide Severity Scale[7]		X[7]	X	X		X		X		X		
Exit Questionnaire										X		
MR-PET Scan[8]		X				X						X[8]
Blood draw for Biomarker Testing[9]		X		X		X		X		X		
Blood draw for PK Analysis[10]		X				X				X[11]		
Blood draw for optional DNA collection[12]		X	X	X		X		X		X		
Adverse Events[13]	X	X	X	X	X	X	X	X	X	X	X	X
Blood draw for TSPO affinity testing[14]	X											
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X
Randomization[15]		X										
Dispense Study Drug[16]		X		X		X		X				
Drug Accountability/ Compliance			X[17]	X	X	X	X	X	X	X		

[1]The Baseline Visit should occur no more than 42 days after the Screening Visit.

[2]A Final Safety Telephone Call will be conducted 28 (+5 days) after the subject takes their last dose of study drug (whether or not the subject has discontinued from the study) to assess for adverse events (AEs), changes in concomitant medications and to administer the ALSFRS-R. This call will only be required for subjects who do NOT enroll in the OLE.

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

[4] The standard Neurological Exam will be used for all subjects. The Upper Motor Neuron Burden Scale (UMN-B) will be included for the MR-PET Sub-Study only and administered at the time of the scan.

[5]Physical Exam will include height and weight. Height will be collected at Screening Visit ONLY.

[6]Safety labs include Hematology (CBC with differential), Complete Chemistry Panel, Liver Function Tests and Urinalysis. Serum pregnancy testing will occur in women of child bearing potential (WOCBP) at the Screening Visit and as necessary during the course of the study.

[7]C-SSRS Baseline version to be completed at Baseline Visit only. C-SSRS Since Last Visit version to be completed at all other visits.

[8]Approximately 20 subjects will receive MR-PET (Magnetic Resonance-Positron Emission Tomography) scanning completed at selected sites. First scan will occur PRIOR to the Baseline Visit (pre-dose) and the second scan will occur between the Week 12 and Week 21 study visits. MR-PET subjects will also provide blood samples for peripheral blood mononuclear cell (PBMC) extraction prior to each MR-PET scan.

[9]Subjects will provide a blood sample for biomarker testing and storage in a biorepository.

[10]All subjects will provide a blood sample for pharmacokinetic (PK) testing at the Baseline Visit (pre-dose). Subjects will also provide a blood sample either 1 hour or 4 hours post-dose (±10 minute window per time point) at the Week 12 and Week 24 Visits. PK times will be randomized such that every subject has a 1-hour draw at one visit and a 4-hour draw at the other.

[11]PK should not be drawn for early termination subjects

[12] If Baseline visit has already occurred or the sample was not collected, DNA should be obtained at next available visit. This is a one-time collection.

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

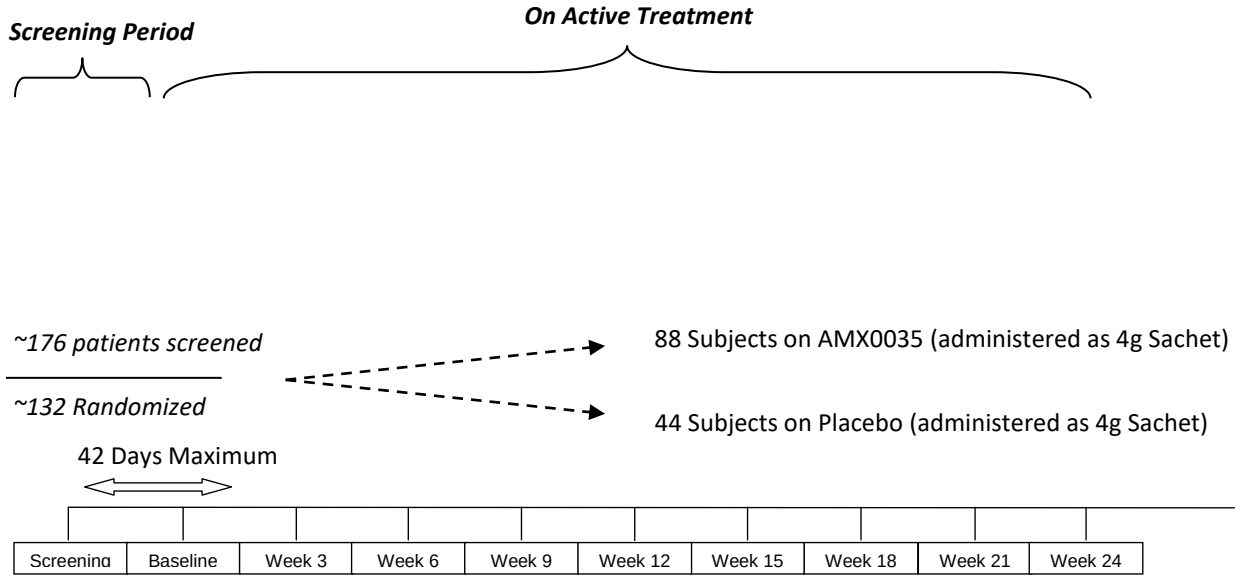
[14]For MR-PET Sub-Study subjects only, blood will be drawn for TSPO testing at the subject's site during the Screening Visit.

[15]Randomization should occur at the Baseline Visit. Randomization will entail entering a subject's kit number into the data capture system.

[16]First dose of study drug will be administered in clinic after ALL Baseline Visit procedures are completed.

[17]Notify subjects of increase from one sachet per day to two sachets per day

Figure 1: Study Workflow



4 RANDOMIZATION AND UNBLINDING PROCEDURES

4.1 Randomization

Approximately, one hundred thirty-two (132) subjects will be randomly assigned in a 2:1 ratio to oral (or feeding tube) twice daily sachet of active therapy or matching placebo. The randomization scheme will be independently developed and will indicate the treatment assignment and the subject numbers to be used by each site. The randomization scheme will be managed by the manufacturer.

4.2 Unblinding

Only in the case of an emergency, when knowledge of whether the subject has received the investigational product is essential for the clinical management or welfare of the subject, may the Investigator request to unblind a subject's treatment assignment. Unblinding at the study site for any other reason will be considered a protocol deviation. If the Investigator needs the blind to be unmasked for a subject for any reason, the Investigator must contact the Medical Monitor to obtain an approval. Breaking the blind must be reported, documenting the date, the site personnel exposed to the treatment assignment, and the reason the blind was broken.

Upon completion of the study and after the database is locked according to the Sponsor (or designee) operating procedures, the final SAP will be signed-off and the SDTM data including the actual randomization codes will be provided to Pentara.

5 EFFICACY/SAFETY ASSESSMENTS

5.1 Primary Endpoint

5.1.1 ALSFRS-R

The primary efficacy outcome measure for the study is the rate of decline (slope of decline) in the ALS functional rating scale (ALSFRS-R). The revised version of the ALSFRS was created to add assessments of respiratory dysfunction, including dyspnea, orthopnea, and the need for ventilatory support. The revised ALSFRS (ALSFRS-R) has been demonstrated to retain the properties of the original scale and show strong internal consistency and construct validity.

Initial validity was established by documenting that in ALS subjects, 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. The ALSFRS-R will therefore also be given to the study subject over the phone. All ALSFRS-R evaluators must be NEALS certified.

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. Higher scores indicate better performance. The maximum score is 48 points. All 12 activities are relevant in ALS. The ALSFRS-R can be broken down into four domains as described below:

1. Bulbar
    a. Speech
    b. Salivation
    c. Swallowing
2. Fine Motor
    a. Handwriting
    b. Cutting Food/Handling Utensils
    c. Dressing and Hygiene
3. Gross Motor
    a. Turning in Bed
    b. Walking
    c. Climbing Stairs
4. Breathing
    a. Dyspnea
    b. Orthopnea
    c. Respiratory Insufficiency

The total ALSFRS-R scale will be the primary efficacy outcome and the four domains described above will be considered exploratory efficacy outcomes.

5.1.2 Tolerability

We will consider a dose tolerable if the proportion of treatment failures (discontinuation of study drug due to an adverse event) is less than 40% with 80% confidence, one-tailed. With 88 treated subjects this would occur if 30 or fewer subjects on AMX0035 fail to complete the 6-month study. By this criterion, we will have 80% power for declaring AMX0035 tolerable at the tested dose if the true treatment failure rate is 30%.

5.2 Secondary Efficacy Endpoints

The secondary outcome measures are listed below in hierarchical order:

- Assessing the impact of AMX0035 on the rate of decline of isometric muscle strength, as measured by the ATLIS;
- Assessing the impact of AMX0035 on phosphorylated axonal neurofilament H subunit (pNF-H) levels;
- Assessing the impact of AMX0035 on disease progression as measured by SVC decline;
- Assessing the impact of AMX0035 on survival, hospitalization and tracheostomies;
- Assessing the concentration-response model of PB and TURSO at steady-state after administration of AMX0035 4 grams twice daily. This analysis and any other pharmacokinetic (PK) analyses will be described in a separate PK SAP.
- Assessing the impact of AMX0035 on TSPO uptake measured by PET scan. Due to small sample size this data will not be analyzed but rather presented in a listing.

