STATISTICAL ANALYSIS PLAN

Protocol:

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) Open Label Extension (OLE)

Amylyx Pharmaceuticals Inc.

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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) Open Label Extension (OLE)

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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01 Nov 2019

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List of Abbreviations

ΔFS	Del-FS Score
AA	Those patients on active in the main study who continue into the extension
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
PA	Those patients who were on placebo in the main study who continue into the 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 in the OLE.
Modified Intent-to-Treat Population (mITT)	All subjects who receive at least one dose of study medication and have at least one ALSFRS-R score recorded after their OLE baseline visit
Treatment	In the OLE, all patients will receive AMX0035 via sachet (3g PB and 1g TURSO) once daily for the first three weeks and increased to two sachets daily if tolerated.
Two groups will be defined in the open label extension	PA defined as those patients who were on placebo in the main study who continue into the extension AA defined as those patients who were on active in the main study who continue into the extension

1 INTRODUCTION

The objective of the Statistical Analysis Plan (SAP) 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 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).

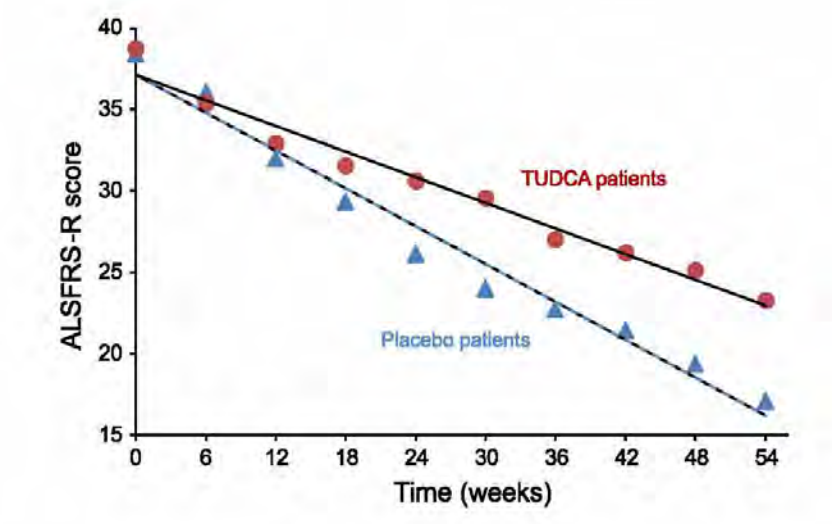

Figure 3: Linear regression analysis of ALSFRS-R mean scores over time for the TURSO (TUDCA in the publication) (circles, slope -0.388) and placebo groups (triangles, slope -0.262).

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 outcome measure will be:

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

2.2 Secondary Objectives

The secondary objectives will be to measure:

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

3 STUDY DESIGN

3.1 Number of Subjects

This study will be conducted in subjects who complete the CENTAUR main study and opt into the extension study. Detailed inclusion exclusion criteria can be found in Appendix VII of the clinical protocol.

All but one site from the CENTAUR main study participated in the open label extension study. Subjects will be assigned to oral (or feeding tube) AMX0035 treatment (1 sachet= 3g PB and 1g TURSO plus excipients) at two sachets daily.

3.2 Sample Size Considerations

Detailed powering calculations were not conducted for the open label extension study.

3.3 Study Design

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 who completed the 24 weeks placebo controlled randomized phase of the trial and elect to continue treatment in this trial extension will be given oral (or feeding tube) twice daily sachets of AMX-0035 (verum) therapy. Patients previously randomized to placebo in the randomized placebo-controlled phase of the trial will be crossed over to AMX0035 therapy. Treatment duration will be up to one hundred and thirty-two (132) weeks starting upon completion of the randomized phase of the trial. Clinic visits will occur at Screening/Baseline (entry in the OLE), 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 in the randomized phase of the trial 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.

