Amylyx Pharmaceuticals, Inc.

Integrated Analysis of Protocols # AMX3500 and AMX3500-OLE
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)

Version 1
Statistical Analysis Plan for Survival

March 27, 2020

Prepared by:

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Abbreviations

ALS	Amyotrophic Lateral Sclerosis
ALSFRS-R	ALS Functional Rating Scale - Revised
CRF	case report form
CSR	clinical study report
FS	functional score
del-FS	delta-FS
HR	hazard ratio
ICH	International Conference on Harmonization
ITT	intent-to-treat
OLE	open label extension
PAV	permanent assisted ventilation
PB	Phenylbutyrate
SAP	statistical analysis plan
TURSO	Taurursodiol

1. Introduction

This statistical analysis plan (SAP) is based on Amylyx Pharmaceutical's Protocols AMX3500 and AMX3500-OLE, "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)", dated January 11, 2019. This SAP focuses specifically on analyses of survival from the time of randomization in Protocol AMX3500 ("main study", also referred to as "CENTAUR") through the open label extension ("OLE") period up to a cutoff date of February 29, 2020. This document is complementary to the main OLE SAP [1].

The statistical principles applied in the design and planned analyses of this study are consistent with the ICH guidelines E9 (Statistical Principles for Clinical Trials) [2].

This document will focus on the outcomes and specifications relevant to analyses of survival through the cutoff date of February 29, 2020. Detailed descriptions of study design and other analyses appear in the main study SAP [3] and the OLE SAP. If there are discrepancies for survival analyses between this SAP and previously written analysis plans, the analyses in this SAP supersede those in previous documents.

2. Summary of study design and treatment

ALS is a progressive neurodegenerative disease that has no cure. The disease exacts a significant economic burden. There are only two medications approved specifically for treating ALS: Rilutek (riluzole), which only provides a modest benefit for subjects, and Radicava (edaravone).

AMX3500 was a Phase II, multicenter, randomized, double-blind, placebo-controlled evaluating the safety, tolerability, efficacy, pharmacokinetics and biological activity of AMX3500 in subjects with Amyotrophic Lateral Sclerosis (ALS).

Subjects were 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 were to take one sachet daily (i.e. half-dose) which, if tolerated, increased to two sachets daily. Subjects were to remain on treatment until the Week 24 visit. An Open Label Extension (OLE) study was available to those subjects who completed the randomized, double-blind study. The duration of the OLE is 132 weeks. In the open label extension study all subjects received active therapy.

If a subject did not enroll in the extension, they were requested to complete 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.

3. Objectives of the Statistical Analysis Plan

The analyses in this SAP are meant to support the objectives of the protocol through a comparison of the survival of the two treatment groups from the time of randomization in the main study until February 29, 2020. Survival is defined in two ways:

- Death alone – all cause mortality
- Death or death equivalent, also referred to as the "combined survival" outcome. The "death equivalent" outcome is comprised of:
  - Tracheostomy
  - Permanent assisted ventilation (PAV), defined as more than 22 hours daily of non-invasive mechanical ventilation for more than one week (7 days).

4. Randomization and masking considerations

4.1. Randomization

The randomization assignments for this analysis were the same as for the main CENTAUR study:

- Original Placebo group, defined as those patients who were randomized to placebo in the main study whether or not they continue into the extension
- Original Active group, defined as those patients who were randomized to AMX0035 in the main study whether or not they continue into the extension.

A status table and/or by-subject listing will summarize subjects who withdrew during the main study and those who did not enter OLE, as well as their status as of February 29, 2020.

4.2. Masking

The authors of this complementary SAP do not have any access to the raw study data, analysis datasets, or the study database. Furthermore, although the subjects who have entered the OLE are all receiving active treatment, the authors are masked to the originally assigned treatment groups in the main study and have no access to any individual-level treatment group data. The SAP authors will continue to remain masked until the document is finalized.

5. General conventions and statistical considerations

General statistical procedures will follow those in the main SAPs. All analyses described in this plan are considered a priori analyses in that they have been defined prior to the authors of the SAP being unblinded to any raw data and to individual treatment assignments. All other analyses, if any, defined subsequent locking the survival database will be considered post-hoc analyses and will be applied using exploratory methodology. The Clinical Study Report will identify all post-hoc analyses as such.

Throughout this analysis plan, the phrase "by treatment regimen" will be understood to mean the two originally assigned groups from the main study protocol.

All significance testing will be two-tailed using $\alpha = 0.05$. Tests will be declared statistically significant if the calculated p-value is ≤ 0.05.

5.1. Baseline

Two baselines are used in the OLE: the baseline from the randomized phase of the trial, and the baseline from the OLE portion of the study. The applicable baseline for survival analyses is the former: the baseline from the randomized phase of the trial, or "study baseline". Unless otherwise noted, baseline is defined as the last non-missing measurement before randomization.