5.2.1 ATLIS

We will measure isometric strength using the ATLIS device developed by Dr. Patricia Andres of Massachusetts General Hospital. The device was specifically designed to alleviate the reproducibility concerns that exist for prior strength measurements such as hand-held dynamometry (HHD). ATLIS does not depend on experimenter strength and has measurement settings to ensure that subjects are in the same position each time they are tested. All ATLIS evaluators must be trained and certified. ATLIS may detect functional decline before the ALSFRS-R, which may have a ceiling effect, and may be able to detect changes in function with greater sensitivity to ALSFRS-R. The measure does show a small training effect, so we will conduct the test at initial screening visit to allow subjects to become acquainted with the device.

ATLIS is an isometric strength measurement device. Each subject's absolute strength in twelve muscle areas will be measured at screening by ATLIS and then normalized to standard values based on Patricia Andres per predicted normal dataset (Andres, P. et al. Developing normalized strength scores for neuromuscular research. Muscle and Nerve. 2013.). Of the twelve muscle areas, 6 are considered lower and the other 6 are considered upper. Average standardized ATLIS scores will be used in the analysis. The coefficients and intercept that will be used to obtain the predicted value for each of the 12 muscle group areas measured in ATLIS is shown in Table 2 below. Two ATLIS trials will be conducted generally, but a third may be conducted if the first two trials vary by over 15%. The highest score of all trials at a time point will be used for analysis.

Table 2: Coefficient and Intercept for ATLIS Standardization

Gender	Maneuver	Age (years) Coefficient	Weight (lbs) Coefficient	Height (in) Coefficient	Intercept
Female	Left Grip	-0.15	0.16	1.18	-28.91
	Right Grip	-0.21	0.18	1.05	-14.01
	Left Elbow Flexion	-0.04	0.14	0.44	-6.03
	Right Elbow Flexion	-0.07	0.13	0.49	-6.95
	Left Elbow Extension	-0.09	0.1	0.09	12.14
	Right Elbow Extension	-0.09	0.08	0.13	13.37
	Left Knee Extension	-0.231	0.231	0.352	21.263
	Right Knee Extension	-0.231	0.165	0.319	32.604
	Left Knee Flexion	-0.14	0.08	0.62	-12.64
	Right Knee Flexion	-0.19	0.09	0.65	-14.23
	Left Ankle Dorsiflexion	-0.13	0.1	0.06	23.63
	Right Ankle Dorsiflexion	-0.08	0.11	0.03	23.28
Male	Left Grip	-0.28	0.17	1.41	-20.59
	Right Grip	-0.27	0.19	1.65	-32.94
	Left Elbow Flexion	-0.14	0.15	0.24	26.61
	Right Elbow Flexion	-0.17	0.16	0.53	5.89
	Left Elbow Extension	-0.26	0.14	-0.21	50.13
	Right Elbow Extension	-0.29	0.13	-0.24	55.17
	Left Knee Extension	-0.011	0.297	-0.594	74.789
	Right Knee Extension	0.022	0.33	-1.056	101.992
	Left Knee Flexion	-0.19	0.18	0.27	-1.07
	Right Knee Flexion	-0.22	0.16	0.15	14.26
	Left Ankle Dorsiflexion	-0.06	0.11	0.06	26.03
	Right Ankle Dorsiflexion	-0.04	0.13	0.02	26.62

For example, the predicted value for left grip maneuver for a 41-year-old female who is 62 inches tall and weighs 126 pounds would be 58.26, see formulas below.

$$Predicted = -28.91 - 0.15 * Age + 0.16 * Weight + 1.18 * Height$$

$$Predicted = -28.91 - 0.15 * 41 + 0.16 * 126 + 1.18 * 62$$

$$Predicted = 58.26$$

ATLIS scores for each subject and visit will go through the following steps in order to be used in analyses:

1. Obtain predicted value for each of the 12 muscle groups using each subject's baseline information (age, height and weight) and the coefficient and intercept estimates provided in Table 2;

2. For each of the 12 muscle groups, divide the maximum observed score for each subject and visit combination by the predicted score. These are the standardized ATLIS scores. If a subject has no motion in a limb and is therefore not tested, his/her score will be recorded as a zero. If he/she had motion, but are for other reasons unable to complete the testing this data will be considered missing. A zero score divided by the predicted score will still be zero, so the zeros are considered "standardized ATLIS scores" in the following calculation steps.

3. Average the 6 standardized upper muscle groups (left grip, right grip, left elbow flexion, right elbow flexion, left elbow extension, right elbow extension) to obtain the "Upper Extremity (Arm) ATLIS" score. Only calculate the average score if at least 4 of the 6 items are observed;

4. Average the 6 standardized lower muscle groups (left knee extension, right knee extension, left knee flexion, right knee flexion, left ankle dorsiflexion, right ankle dorsiflexion) to obtain the "Lower Extremity (Leg) ATLIS" score. Only calculate the average score if at least 4 of the 6 items are observed;

5. Average the lower and upper ATLIS scores (numbers 3 and 4 above) to obtain the "Total ATLIS" score. Only calculate the average score if both averaged standardized muscle groups are observed.

5.2.2 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. All VC evaluators must be NEALS certified. Three VC trials are required for each testing session, however up to 5 trials may be performed if the variability between the highest and second highest VC is 10% or greater for the first 3 trials. Only the 3 best trials are recorded on the CRF. The highest VC recorded is utilized for analysis, regardless of the number of trials performed.

5.2.3 Survival, Hospitalization and Tracheostomies

Survival endpoint will be defined as death, tracheostomy or permanent assisted ventilation (PAV). 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.

5.2.4 Biomarkers and Pharmacokinetics

Subjects will have blood drawn to assess AMX0035 concentrations for pharmacokinetics (PK) pre-dose at the Baseline Visit and then again at either 1 hour or 4 hours (± 10 minutes) post-dose at the Week 12 and 24 visits. Every attempt should be made to collect samples within the allotted timeframes; however, all samples should be analyzed regardless of actual collection time. The time of administration will be noted. The time of the last meal prior to administration and the time of the drug administration(s) in the previous 24 hours will also be noted.

Additionally, blood will be collected for biomarker analysis of heavy neurofilament testing (pNF-H). Light neurofilament testing (NF-L) may also be conducted. Neurofilaments will be used as a mechanistic measure of neuronal death. These proteins are greatly elevated in ALS subjects and promising results from multiple trials suggest this marker may be prognostic of clinical decline. NF-L and pNF-H will be tested over multiple time points with the intention of generating a longitudinal dataset correlating neurofilament levels to observed clinical outcomes. This dataset will help to validate AMX0035 therapeutic mechanism and provide a dataset for the ALS field.

5.2.3 Imaging

Imaging results were collected on a small subset of subjects. These results will not be analyzed due to the small sample size, but will be provided in a listing.

5.4 Safety Assessments

Safety assessments include the following:

- Adverse events (AEs);
- Vital signs;
- 12-lead ECG;
- Hematology, chemistry and urinalysis;
- Physical and neurological examinations;
- Columbia Suicide Severity Rating Scale (C-SSRS).

5.4.1 Adverse Events

An adverse event (AE) or adverse experience is any untoward medical occurrence in a subject or clinical investigation subject who is administered a medicinal product that does not necessarily have a causal relationship with this treatment that occur after informed consent is signed and up to 28 days (+5 days) after the study drug has been discontinued. For the purposes of this study, symptoms of progression/worsening of ALS, including ‘normal’ progression, will be recorded as 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.

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 adverse drug reaction [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)

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 in-subject 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 in-subject hospitalization, or the development of drug dependency or drug abuse.

5.4.2 Vital Signs

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.

5.4.3 ECG

A standard 12-lead ECG will be performed. Tracings will be reviewed by a central ECG reader and a copy of the tracings will be kept on site as part of the source documents. The central ECG vendor will provide standard ECG devices for every site and provide training as necessary.

5.4.4 Clinical Laboratory Assessments

The following laboratory tests will be performed for safety:

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

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

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

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

All subjects will have safety laboratory tests at the designated visits outlined in the protocol. These samples will be analyzed at a central laboratory. The Site Investigator (SI) may order additional testing, 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.

Specific instructions regarding the collection, processing, storage and shipment of these samples will be provided in the Site Manual of Procedures (MOP).

5.4.5 Physical Examination

A comprehensive physical examination will be performed and recorded.

5.4.6 Neurological Examination

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

5.4.7 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). 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) [Posner K, 2007]. The C-SSRS involves a series of probing questions to inquire about possible suicidal thinking and behavior.

At the Baseline Visit, the C-SSRS Baseline version will be administered. This version is used to assess suicidality over the subject’s lifetime.

At all clinic visits after the Baseline Visit, 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.