Table 1: Schedule of Activities

ACTIVITY	Screening/ Baseline Visit	Study Drug Administration (weeks)									
		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 Visit
	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. During the OLE, safety laboratory tests will be performed by local laboratories.

[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.

4 RANDOMIZATION AND UNBLINDING PROCEDURES

4.1 Randomization

This is an open label extension so subjects will all be on active therapy. However, two groups will be defined in the OLE:

- PA group defined as those patients who were randomized to placebo in the main study who continue into the extension
- AA group defined as those patients who were randomized to AMX-0035 verum in the main study who continue into the extension

5 EFFICACY/SAFETY ASSESSMENTS

5.1 Efficacy Endpoints

The efficacy outcome measures are listed below in hierarchical order:

- Rate of progression on the ALSFRS-R total score;
- The rate of key study events including tracheostomy, hospitalization, and death;
- Rate of progression in upper and lower ATLIS scores;
- Rate of progression of slow vital capacity;
- Rate of progression on ALSFRS-R domains;
- Rate of progression on total ATLIS score.

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 [redacted].

5.1.2 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.

5.1.3 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.

5.1.4 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 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).

5.2.1 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 for the extension study is signed and started more than 33 days after the last treatment was received in the main study up to 28 days (+5 days) after the study drug has been discontinued in the extension study. AEs starting within 28 days (+5 days), i.e. 33 days, after the last treatment was received in the main study were included and summarized as part of the main study. 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.
   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.
   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.
   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.

5.2.2 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.

5.2.3 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.

5.2.4 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).

5.2.5 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.

5.3 Other Evaluations

Additional evaluations include the following:

1. Demographics;
2. Baseline disease characteristics;
3. Medical history;
4. Days hospitalized;
5. Prior medications.

5.4 Interim of Analysis

An interim analysis of the OLE will occur using data collected up to the time of the last-patient last-visit (LPLV) in the randomized phase of the trial.

6 ANALYSIS POPULATIONS AND GENERAL STATISTICAL PROCEDURES

6.1 Definition of OLE Analysis Populations

Note: All definitions below refer to OLE study period and the baseline is the last visit of the randomized phase of the study. 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 in the OLE. 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-OLE baseline total ALSFRS-R score available. Subjects in this population will be analyzed based on the treatment they were assigned to.

The PP population will include all mITT subjects up until the point they did not take study drug for 30 days or had a major protocol violation which will exclude subsequent visits from PP analysis. 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.

6.2 Application of Analysis Populations

The primary population for efficacy analysis is the mITT population. Analysis of 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.

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.

As applicable, two baselines will be used for analysis: the baseline from the randomized phase of the trial and the baseline from the OLE portion of the study. 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 or the last pre-OLE measure is defined as the baseline value. For efficacy measures baseline is defined as the last pre-randomization measurement or the last pre-OLE measure as applicable.

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 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 in OLE). 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.

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.

There are two groups in the OLE study which will be referred to as "Treatment" and they are:

1. PA group defined as those patients who were randomized to placebo in the main study who continue into the extension;
2. AA group defined as those patients who were randomized to AMX-0035 verum in the main study who continue into the extension.

Sensitivity analyses similar to in the main study may be performed.

7 SUBJECT DISPOSITION, DEMOGRAPHICS AND BASELINE CHARACTERISTICS EVALUATIONS

7.1 Subject Enrollment

Subject enrollment in the OLE will be summarized by treatment group (AA and PA) 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 subjects in the OLE.

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 (AA and PA) for all populations. The number and percentage of subjects who remain in the study at the time of data freeze (same time as last patient last visit of main study) and discontinuing from the study will be presented by treatment (AA and PA) 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 0) 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. Baseline disease characteristics and demographics at both baselines (randomized phase and OLE) will be provided in separate summary tables.

Demographic information to be assessed is age, gender, ethnicity, racial group, height and weight. Subject demographics will be summarized by treatment (AA and PA) 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.