Baseline age, baseline ALSRS-R, and del-FS, the rate of disease progression prior to entering the study, will be covariates in the survival analysis.

5.2. Data sources

Three sources of data will contribute to the analyses.

1. *Main study data*: a cleaned, locked CRF database from the main CENTAUR study.
2. *OLE data*: An interim snapshot of the OLE's database; data collection and monitoring will be ongoing.
3. *Survival sweep data*: an independent assessment of vital status which will capture updated data on deaths and corresponding death dates through February 29, 2020.

5.3. Changes to planned analyses

Changes to the analyses described in this plan will be fully documented in a revised version of the plan prior to locking the study database and conducting the survival analyses. Changes made after locking both the OLE snapshot and survival sweep databases will be described in the clinical study report and characterized as "exploratory".

6. Survival analyses

Survival analyses will use the intent-to-treat population, i.e. all 137 randomized subjects.

The analysis will include all events captured in the three data sources from study start through February 29, 2020.

The analysis will include all subjects, i.e., those who, up through February 29, 2020:

- Died during the main study period
- Died during the OLE up to and including February 29, 2020
- Withdrew or were lost to follow-up
- Did not enroll in the OLE.

Outside of the vital status follow-up activities listed in the protocol, which included searches of public records for vital status, Amylyx is currently conducting a separate, comprehensive

vital status "sweep" assessment for each randomized subject through February 29, 2020. The assessment will include those subjects who withdrew or were lost to follow-up at any time prior to this date, as well as those who did not enroll in the OLE. The organization conducting the assessment estimates identifying the vital status of 95% subjects in a trial, meaning the survival sweep will likewise identify the updated vital status (alive or dead) for approximately 95% of the original study population. As a result, the analysis for survival outcomes will adopt an administrative censoring approach with a cutoff date of February 29, 2020. Amylyx chose this date because it is Rare Disease Day [4] and prior to the rapid increase in cases of COVID-19 in the United States.

A table will show the concordance between deaths identified in the CRF databases and those identified in the survival sweep.

6.1. Survival outcomes

6.1.1. Main analysis: Death

The main analysis will compare time to death between the two treatment regimens. The date of death will initially be recorded on the CRF. The vital status sweep may capture additional deaths and associated death dates through the cut-off date of February 29, 2020. Some of these additional deaths may have previously been recorded as alive or lost to follow-up. For the analysis of survival defined by death alone, the following conventions will apply:

- The vital status sweep may capture additional deaths for those who were previously recorded as alive or lost to follow-up on the CRF. The analysis will use the updated death status and dates of death from the survival sweep if they occur before February 29, 2020.
- If a date of death from the vital status sweep and a death from either CRF database conflict, the date of death from the survival sweep will be used as the definitive date if occurs before February 29, 2020.
- The OLE snapshot may capture death dates that are later than February 29, 2020. In these cases, subjects will be considered alive for the analysis and censored on February 29, 2020.

- Otherwise, surviving subjects who were not recorded as having died during the analysis period will be censored on February 29, 2020
- Survival time = (earliest date of death or censoring -randomization date) + 1.

The median duration of survival and the associated 95% confidence interval will be estimated overall using the Kaplan-Meier method.

The hazard ratio of death comparing the Original Active group to the Original Placebo group will be estimated using a Cox proportional hazards model (proc phreg in SAS and coxph() in R) with treatment and covariates of del-FS, baseline ALSFRS-R, and age at baseline. Inference for treatment effect adjusted for the model covariates will use the likelihood ratio test, which has been shown to be more stable for smaller sample sizes and extreme data situations and was referenced in the previous main study and open label extension SAP [5]. When the sample size is large, the likelihood ratio test will give results similar to those of the Wald test, which is the proc phreg default. Because the likelihood ratio test is not produced by default in SAS or R for model estimates, the analysis will need to run two models to calculate the likelihood ($\hat{L}$) for:

- A full model with treatment and the three covariates
- A reduced model with del-FS, baseline ALSFRS-R, and age at baseline (no treatment).

If the coefficient for treatment in the full model is $\hat{b}$, then the hypothesis H_0: $\hat{b} = 0$ can be tested by comparing the likelihoods of the full and reduced models, which are considered nested models. The likelihood ratio test statistic for the treatment effect adjusted for the model covariates is the difference between -2×log likelihood for the reduced model and -2×log likelihood for the full model, or $-2[\log(\hat{L}_{full}) - \log(\hat{L}_{reduced})]$. Under H_0, this test statistic is approximately distributed as χ^2 with 1 degree of freedom.

The analysis will use the Efron likelihood for handling ties, which is the default method in R's coxph(). The Breslow likelihood, which is the default in SAS's proc phreg, may be biased in the presence of heavy ties.

Figures for the Kaplan-Meier estimates and the hazard function will be presented showing the survival of Original Active and Original Placebo subjects, in addition to hazard ratios and Wald p-values for each covariate.