5.5 Other Evaluations

Additional evaluations include the following:

1. Demographics;
2. Baseline disease characteristics;
3. Medical history;
4. Days hospitalized;
5. Prior medications.

6 ANALYSIS POPULATIONS AND GENERAL STATISTICAL PROCEDURES

6.1 Definition of Analysis Populations

Statistical analysis and data tabulation will be performed using the following subject populations unless specified otherwise:

1. Safety Population;
2. Modified Intent-to-Treat (mITT) Population;
3. Per Protocol (PP) Population.

The safety population will include all subjects who received at least one dose of study medication. Subjects in this population are analyzed based on the actual treatment they received.

The mITT population will include all subjects who receive at least one dose of study medication and have at least one post-baseline total ALSFRS-R score. Subjects in this population will be analyzed based on the treatment they were assigned to.

The PP population will include all mITT subjects who took the assigned medication for 24 weeks and did not have any major protocol violations which exclude them from PP analysis (as determined by committee prior to database lock). PP assignments will be based on a by-visit basis, removing visits that could have been affected by major protocol deviations and all visits thereafter. Subjects will remain in the analysis up until the time that they had a major protocol violation or have not taken study medication for 30 days. The date of the major protocol deviation or month drug interruption (whichever comes first) for each subject will be used to filter the data collected after these events and exclude it from the PP population.

Major protocol deviations will be reviewed on a case-by-case basis by a committee without knowledge of the treatment assigned and before unblinding the study. The goal of the committee will be to determine those protocol deviations which could bias or confound interpretation of results. The committee will document their decisions regarding these protocol deviations.

6.2 Application of Analysis Populations

The primary population for efficacy analysis is the mITT population. Analysis of primary and secondary efficacy endpoints will be performed in the mITT and PP populations. The safety population will be used for analyses of safety endpoints.

Subject enrollment, disposition, drug exposure, demographics and baseline disease characteristics will be shown for all populations.

Efficacy analyses will be performed on both the mITT and PP populations, unless otherwise stated.

All safety evaluations, medical history and medication use will be based on the safety population.

6.3 General Statistical Procedures

All analyses described in this plan are considered *a priori* analyses in that they have been defined prior to breaking the study blind. All other analyses, if any, defined subsequent to breaking the study blind will be considered *post hoc* analyses and will be applied using exploratory methodology. All *post hoc* analyses will be identified as such in the Clinical Study Report.

Descriptive statistics for continuous variables will include number of subjects (N), arithmetic mean, standard deviation (SD), median, minimum, maximum and first and third quartile limits unless otherwise noted. Frequency and percentage will be calculated for categorical variables. Unless stated otherwise, all summary tables will present descriptive statistics and/or frequencies either by treatment or overall, and all data listings will be sorted by subject number.

Unless otherwise specified, all significance testing will be 2-tailed using $\alpha = 0.05$. Tests will be declared statistically significant if the calculated p-value is ≤ 0.05. The primary study hypothesis will be deemed satisfied if the ALSFRS-R slope over time is significantly better than the placebo slope at the 0.05 level.

Change from baseline is calculated by subtracting the baseline score from the observed value at any subsequent visit. For safety summaries, the last pre-randomization measurement is defined as the baseline value. For efficacy measures baseline is defined as the last pre-randomization measurement. Most efficacy analyses will be performed using the actual number of days relative to dosing where Day 0 is the day of the first dose. If the baseline record is missing for an outcome than the screening record may be used as "baseline." Efficacy results from the MR-PET visit or the follow-up phone call will not be included in the efficacy analyses. MR-PET visits were not conducted at the same location as the other visits, so this information is expected to be inconsistent with the other results.

Visit windowing will be applied for analyses which use visit categories instead of actual number of days relative to dosing for each assessment. For categorical visit summaries, all visits including early termination assessments and unscheduled visits but excluding MR-PET visit will be included with the closest scheduled post-baseline visit that includes the efficacy or safety assessment, based on number of days since Day 0 (first dose). Any visit >14 days after the week 24 visit date (Day 168) will be categorized as a follow up visit. Follow up visits will not be included in efficacy modeling but will be included in safety modeling. This convention results in sequential visit windows so that no data is excluded from analysis. If an early termination visit and a regular visit (other than baseline) both fall within the same visit window, any non-missing efficacy assessments will be averaged and a worst-case approach will be used for safety data. Follow up visits were not conducted for subjects who continued into the extension and therefore there are very few follow up results available. Summaries of follow up results will be interpreted accordingly.

Safety analyses will be used based on visit category as recorded. In the case that there is more than one result at the same visit, a worst-case approach will be used and the "worst" value will be used for summary statistics and analyses.

Percentages are based on the number of subjects in each treatment group and overall in the given population for medical history, prior and concomitant medications and AE summary tables. For

all other tables, percentages are based on the number of subjects with non-missing data in each treatment group and overall for the given population.

If partial dates are recorded for efficacy outcomes then partially missing start/beginning date (e.g. AE/Concomitant medication start date) will fill in the missing month with January and missing day with 1. For example, if month and day were both missing, then the date would be filled in with January 1st. Partially missing end/finishing date (e.g. AE/Concomitant medication end date) will be filled in with December and missing day with the last day of the month. For example, if month and day were both missing, then the date would be filled in with December 31st. For other outcomes (e.g. date of vital signs collection) fill in missing month with June (middle month) and missing day with the middle day of the month. For example, if month and day were both missing, then the value would be filled in with June 15th.

Days will be converted to weeks by dividing by seven. Days will be converted to months by dividing by 30.417. Days will be converted to years by dividing by 365.25. All analyses will be conducted with R v3.3.1 or SAS® v9.4 or later using procedures appropriate for the particular analysis. All data collected during the study will be analyzed and reported unless stated otherwise.

6.4 Procedures for Handling Intercurrent Events and Missing Data

Subjects who drop out will have all available baseline and post-baseline data included in the analysis. The main efficacy analysis is a mixed model with repeated measures (MMRM). This model will be implemented using PROC MIXED in SAS®. PROC MIXED handles missing values and accounts for them using a MAR assumption.

It is assumed that missing values for reasons other than death or death equivalent events are missing at random. However, an exploratory analysis will be conducted to evaluate death or equivalent events and a sensitivity analysis will be performed to assess the whether or not results are MAR or missing not at random (MNAR).

6.5 Interim Analysis

No interim analysis of the data was planned or performed.

7 SUBJECT DISPOSITION, DEMOGRAPHICS AND BASELINE CHARACTERISTICS EVALUATIONS

7.1 Subject Enrollment

Subject enrollment will be summarized by treatment and center for all populations. The number of subjects overall and at each center for each analysis population will be presented.

Study timelines will also be summarized by treatment and overall for all randomized subjects. This summary will include the earliest and latest screening and dosing dates among subjects within each treatment group and overall. It will also list the last subject and the date of his/her last visit within each treatment group and overall. Study duration will be presented in weeks and will be calculated using the following formula:

$$\frac{(Latest\ Week\ 24\ Visit\ Date - Earliest\ Screening\ Date + 1)}{7}.$$

Enrollment information will be provided in a data listing by subject.

7.2 Subject Disposition

Subject disposition will be summarized overall and by treatment group for all populations. The number and percentage of subjects completing the study and discontinuing from the study will be presented by treatment and overall and by reason for termination.

Subject disposition will be provided in a data listing by subject.

7.3 Drug Exposure

Duration of exposure is defined as the total time a subject is exposed to any study drug. The duration of exposure in weeks will be calculated by dividing the total number of days from the first dose date (Day 1) to the last dose date by 7 days/week. If the last dose date is missing or a subject is lost to follow-up, but the study medication administration log confirms that the subject has taken study drug, the date of the last completed study medication administration will be used.

Extent of exposure to study drug will also be characterized by calculating the cumulative number of grams taken by subjects. The duration and extent of exposure to study drug will be summarized by treatment group for both the safety and mITT populations. N and percentage of subjects in each population will be displayed. The duration and extent of exposure to study drug will be summarized using descriptive statistics.

7.4 Subject Demographic and Baseline Data

Subjects will be described using demographic information and baseline characteristics recorded during the screening phase.

Demographic information to be assessed is age, gender, ethnicity, racial group, height and weight. Subject demographics will be summarized by treatment for the safety, mITT and PP populations.

Racial group, ethnicity, gender and other categorical questions will be summarized using the number and percentage of subjects with a particular attribute. The denominators for calculating the percentages will be the number of subjects in each treatment for the safety, mITT, and PP populations. Age, weight, height and other numeric responses will be summarized using descriptive statistics.

Baseline disease characteristics will be provided in a separate summary table. Prior/current ALS therapy, length of time on specified ALS therapy (edaravone and riluzole), time since diagnosis, time since symptom onset, and baseline efficacy variables will be summarized using descriptive statistics by treatment for the safety, mITT and PP populations.

Demographics and baseline disease characteristics will be provided in a data listing by subject.

7.5 Medical History

Medical history will be summarized by treatment for each System/Category for the safety population. The number and percentage of subjects with significant medical history will be presented for each system organ class and preferred term. The denominators for calculating the percentages will be based on the number of subjects in each treatment group in the mITT population.

Medical history will be provided in a data listing by subject.