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 (AA and PA) 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 OLE baseline) medication use and concomitant (after OLE baseline) 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 (AA and PA) and overall.

Protocol deviations will be provided in a data listing by subject.

8 EFFICACY EVALUATIONS

Overall efficacy evaluation for the OLE will follow the methods outlined in the SAP for the main study. Two sets of analyses will be performed: one using the original baseline and following patients through to the end of the observation time in the OLE, and a second analysis using only data from the OLE phase corrected for the new baseline at the beginning of the OLE phase of the study.

Specifically, all continuous efficacy measures will use the same statistical model (ALSFRS-R total, ATLIS lower, ATLIS upper, ATLIS total, SVC, and 4 ALSFRS-R domains) and will be presented in hierarchical order. 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 total) interacting with time will be included in the analysis. Del-FS from the original baseline will be included as a covariate in the OLE phase model in addition to rate of disease progression from the double-blind phase. It is acknowledged that any model that corrects for a post-randomization covariate may interfere with assessment of the treatment effect since the covariate would likely have been influenced by the treatment effect in the double-blind phase.

8.1 Efficacy Analyses

All analyses defined in the original SAP for the main study will also be performed for the OLE study. Two groups of patients will be compared, those who have previously been in the placebo group of the main study (PA group) and those who received AMX-0035 in the main study (AA group). The following evaluations will be done:

1. Slope during the extension will be compared to slope during the main study to determine whether the change from the double-blind phase to the OLE phase differs between treatment groups.
2. Within group comparisons of the difference in slope between the extension phase and the main study will be performed for patients who received placebo to determine whether initiation of treatment influenced the placebo slope and for patients who received AMX0035 during the main study to determine robustness and extent of duration of effect.
3. Slope over the entire duration of the main and extension study periods will be assessed using the original model from the main study SAP, but extended throughout the observation time of the OLE study.
4. The first 6 months of active treatment will be pooled for patients in the PA and AA groups and received treatment during the OLE phase. This pooled group will be compared to the placebo arm from the main study. An assessment of the relationship between the covariates and the slope over time will be performed to make sure that the range of covariate values for the two active groups overlap sufficiently to allow

inclusion of both groups in the same model. The second phase of the active arm may also be included in this model as a third treatment arm resulting in a comparison of these 3 arms: placebo, first 6 months of treatment and second 6 months of treatment.

[redacted]

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.

The original analysis will be continued out to the end of the OLE study observation, and the analysis will also be performed beginning at the initiation of the OLE study. For the analysis beginning at the initiation of the OLE study, only events occurring more than 28 weeks after the main study (24 weeks + 28 days) will be included since events which occurred within 28 weeks are addressed in the main study analysis. An additional analysis including 2 treatment arms will also be performed: a pooled active arm including the time from initiation of active treatment and a placebo arm from baseline out to 6 months.

Figures for the hazard function will be presented showing the survival of AA subjects vs. PA 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." This analysis will be performed for the entire study population of the main study (mITT and PP) using as baseline the initiation of treatment in the randomized phase of the trial, and will be repeated for the OLE population (OLE mITT and OLE PP) using as baseline the first day of treatment in the OLE.

[redacted]

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 may 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. The parameters for subjects who never took any medication will be taken from the

placebo arm of the double-blind treatment phase of the study. Subjects with outcomes that are more extreme than all measurements in the placebo arm of the double-blind treatment phase may be excluded.

8.5 Subject Discontinuation Rate

Counts of subjects who discontinue from the OLE 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.

8.6 Additional Analyses

Additional efficacy analyses may include: comparison of the full open label extension population to appropriate historical or virtual controls or a crossover analysis of the PA patients.

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.

In addition, the event rate per 100 person years will also be summarized to allow comparison of event rates between the placebo group and the active treatment periods which will have much longer exposure time.

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 and duration of treatment 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 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.

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 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.

9.6 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 PA treatment and AA 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. 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;
- Concomitant medications;