6.1.2. Supportive analysis: Death or death equivalent

A supportive analysis will compare time to death or death equivalent between the two treatment regimens. Death and death equivalent events will be tabulated by treatment regimens. As mentioned in Section 5.2, the data for death equivalent events will be recorded in the locked CENTAUR database and an interim snapshot of the OLE's database. The OLE database will still be undergoing data collection at the time of the data snapshot for analysis.

For death equivalent events:

- Tracheostomy: analyses will use the recorded date of tracheostomy as the event date.
- PAV: 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.

For the analysis of the combined survival defined by death or equivalent, the following conventions will apply:

- The vital status sweep may capture additional deaths for those who were previously recorded as alive or lost to follow-up. The analysis will use the updated death status and dates of death from the survival sweep.
- If a date of death from the vital status sweep and a death from either CRF database conflict, the date of death from the survival sweep will be used as the definitive date.
- The OLE snapshot may capture death dates or death equivalent dates that are later than February 29, 2020. In these cases, subjects will be considered alive for the analysis and censored on February 29, 2020.
- It is possible that some of the participants who were lost to follow-up or who did not enter the OLE experienced a death equivalent event after they were no longer being followed. Such events will likely not appear in any of the data sources; the analysis

will assume that these subjects did not have a death equivalent event in this period and will assume a censoring date of February 29, 2020.

- Subjects who are determined to be alive from the survival sweep, but who are recorded as having a death equivalent event in either CRF database prior to February 29, 2020, will be considered as having a death equivalent event in the analysis.
- Surviving subjects who were not recorded as experiencing death, tracheostomy, or PAV during the analysis period will be censored on February 29, 2020.
- Survival time = (earliest of death date, tracheostomy date, or PAV date as defined above – randomization date) + 1.

The median duration of the combined survival endpoint and the associated 95% confidence interval will be estimated overall using the Kaplan-Meier method.

The hazard ratio of death or equivalent comparing the Original Active group to the Original Placebo group will be estimated using a Cox proportional hazards model (proc phreg in SAS or coxph() in R) with treatment and covariates of del-FS, baseline ALSFRS-R, and age at baseline. Inference for treatment effect adjusted for the model covariates will use the likelihood ratio test as described above in section 6.1.1.

Figures for the Kaplan-Meier estimates and the hazard function will be presented showing the survival of Original Active and Original Placebo subjects in addition to hazard ratios and p-values for each covariate.

6.2. Covariates

The covariates of baseline ALSFRS-R, baseline age, and del-FS will enter the Cox models as covariates.

"Del-FS", short for "delta-FS", is the rate of disease progression prior to entering the trial. As a measure of decline in the subject since symptom onset, del-FS is measured at the baseline visit. Del-FS = (48 – ALSFRS-R at first available visit)/ time in months from symptom onset to first available visit.

6.3. Details of the Cox proportional hazard model

Details of the Cox model are adapted from the main AMX3500 SAP.

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:

$$h(t) = h_o(t) \times \exp\left(b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4\right).$$

- *t* represents the survival time;
- *h(t)* is the hazard function determined by a set of covariates (x_1, x_2, x_3, *and* x_4);
- the coefficients (b_1, b_2, b_3, *and* b_4) measure the impact (i.e., the effect size) of the covariates;
- x_1 represents treatment (active-active or placebo-active);
- x_2 represents del-FS;
- x_3 represents age at baseline;
- x_4 represents ALSFRS-R at baseline
- the term $h_o(t)$, which is called the baseline hazard, 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 event hazard increases and the length of survival decreases. A hazard ratio above 1 indicates a covariate that is positively associated with the event probability and therefore negatively associated with the length of survival.

The hazard ratio will be examined to determine how the active treatment influenced the hazard.

- HR=1: No effect;
- HR<1: Active treatment reduces the hazard
- HR>1: Active treatment increases the hazard

6.4. Sensitivity analyses

Sensitivity analyses will include the following models for the outcomes of death and combined survival (death and equivalent):

- Analysis using a Cox proportional hazards model with treatment and covariates of del-FS and baseline age (as specified in the original main study SAP).
- Analysis using a Cox proportional hazards model with only treatment (no covariate).

7. References

1. Statistical Analysis Plan, AMX3500-OLE, Amylyx Pharmaceuticals, Inc.; October 2019 v3.

2. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. Statistical principles for clinical trials (E9). International Conference on Harmonization; 1998.

3. Statistical Analysis Plan, AMX3500, Amylyx Pharmaceuticals, Inc.; October 2019 v3.

4. https://www.rarediseaseday.org/article/about-rare-disease-day

5. Hsieh, FY. A Cautionary Note on the Analysis of Extreme Data with Cox Regression. The American Statistician, May 1995, Vol. 49, No. 2, 1995.