7.6 Medications

Medication summaries will present the number and percentage of subjects taking medications for the safety population. Summaries will be presented for prior (prior to Day 0) medication use and concomitant (Day 0 or later) medication use, if applicable.

All summaries will present the number and percentage of subjects by treatment. Prior, concomitant and ALS medication will be provided in a data listing by subject.

7.7 Protocol Deviations

Major protocol deviations are defined to be those deviations that could potentially bias the conclusions of the study. Minor deviations are defined to be those deviations not deemed major.

The protocol deviations summary will present the number and percentage of subjects with each deviation category and specific deviation term within each treatment group and overall. In this summary, the total number of protocol deviations and number of subjects with at least one protocol deviation will be tabulated by treatment group and overall.

Protocol deviations will be provided in a data listing by subject.

8 EFFICACY EVALUATIONS

8.1 Primary Efficacy Analyses

All continuous primary, secondary and exploratory efficacy measures will use the same statistical model (ALSFRS-R, ATLIS lower, ATLIS upper, ATLIS total, SVC, pNF-H and 4 ALSFRS-R domains) and will be presented in hierarchical order. The placebo and active arms will be compared by a shared baseline, mixed effects analysis. Covariates of age, rate of disease progression prior to entering trial ΔFS (del-FS) and del- of the efficacy outcome being measured (if other than ALSFRS-R) interacting with time will be included in the analysis. Time will be a quantitative measure in the primary analysis, with day 0 being the baseline/randomization visit. Time for subsequent visits will be the number of days since randomization. All post-baseline visits will be included in the efficacy analysis, even if they are categorized as the same nominal visit. This means that post-baseline unscheduled visits or telephone calls will be included in the model. Historical studies have shown that the efficacy assessments collected over the phone in a telephone interview are consistent with those collected in an in-office visit. For this reason, it is acceptable that in-office and telephone interview records be included in the same analysis [Kaufmann, 2007]. Efficacy data from the follow-up telephone interview will not be included since subjects were off study drug at this assessment. Any pre-treatment record(s) that is (are) not the baseline (see Section 6.3) record will not be included in the analysis. Efficacy results from the MR-PET Visit 1 will also not be included in the analyses because these measurements were done at a different facility and rated by different monitors.

Historical analyses have shown that del-FS is a strong predictor of future progression since ALS has a linear disease progression [Karanevich, 2018]. Del-FS is derived based on the baseline ALSFRS-R score combined with time since symptom onset. Del-FS is a measurement of decline in the subject since symptom onset. The del-FS calculation is made at the baseline visit and the following formula is used:

$$Del - FS = \frac{48 - ALSFRS\ R\ at\ First\ Available\ Visit}{Time\ in\ Months\ from\ Symptom\ Onset\ to\ First\ Available\ Visit}.$$

Note that the maximum score for the ALSFRS-R is 48 and that the "First Available Visit" is the same as the "Baseline" record as described in Section 6.3. Analyses performed on study subjects showed that decline since symptom onset was a significant predictor not only for ALSFRS-R, but also for other efficacy outcomes like ATLIS. For this reason, each model will include a del- score based on the outcome variable and the same formula will apply:

$$Del - Efficacy = \frac{Ceiling\ Maximum\ Efficacy\ Score - Efficacy\ Score\ at\ First\ Available\ Visit}{Time\ in\ Months\ from\ Symptom\ Onset\ to\ First\ Available\ Visit}.$$

When there is no defined maximum, like SVC, the observed maximum score across all active and placebo subjects will be used in the derivation for "Maximum Efficacy Score." Analyses performed on study subjects also showed that del-FS was a significant predictor of decline for outcomes other than ALSFRS-R. For this reason, all efficacy models will include del-FS in

addition to the del- associated with the response variable. It is understood that del-FS and the other del- term in the model will be collinear. However, the inclusion of the del- terms in the efficacy model is for correction and not for estimation, meaning that the collinearity of the items will not affect our estimates from the model but help to remove sources of variation.

The mixed-effects model accounts for both the variance between subjects and the deviation within subjects from their average rate of decline. The model used is as follows:

$$Y_{i,t} = \mu + u_i + (\beta_0 + b_i) \times t + \beta_1 \times z_i \times t + \beta_2 \times Age_i \times t + \beta_3 \times DelFS_i \times t + \beta_4 \times DelY_i \times t + \varepsilon_{i,t}$$

- *i* represents the i[th] subject, *i* ranges from 1 to the number of subjects in the mITT population;
- *t* represents the actual time in weeks of each observation, time since "baseline" assessment;
- $Y_{i,t}$ is the dependent variable observed at time *t*, i.e. the actual efficacy score at time t
- *z* is a treatment indicator which is 0 in the control group and 1 in the treatment group;
- u_i is the random intercept for each subject and has an unspecified bivariate normal distribution;
- b_i is the random slope in the efficacy outcome for each subject over time and has an unspecified bivariate normal distribution;
- *Age x t* is the interaction representing the effect of age on progression over time. It is expected that older subjects will decline faster;
- $DelFS_i$ *x t* is the interaction representing the effect of previous progression measured by ALSFRS-R on progression over time. It is expected that subjects who were progressing quickly since symptom onset will continue to progress quickly;
- $DelY_i$ *x t* is the interaction representing the effect of previous progression measured by the efficacy outcome of interest (response variable) on progression over time. It is expected that subjects who were progressing quickly since symptom onset will continue to progress quickly;
- μ is the estimated intercept of the efficacy outcome across all subjects;
- β_0 is the estimated slope for time;
- β_1 is the estimated slope for treatment;
- β_2 is the estimated slope for age at baseline (years);
- β_3 is the estimated slope for del-FS;
- β_4 is the estimated slope for del-efficacy (corresponding to $Y_{i,t}$);
- $\varepsilon_{i,t}$is the random error which shows the amount by which the observed value differs from its expected value.

Each estimated slope is the expected increase in the efficacy outcome for a one unit increase in the explanatory variable for a one-week increment in time (all slopes are for interaction terms with time). For example, β_2 is the expected increase in the efficacy outcome for a one-year increase in age over 1 week.

Historical and pre-SAP analyses have shown ALS to be a disease with linear progression over time. However, linearity cannot be assumed at this point for the study given the unknown effect of the treatment. In order to confirm linearity, the model described above will be modified to include quadratic terms for time

If the quadratic terms for time are insignificant (p-values >0.10) then linearity will be assumed and the linear primary model will be used for analysis. If at least one of the interaction terms is significant (p-value<0.10) then the quadratic version of the primary model will be used for analysis. P-values for the quadratic terms in the model will be presented. If at least one of the quadratic terms is significant than the summary statistics described in the subsequent paragraph will be presented and the same statistics will be presented for the linear primary model.

The difference in treatment and placebo slope will be calculated in addition to a p-value for the comparison and a 95% confidence interval for the estimated difference. Least-squares means (LSMEANs) and standard errors will be estimated for active treatment and placebo at each scheduled time point for the mean level of baseline covariates across all subjects included in the analysis. The least-squares difference and standard error in predicted values between treatments at each scheduled time points will also be presented. The LS mean at each time point is the expected efficacy result for each treatment for a subject with mean baseline covariates across all subjects in the study. In addition, treatment differences, p-values, 95% confidence intervals for the difference, and effect size will be displayed for treatment comparisons. The number of subjects with an observed efficacy outcome, mean, standard deviation, median, 25th percentile (Q1), 75th percentile (Q3), minimum and maximum will all be reported and accompany the estimates from the MMRM outlined in this section.

The analyses in this section will be applied to the mITT and PP populations

8.2 Survival Analyses

Survival analyses will be performed using a Cox proportional hazards model with covariates of del-FS and age at baseline. There are 3 survival outcomes: 1) death, 2) tracheostomy and 3) PAV. Any of these events that occurred within 28 weeks (24 weeks + 28 days) will be included in this analysis. Events which occurred after 28 weeks will be addressed in the extension analysis. In addition, a combined survival analysis will be performed where any one of the 3 events will be considered a failure and the time for the first occurrence of any of the 3 events will be analyzed. This combined survival analysis will be referred to as time to "Death or Equivalent." For the survival analyses, death of subjects who dropped-out but died within the original study window (24 weeks after baseline) will be included in the analysis.

The Cox model is expressed by the hazard function denoted by *h(t)*. The hazard function can be interpreted as the risk of dying at time *t*, which can be estimated as follows:

$$h(t) = h_o(t) \times \exp\left(b_1 x_1 + b_2 x_2 + b_3 x_3\right).$$

- *t* represents the survival time;
- *h(t)* is the hazard function determined by a set of covariates (x_1, x_2 *and* x_3);
- the coefficients (b_1, b_2 and b_3) measure the impact (i.e. the effect size) of the covariates;
- x_1 represents treatment (active or placebo);
- x_2 represents del-FS;
- x_3 represents age at baseline;
- the term $h_o(t)$ is called the baseline hazard. It corresponds to the value of the hazard function if all *x* values are equal to zero.

The quantiles *exp(b_i)* are called hazard ratios (HR). A value of b_i greater than zero, or equivalently a hazard ratio greater than one, indicates that as the value of the i^{th} covariate increases, the even hazard increases and the length of survival decreases. A hazard ratio above 1 indicates a covariate that is positively associated with the even probability and therefore negatively associated with the length of survival.

The likelihood-ratio test will be used to determine whether or not the covariates have a statistically significant impact on the hazard of the event. The likelihood-ratio test has better behavior for small sample sizes. When there are large sample sizes, the likelihood-ratio test will give similar results to the other two hazard tests (Wald test and log-rank statistics). In order to be conservative, the likelihood-ratio test has been selected as the primary test statistic to determine whether or not the treatment had a significant impact on the hazard. If the p-value for the effect of treatment is significant (p-value<0.05), then the hazard ratio will be examined to determine which treatment increased the hazard:

- HR=1: No effect;
- HR<1: Reduction in the hazard;
- HR>1: Increase in the hazard.

Figures for the hazard function will be presented showing the survival of active subjects vs. placebo subjects in addition to hazard ratios and p-values for each covariate. A separate survival analysis will be performed for each of the 3 survival outcomes listed above and the combined analysis of "Death or Equivalent."

8.3 Interaction with Important Concomitant Medications

Two medications of interest could be taken during the clinical trial: edaravone (radicava) and riluzole. Efficacy outcomes will be analyzed by comparing efficacy scores over time between treatment groups while accounting for time on concomitant medications of interest. The main efficacy model will be used and a terms to account for time on concomitant medication will be added:

$$Y_{(i,t)} = \mu + u_i + (\beta_0 + b_i) \times t + \beta_1 \times z_i \times t + \beta_2 \times Age_i \times t + \beta_3 \times DelFS_i \times t + \beta_4 \times DelY_i \times t + \beta_5 \times \boldsymbol{I(t - CMT)} + \beta_6 \times \boldsymbol{z\_i} \times \boldsymbol{I(t - CMT)} + \varepsilon_{(i,t)}$$

CMT is the time from the start of the study that the medication started and the function I is a function that is zero for a negative number and the identity function for positive numbers. If the medication never started this term is zero. The overall effect is to create "hockey stick" trajectory where the subjects ALSFS slope changes when the subject starts the medication. The first of these terms measures the effect of the medication on placebo and the sum of both of them measures the effect of the medication when combined with the active drug.

This analysis will be conducted for edaravone only, riluzole only, edaravone *or* riluzole and edaravone *and* riluzole. In the edaravone *or* riluzole variation, the maximum time on either medication will be used in the analysis (CMTime will be the longest time on either concomitant medication). The edaravone *or* riluzole variation is meant to explore how use of *either* of the

concomitant medications of interest affect progression over time across treatment groups. The edaravone *and* riluzole variation is meant to explore how use of *both* concomitant medications of interest affect progression over time across treatment groups.

If there is an interaction between treatment and the start of a medication β_6 is significant. It remains to be seen whether the active medication is antagonistic or synergistic. If the latter is the case, the interaction is not material to the question of whether the treatment is effective. If the former is the case, we need to determine whether, the treatment would be effective if everyone had started the concomitant medication, if no one had started the concomitant medication, and which medication would be preferable if they both should not be given together. These analyses can be conducted using the fitted models to calculate the average slope of the ALSFRS in all subjects under these counterfactual scenarios using the OUTP option in the Model Statement.

The estimated slope and p-value for the interaction involving time on concomitant medication and treatment over time will be presented. If the p-value for this three-way interaction term is significant after correcting for all other factors (p-value<0.10), then it will be concluded that there is a significant interaction between time on concomitant medication and treatment over time. The same summary statistics described in Section 8.1 will be presented in addition to estimated slopes at varying levels of time on concomitant medication.

8.4 Sensitivity Analysis for MAR Assumption

A MMRM, using multiple imputation from the control arm to complete assessments missing after discontinuation of study drug will be performed. This analysis assumes subjects who discontinue medication and are no longer assessed immediately become similar to subjects who never took any medication, and so provides a lower bound on efficacy, again under the MAR assumption that the time of stopping study medication depends only on past history and covariates.

8.5 Left Censoring for Intercurrent Event of Death and Death Equivalent Events

The primary analysis, linear or quadratic depending on the results, will be repeated using the left censored values for all ALSFRS-R, ATLIS, and SVC. In this analysis, all values that are censored by an intercurrent event of death and death equivalent events will be assume to be lower than all observed values, such that the contribution to the likelihood for each subject is the product of the density of all the observed outcomes and of the conditional distribution of the censored outcomes. The left-censoring analyses will be carried out using PROC NLMIXED. The starting values for the fixed variables will be the point estimates from the primary analysis. All variance parameters will have a lower bound of 0.

Let:

$$\theta_{i,t} = \mu + u_i + (\beta_0 + b_i) \times t + \beta_1 \times z_i \times t + \beta_2 \times Age_i \times t + \beta_3 \times DelFS_i \times t + \beta_4 \times DelY_i \times t$$

Then the likelihood for observed outcomes will be

$$\frac{1}{\sqrt{2\pi\sigma_e^2}} \exp\left(-\frac{\left(Y_{i,t} - \theta_{i,t}\right)^2}{2\sigma_e^2}\right)$$

And the likelihood for censored outcomes will be

$$\Phi\left(\frac{Y_{i,t} - \theta_{i,t}}{\sigma_e}\right)$$

8.6 Subject Discontinuation Rate

Counts of subjects who discontinue from the study early will be compared between treatment groups for the safety, mITT and PP populations using Fisher's Exact tests. In addition, time to discontinuation will be displayed. These same analyses will be repeated for any of the following discontinuation reasons with sufficient numbers of subjects:

- Death;
- Subject early terminated;
- Subject withdrew consent prior to end of study;
- Subject lost to follow-up;
- Other.

Time to discontinuation overall and by reason will be analyzed with a Gehan-Wilcoxon test and the corresponding Kaplan-Meier Plots will be displayed. Subjects discontinuing for one of the other reasons will be censored and “time to event” will be used.

9 SAFETY EVALUATIONS

9.1 Adverse Events

AEs reported on CRFs will be coded into system organ classes and preferred terms using the Medical Dictionary for Regulatory Activities (MedDRA v16.1). A treatment-emergent adverse event (TEAE) is defined as an AE with an onset date on or after the start of dosing. The adverse event summary will include only TEAEs. Any AEs that are not considered treatment-emergent will be provided in data listings only.

The incidence of AEs will be summarized for the safety population. Although a preferred term or system organ class may be reported more than once for a subject, each subject will only be counted once in the incidence count for each category. If a subject has the same AE on multiple occasions, the highest severity (severe > moderate > mild) or drug relationship (definite > probable > possible > unlikely > not related) recorded for the event will be presented.

Severity levels include: mild, moderate and severe. Relationships will be grouped into two categories for analysis: related and unrelated. Not related and unlikely will be categorized as "unrelated." Possible, probable and definite will be categorized as "related." If severity or drug relationship is missing no data imputation will be performed and no category of missing will be presented.

Summary tables showing the number of subjects and percent within each category will be generated for each of the following types of adverse events:

- All AEs;
- Fatal Adverse Events;
- AEs for Subjects who Died.

These summaries will present the number and percentage of subjects reporting an adverse event for each classification level. The denominators for calculating the percentages overall will be based on the number of subjects in the safety population. The denominators for calculating the percentages by treatment will be based on the number of subjects exposed to each treatment in the safety population. In addition to these summaries, all AEs will be summarized by action taken, seriousness, severity, and relationship to study drug.

All AEs that occurred in 5% or more of all subjects (active and placebo) will be tabulated for the safety population. These results will be analyzed descriptively and their incidence rate and two-sided 95% confidence intervals will be summarized. In addition, the risk ratio and its 95% confidence intervals between active and placebo will be calculated in order to estimate the occurrence of side effects and adverse events.

All SAEs, AEs leading to premature discontinuation from the study, AEs with fatal outcome, and AEs for subjects who died will also be provided in data listings by subject and preferred term.

9.2 Vital Signs

Each vital sign will be summarized by treatment and overall by visit, using descriptive statistics (mean, median, SD, minimum, maximum, and number of subjects) for the safety population. Additionally, descriptive summaries will be provided for CFB values for each treatment by visit for vital sign measurements collected during the study.

The latest non-missing vital sign value collected prior to dosing will be used as the baseline values. The baseline values will usually be the vital signs recorded at the baseline visit. In the case of repeated vital signs, the last collected values within that visit will be used for the summary tables.

Vital signs will be provided in a data listing by subject, visit, and parameter.

9.3 Electrocardiogram

ECG values and change from baseline values will be summarized by visit using descriptive statistics. ECG abnormalities will be summarized as the count and percentage of subjects in each treatment group. CFB will be summarized in a shift table crossing baseline and each visit result. The denominators for calculating the percentages will be the number of subjects in each treatment group who have an evaluation for both the screening and each visit in the safety population. These results will be analyzed descriptively and their incidence rate and two-sided 95% confidence intervals will be summarized.

9.4 Clinical Laboratory Evaluations

Continuous clinical laboratory analytes absolute values and change from baseline values will be summarized by analyte and visit using descriptive statistics (mean, median, SD, minimum, maximum, and number of subjects). Mean line plots over time will be displayed for each analyte with separate lines for each treatment. Categorical laboratory analytes, classified as normal or abnormal, will be summarized by analyte and visit using the number and percentage of subjects in each category. The denominators for calculating the percentages will be based on the number of subjects with non-missing assessments at a particular visit for the safety population. The latest non-missing clinical laboratory tests collected prior to dosing will be used as the baseline values.

Shifts to values outside of the normal range will be presented by analyte and will be summarized by the number and percentage of subjects with shifts. Shifts will be determined for analytes in which both the baseline value and the termination value are recorded. The denominators for calculating the percentages will be based on the number of subjects with non-missing assessments for a particular analyte.

Clinical laboratory results will be provided in data listings by subject, visit and analyte. Abnormal lab results will be provided in a separate listing by subject, center, analyte and visit.

9.5 Physical and Neurological Exams

Physical and neurological examination findings will be summarized as the count and percentage of subjects in each treatment group. CFB will be summarized in a shift table crossing baseline and each visit results. The denominators for calculating the percentages will be the number of subjects in each treatment group who have an evaluation for both the screening and each visit in the safety population. These results will be analyzed descriptively and their incidence will be summarized

9.6 C-SSRS

The C-SSRS responses will be tabulated by visit, treatment group, question and response. All C-SSRS responses will also be provided in a data listing.

9.7 Days Hospitalized

The number of days hospitalized will be calculated using the start and stop date of a severe or serious AE that resulted in hospitalization. The total number of days each subject was hospitalized over the course of the trial will be calculated by summing all periods of hospitalization. An analysis of covariance (ANCOVA) will be performed to analyze the total days of hospitalization. The model will have total days of hospitalization as the response variable and del-FS and treatment as the explanatory variables.

LSMEANs and standard errors will be estimated for active treatment and placebo at the mean level of del-FS across all subjects. The least-squares difference and standard error in predicted values between treatments will also be presented. The LS mean is the expected days of hospitalization over a 24 week period for each treatment for a subject with mean del-FS across all subjects in the study. In addition, treatment differences, p-values, 95% confidence intervals for the difference, and effect size will be displayed for treatment comparisons. The number of subjects included in the analysis, mean, standard deviation, median, 25^{th} percentile (Q1), 75^{th} percentile (Q3), minimum and maximum total number of days hospitalized will all be reported and accompany the estimates from the ANCOVA outlined in this section.

10 OTHER LISTINGS

The following additional listings will be provided:

- Subjects excluded from the safety, mITT, and PP populations;
- Clinical laboratory results for hematology, blood chemistry and urinalysis;
- Abnormal laboratory results;
- Physical examination assessments;
- Neurological examination assessments;
- Concomitant medications;
- Dose administration dates and times.

11. TABLES, LISTINGS AND GRAPHS REFERRED TO BUT NOT PRESENTED IN THE TEXT

14.1 Demographic and Baseline/Other Data Summaries

Table 14.1.1 Summary of Subject Disposition (Part 1: Safety, Part 2: mITT, Part 3: PP)

Table 14.1.2 Summary of Drug Exposure, Compliance and Tolerability (Part 1: Safety, Part 2: mITT)

Table 14.1.3 Summary of Study Timelines - All Randomized Subjects

Table 14.1.4 Summary of Protocol Deviations - All Randomized Subjects

Table 14.1.5 Summary of Subject Demographics and Baseline Characteristics (Part 1: Safety, Part 2: mITT, Part 3: PP)

Table 14.1.6 Summary of Subject Baseline Disease Characteristics (Part 1: Safety, Part 2: mITT, Part 3: PP)

Table 14.1.7 Summary of Medical History (Part 1: Ongoing, Part 2: Resolved) - Safety

Table 14.1.8 Summary of Prior Medications - Safety

Table 14.1.9 Summary of Concomitant Medications - Safety

Table 14.1.10 Treated Subjects by Center (Part 1: Safety, Part 2: mITT, Part 3: PP)

Figure 14.1.1 Kaplan-Meier Plot for Time to Discontinuation - Safety

14.2 Efficacy Analyses

14.2.1 Primary Shared-Baseline, Mixed Effects

Table 14.2.1.1 Summary of Primary Efficacy Analysis for All Efficacy Outcomes - mITT

Table 14.2.1.2 Summary of Primary Efficacy Analysis for All Efficacy Outcomes - PP

Table 14.2.1.3 Primary Efficacy Analysis for ALSFRS-R (Part 1: Shared-Baseline Mixed Effects Model, Part 2: Raw Summary Statistics) - mITT

Table 14.2.1.4 Primary Efficacy Analysis for ALSFRS-R (Part 1: Shared-Baseline Mixed Effects Model, Part 2: Raw Summary Statistics) - PP

Table 14.2.1.5 Primary Efficacy Analysis for Upper ATLIS (Part 1: Shared-Baseline Mixed Effects Model, Part 2: Raw Summary Statistics) - mITT

Table 14.2.1.6 Primary Efficacy Analysis for Upper ATLIS (Part 1: Shared-Baseline Mixed Effects Model, Part 2: Raw Summary Statistics) - PP

Table 14.2.1.7 Primary Efficacy Analysis for Lower ATLIS (Part 1: Shared-Baseline Mixed Effects Model, Part 2: Raw Summary Statistics) - mITT

Table 14.2.1.8 Primary Efficacy Analysis for Lower ATLIS (Part 1: Shared-Baseline Mixed Effects Model, Part 2: Raw Summary Statistics) - PP

Table 14.2.1.9 Primary Efficacy Analysis for Total ATLIS (Part 1: Shared-Baseline Mixed Effects Model, Part 2: Raw Summary Statistics) - mITT

Table 14.2.1.10 Primary Efficacy Analysis for Total ATLIS (Part 1: Shared-Baseline Mixed Effects Model, Part 2: Raw Summary Statistics) - PP

Table 14.2.1.11 Primary Efficacy Analysis for SVC (Part 1: Shared-Baseline Mixed Effects Model, Part 2: Raw Summary Statistics) - mITT
Table 14.2.1.12 Primary Efficacy Analysis for SVC (Part 1: Shared-Baseline Mixed Effects Model, Part 2: Raw Summary Statistics) - PP
Table 14.2.1.13 Primary Efficacy Analysis for pNF-H (Part 1: Shared-Baseline Mixed Effects Model, Part 2: Raw Summary Statistics) - mITT
Table 14.2.1.14 Primary Efficacy Analysis for pNF-H (Part 1: Shared-Baseline Mixed Effects Model, Part 2: Raw Summary Statistics) - PP
Table 14.2.1.15 Primary Efficacy Analysis for ALSFRS-R Bulbar (Part 1: Shared-Baseline Mixed Effects Model, Part 2: Raw Summary Statistics) - mITT
Table 14.2.1.16 Primary Efficacy Analysis for ALSFRS-R Bulbar (Part 1: Shared-Baseline Mixed Effects Model, Part 2: Raw Summary Statistics) - PP
Table 14.2.1.17 Primary Efficacy Analysis for ALSFRS-R Fine Motor (Part 1: Shared-Baseline Mixed Effects Model, Part 2: Raw Summary Statistics) - mITT
Table 14.2.1.18 Primary Efficacy Analysis for ALSFRS-R Fine Motor (Part 1: Shared-Baseline Mixed Effects Model, Part 2: Raw Summary Statistics) - PP
Table 14.2.1.19 Primary Efficacy Analysis for ALSFRS-R Gross Motor (Part 1: Shared-Baseline Mixed Effects Model, Part 2: Raw Summary Statistics) - mITT
Table 14.2.1.20 Primary Efficacy Analysis for ALSFRS-R Gross Motor (Part 1: Shared-Baseline Mixed Effects Model, Part 2: Raw Summary Statistics) - PP
Table 14.2.1.21 Primary Efficacy Analysis for ALSFRS-R Breathing (Part 1: Shared-Baseline Mixed Effects Model, Part 2: Raw Summary Statistics) - mITT
Table 14.2.1.22 Primary Efficacy Analysis for ALSFRS-R Breathing (Part 1: Shared-Baseline Mixed Effects Model, Part 2: Raw Summary Statistics) - PP

14.2.2 Quadratic Shared-Baseline, Mixed Effects

Table 14.2.2.1 Summary of Quadratic Efficacy Analysis for All Efficacy Outcomes - mITT
Table 14.2.2.2 Summary of Quadratic Efficacy Analysis for All Efficacy Outcomes - PP
Table 14.2.2.3 Quadratic Efficacy Analysis for ALSFRS-R - mITT
Table 14.2.2.4 Quadratic Efficacy Analysis for ALSFRS-R - PP
Table 14.2.2.5 Quadratic Efficacy Analysis for Upper ATLIS - mITT
Table 14.2.2.6 Quadratic Efficacy Analysis for Upper ATLIS – PP
Table 14.2.2.7 Quadratic Efficacy Analysis for Lower ATLIS - mITT
Table 14.2.2.8 Quadratic Efficacy Analysis for Lower ATLIS – PP
Table 14.2.2.9 Quadratic Efficacy Analysis for Total ATLIS - mITT
Table 14.2.2.10 Quadratic Efficacy Analysis for Total ATLIS - PP
Table 14.2.2.11 Quadratic Efficacy Analysis for SVC - mITT
Table 14.2.2.12 Quadratic Efficacy Analysis for SVC - PP
Table 14.2.2.13 Quadratic Efficacy Analysis for pNF-H - mITT
Table 14.2.2.14 Quadratic Efficacy Analysis for pNF-H - PP

Table 14.2.2.15 Quadratic Efficacy Analysis for ALSFRS-R Bulbar - mITT
Table 14.2.2.16 Quadratic Efficacy Analysis for ALSFRS-R Bulbar - PP
Table 14.2.2.17 Quadratic Efficacy Analysis for ALSFRS-R Fine Motor - mITT
Table 14.2.2.18 Quadratic Efficacy Analysis for ALSFRS-R Fine Motor - PP
Table 14.2.2.19 Quadratic Efficacy Analysis for ALSFRS-R Gross Motor - mITT
Table 14.2.2.20 Quadratic Efficacy Analysis for ALSFRS-R Gross Motor - PP
Table 14.2.2.21 Quadratic Efficacy Analysis for ALSFRS-R Breathing - mITT
Table 14.2.2.22 Quadratic Efficacy Analysis for ALSFRS-R Breathing - PP

14.2.3 Left Censoring for Intercurrent Event of Death and Death Equivalent Events

Table 14.2.3.1 Summary of Primary Efficacy Analysis with Left Censoring for Intercurrent Event of Death and Death Equivalent Events for All Efficacy Outcomes - mITT
Table 14.2.3.2 Summary of Primary Efficacy Analysis with Left Censoring for Intercurrent Event of Death and Death Equivalent Events for All Efficacy Outcomes - PP
Table 14.2.3.3 Primary Efficacy Analysis with Left Censoring for Intercurrent Event of Death and Death Equivalent Events for ALSFRS-R - mITT
Table 14.2.3.4 Primary Efficacy Analysis with Left Censoring for Intercurrent Event of Death and Death Equivalent Events for ALSFRS-R - PP
Table 14.2.3.5 Primary Efficacy Analysis with Left Censoring for Intercurrent Event of Death and Death Equivalent Events for Upper ATLIS - mITT
Table 14.2.3.6 Primary Efficacy Analysis with Left Censoring for Intercurrent Event of Death and Death Equivalent Events for Upper ATLIS - PP
Table 14.2.3.7 Primary Efficacy Analysis with Left Censoring for Intercurrent Event of Death and Death Equivalent Events for Lower ATLIS - mITT
Table 14.2.3.8 Primary Efficacy Analysis with Left Censoring for Intercurrent Event of Death and Death Equivalent Events for Lower ATLIS – PP
Table 14.2.3.9 Primary Efficacy Analysis with Left Censoring for Intercurrent Event of Death and Death Equivalent Events for Total ATLIS - mITT
Table 14.2.3.10 Primary Efficacy Analysis with Left Censoring for Intercurrent Event of Death and Death Equivalent Events for Total ATLIS - PP
Table 14.2.3.11 Primary Efficacy Analysis with Left Censoring for Intercurrent Event of Death and Death Equivalent Events for SVC - mITT
Table 14.2.3.12 Primary Efficacy Analysis with Left Censoring for Intercurrent Event of Death and Death Equivalent Events for SVC - PP

14.2.4 Survival Analyses

Table 14.2.4.1 Cox Proportional Hazards Analysis for Death or Equivalent - mITT
Table 14.2.4.2 Cox Proportional Hazards Analysis for Death or Equivalent – PP
Table 14.2.4.3 Cox Proportional Hazards Analysis for Death - mITT

Table 14.2.4.4 Cox Proportional Hazards Analysis for Death - PP
Table 14.2.4.5 Cox Proportional Hazards Analysis for Tracheostomy - mITT
Table 14.2.4.6 Cox Proportional Hazards Analysis for Tracheostomy - PP
Table 14.2.4.7 Cox Proportional Hazards Analysis for PAV - mITT
Table 14.2.4.8 Cox Proportional Hazards Analysis for PAV - PP

14.2.5 Time on Edaravone

Table 14.2.5.1 Summary of Analysis with Time on Edaravone for All Efficacy Outcomes - mITT
Table 14.2.5.2 Summary of Analysis with Time on Edaravone for All Efficacy Outcomes - PP
Table 14.2.5.3 Analysis with Time on Edaravone for ALSFRS-R - mITT
Table 14.2.5.4 Analysis with Time on Edaravone for ALSFRS-R - PP
Table 14.2.5.5 Analysis with Time on Edaravone for Upper ATLIS - mITT
Table 14.2.5.6 Analysis with Time on Edaravone for Upper ATLIS – PP
Table 14.2.5.7 Analysis with Time on Edaravone for Lower ATLIS - mITT
Table 14.2.5.8 Analysis with Time on Edaravone for Lower ATLIS - PP
Table 14.2.5.9 Analysis with Time on Edaravone for Total ATLIS - mITT
Table 14.2.5.10 Analysis with Time on Edaravone for Total ATLIS - PP
Table 14.2.5.11 Analysis with Time on Edaravone for SVC - mITT
Table 14.2.5.12 Analysis with Time on Edaravone for SVC - PP
Table 14.2.5.13 Analysis with Time on Edaravone for pNF-H - mITT
Table 14.2.5.14 Analysis with Time on Edaravone for pNF-H - PP
Table 14.2.5.15 Analysis with Time on Edaravone for ALSFRS-R Bulbar - mITT
Table 14.2.5.16 Analysis with Time on Edaravone for ALSFRS-R Bulbar - PP
Table 14.2.5.17 Analysis with Time on Edaravone for ALSFRS-R Fine Motor - mITT
Table 14.2.5.18 Analysis with Time on Edaravone for ALSFRS-R Fine Motor - PP
Table 14.2.5.19 Analysis with Time on Edaravone for ALSFRS-R Gross Motor - mITT
Table 14.2.5.20 Analysis with Time on Edaravone for ALSFRS-R Gross Motor - PP
Table 14.2.5.21 Analysis with Time on Edaravone for ALSFRS-R Breathing - mITT
Table 14.2.5.22 Analysis with Time on Edaravone for ALSFRS-R Breathing - PP

14.2.6 Time on Riluzole

Table 14.2.6.1 Summary of Analysis with Time on Riluzole for All Efficacy Outcomes - mITT
Table 14.2.6.2 Summary of Analysis with Time on Riluzole for All Efficacy Outcomes - PP
Table 14.2.6.3 Analysis with Time on Riluzole for ALSFRS-R - mITT
Table 14.2.6.4 Analysis with Time on Riluzole for ALSFRS-R - PP
Table 14.2.6.5 Analysis with Time on Riluzole for Upper ATLIS - mITT

Table 14.2.6.6 Analysis with Time on Riluzole for Upper ATLIS - PP
Table 14.2.6.7 Analysis with Time on Riluzole for Lower ATLIS - mITT
Table 14.2.6.8 Analysis with Time on Riluzole for Lower ATLIS - PP
Table 14.2.6.9 Analysis with Time on Riluzole for Total ATLIS - mITT
Table 14.2.6.10 Analysis with Time on Riluzole for Total ATLIS - PP
Table 14.2.6.11 Analysis with Time on Riluzole for SVC - mITT
Table 14.2.6.12 Analysis with Time on Riluzole for SVC - PP
Table 14.2.6.13 Analysis with Time on Riluzole for pNF-H - mITT
Table 14.2.6.14 Analysis with Time on Riluzole for pNF-H - PP
Table 14.2.6.15 Analysis with Time on Riluzole for ALSFRS-R Bulbar - mITT
Table 14.2.6.16 Analysis with Time on Riluzole for ALSFRS-R Bulbar - PP
Table 14.2.6.17 Analysis with Time on Riluzole for ALSFRS-R Fine Motor - mITT
Table 14.2.6.18 Analysis with Time on Riluzole for ALSFRS-R Fine Motor - PP
Table 14.2.6.19 Analysis with Time on Riluzole for ALSFRS-R Gross Motor - mITT
Table 14.2.6.20 Analysis with Time on Riluzole for ALSFRS-R Gross Motor - PP
Table 14.2.6.21 Analysis with Time on Riluzole for ALSFRS-R Breathing - mITT
Table 14.2.6.22 Analysis with Time on Riluzole for ALSFRS-R Breathing - PP

14.2.7 Time on Edaravone/Riluzole

Table 14.2.7.1 Summary of Analysis with Time on Edaravone/Riluzole for All Efficacy Outcomes - mITT
Table 14.2.7.2 Summary of Analysis with Time on Edaravone/Riluzole for All Efficacy Outcomes - PP
Table 14.2.7.3 Analysis with Time on Edaravone/Riluzole for ALSFRS-R - mITT
Table 14.2.7.4 Analysis with Time on Edaravone/Riluzole for ALSFRS-R - PP
Table 14.2.7.5 Analysis with Time on Edaravone/Riluzole for Upper ATLIS - mITT
Table 14.2.7.6 Analysis with Time on Edaravone/Riluzole for Upper ATLIS – PP
Table 14.2.7.7 Analysis with Time on Edaravone/Riluzole for Lower ATLIS - mITT
Table 14.2.7.8 Analysis with Time on Edaravone/Riluzole for Lower ATLIS - PP
Table 14.2.7.9 Analysis with Time on Edaravone/Riluzole for Total ATLIS - mITT
Table 14.2.7.10 Analysis with Time on Edaravone/Riluzole for Total ATLIS - PP
Table 14.2.7.11 Analysis with Time on Edaravone/Riluzole for SVC - mITT
Table 14.2.7.12 Analysis with Time on Edaravone/Riluzole for SVC - PP
Table 14.2.7.13 Analysis with Time on Edaravone/Riluzole for pNF-H - mITT
Table 14.2.7.14 Analysis with Time on Edaravone/Riluzole for pNF-H - PP
Table 14.2.7.15 Analysis with Time on Edaravone/Riluzole for ALSFRS-R Bulbar - mITT
Table 14.2.7.16 Analysis with Time on Edaravone/Riluzole for ALSFRS-R Bulbar - PP
Table 14.2.7.17 Analysis with Time on Edaravone/Riluzole for ALSFRS-R Fine Motor - mITT

Table 14.2.7.18 Analysis with Time on Edaravone/Riluzole for ALSFRS-R Fine Motor - PP
Table 14.2.7.19 Analysis with Time on Edaravone/Riluzole for ALSFRS-R Gross Motor - mITT
Table 14.2.7.20 Analysis with Time on Edaravone/Riluzole for ALSFRS-R Gross Motor - PP
Table 14.2.7.21 Analysis with Time on Edaravone/Riluzole for ALSFRS-R Breathing - mITT
Table 14.2.7.22 Analysis with Time on Edaravone/Riluzole for ALSFRS-R Breathing - PP

14.2.8 Time on Edaravone/Riluzole

Table 14.2.8.1 Summary of Analysis with Time on Edaravone/Riluzole for All Efficacy Outcomes - mITT
Table 14.2.8.2 Summary of Analysis with Time on Edaravone/Riluzole for All Efficacy Outcomes - PP
Table 14.2.8.3 Analysis with Time on Edaravone/Riluzole for ALSFRS-R - mITT
Table 14.2.8.4 Analysis with Time on Edaravone/Riluzole for ALSFRS-R - PP
Table 14.2.8.5 Analysis with Time on Edaravone/Riluzole for Upper ATLIS - mITT
Table 14.2.8.6 Analysis with Time on Edaravone/Riluzole for Upper ATLIS – PP
Table 14.2.8.7 Analysis with Time on Edaravone/Riluzole for Lower ATLIS - mITT
Table 14.2.8.8 Analysis with Time on Edaravone/Riluzole for Lower ATLIS - PP
Table 14.2.8.9 Analysis with Time on Edaravone/Riluzole for Total ATLIS - mITT
Table 14.2.8.10 Analysis with Time on Edaravone/Riluzole for Total ATLIS - PP
Table 14.2.8.11 Analysis with Time on Edaravone/Riluzole for SVC - mITT
Table 14.2.8.12 Analysis with Time on Edaravone/Riluzole for SVC - PP
Table 14.2.8.13 Analysis with Time on Edaravone/Riluzole for pNF-H - mITT
Table 14.2.8.14 Analysis with Time on Edaravone/Riluzole for pNF-H - PP
Table 14.2.8.15 Analysis with Time on Edaravone/Riluzole for ALSFRS-R Bulbar - mITT
Table 14.2.8.16 Analysis with Time on Edaravone/Riluzole for ALSFRS-R Bulbar - PP
Table 14.2.8.17 Analysis with Time on Edaravone/Riluzole for ALSFRS-R Fine Motor - mITT
Table 14.2.8.18 Analysis with Time on Edaravone/Riluzole for ALSFRS-R Fine Motor - PP
Table 14.2.8.19 Analysis with Time on Edaravone/Riluzole for ALSFRS-R Gross Motor - mITT
Table 14.2.8.20 Analysis with Time on Edaravone/Riluzole for ALSFRS-R Gross Motor - PP
Table 14.2.8.21 Analysis with Time on Edaravone/Riluzole for ALSFRS-R Breathing - mITT
Table 14.2.8.22 Analysis with Time on Edaravone/Riluzole for ALSFRS-R Breathing - PP

14.2.9 Sensitivity Analysis for Missing at Random Assumption

Table 14.2.9.1 Summary of Sensitivity Analysis for MAR Assumption for Select Efficacy Outcomes - mITT
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Table 14.2.9.3 Sensitivity Analysis for MAR Assumption for ALSFRS-R - mITT
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16.1.9.1.1 Statistical Analysis Plan

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AMX0035 SAP Quadratic Model Clarification Note to File

Description	Note to file to clarify SAP language regarding the quadratic model for efficacy analyses
Date	21NOV2019
Prepared By, Title	Noel Ellison, Director of Biostatistics

Final SAP Language

The SAP was finalized on October 9th, 2019 and has the following text regarding the quadratic model for efficacy analyses:

Historical and pre-SAP analyses have shown ALS to be a disease with linear progression over time. However, linearity cannot be assumed at this point for the study given the unknown effect of the treatment. In order to confirm linearity, the model described above will be modified to include quadratic terms for time.

If the quadratic terms for time are insignificant (p-values >0.10) then linearity will be assumed and the linear primary model will be used for analysis. If at least one of the interaction terms is significant (p-value<0.10) then the quadratic version of the primary model will be used for analysis. P-values for the quadratic terms in the model will be presented. If at least one of the quadratic terms is significant than the summary statistics described in the subsequent paragraph will be presented and the same statistics will be presented for the linear primary model.

Clarification

The purpose of this note to file is to clarify the quadratic terms for time that will be included in the efficacy analysis accounting for potential non-linearity of disease progression over time. The quadratic model will include interaction with quadratic time for all terms in the linear model except for treatment by quadratic time interaction. The model will be as follows:

$$Y_{i,t} = \mu + u_i + (\beta_0 + b_i) \times t + \beta_1 \times z_i \times t + \beta_2 \times Age_i \times t + \beta_3 \times DelFS_i \times t + \beta_4 \times DelY_i \times t + (\beta_5 + a_i) \times t^2 + \beta_6 \times Age_i \times t^2 + \beta_7 \times DelFS_i \times t^2 + \beta_8 \times DelY_i \times t^2 + \varepsilon_{i,t}$$

- *i* represents the i[th] subject, *i* ranges from 1 to the number of subjects in the mITT population;
- *t* represents the actual time in weeks of each observation, time since "baseline" assessment;
- $Y_{i,t}$ is the dependent variable observed at time *t*, i.e. the actual efficacy score at time t
- *z* is a treatment indicator which is 0 in the control group and 1 in the treatment group;
- u_i is the random intercept for each subject and has an unspecified bivariate normal distribution;
- b_i is the random slope in the efficacy outcome for each subject over time and has an unspecified bivariate normal distribution;

- a_i is the random slope in the efficacy outcome for each subject over time-squared and has an unspecified bivariate normal distribution;
- *Age x t* and *Age x* t^2 are the interactions representing the effect of age on progression over time and time-squared. It is expected that older subjects will decline faster;
- $DelFS_i$ *x t* and $DelFS_i$ *x* t^2 are the interactions representing the effect of previous progression measured by ALSFRS-R on progression over time and time-squared. It is expected that subjects who were progressing quickly since symptom onset will continue to progress quickly;
- $DelY_i$ *x t* and $DelY_i$ *x* t^2 are the interactions representing the effect of previous progression measured by the efficacy outcome of interest (response variable) on progression over time. It is expected that subjects who were progressing quickly since symptom onset will continue to progress quickly;
- μ is the estimated intercept of the efficacy outcome across all subjects;
- β_0 is the estimated slope for time;
- β_1 is the estimated slope for treatment;
- β_2 is the estimated slope for age at baseline (years);
- β_3 is the estimated slope for del-FS;
- β_4 is the estimated slope for del-efficacy (corresponding to $Y_{i,t}$);
- β_5 is the estimated coefficient for time-squared;
- β_6 is the estimated coefficient for the interaction of age at baseline (years) and time-squared;
- β_7 is the estimated coefficient for the interaction of del-FS and time-squared;
- β_8 is the estimated coefficient for the interaction of del-efficacy (corresponding to $Y_{i,t}$) and time-squared;
- $\varepsilon_{i,t}$ is the random error which shows the amount by which the observed value differs from its expected value.

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