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APPLICATION NUMBER:

216660Orig1s000

CLINICAL AND STATISTICAL REVIEW(S)

CLINICAL AND STATISTICAL REVIEW

Application Type	NDA
Application Number(s)	216660
Priority or Standard	Priority
Submit Date(s)	October 29, 2021
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Division/Office	DN1/ON
Reviewer Name(s)	Veneeta Tandon (Clinical), Tristan Massie (Statistics)
Review Completion Date	September 28, 2022
Established/Proper Name	AMX0035 (phenylbutyrate-taurursodiol)
(Proposed) Trade Name	RELYVRIO
Applicant	Amylyx Pharmaceuticals, Inc
Dosage Form(s)	3 g/1 g powder-filled sachets for suspension administered orally (or through a feeding tube): Phenylbutyrate (PB; 3 g) and ursodoxicoltaurine (taurursodiol; 1 g)
Applicant Proposed Dosing Regimen(s)	Run-in: 1 sachet daily (as once daily) for (b) (4) 21 days Maintenance: 2 sachets daily after 21 days (as 1 sachet twice daily).
Applicant Proposed Indication(s)/Population(s)	Treatment of Amyotrophic lateral sclerosis (ALS)
Recommendation on Regulatory Action	Approve
Recommended Indication(s)/Population(s) (if applicable)	Treatment of Amyotrophic lateral sclerosis (ALS)

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Glossary

	Definition
AE	Adverse event
ALS	Amyotrophic Lateral Sclerosis
ALSFRS-R	ALS Functional Rating Scale-Revised
APIs	active pharmaceutical ingredients
ATLIS	Accurate Test of Limb Isometric Strength
BID	Twice daily
BRF	Benefit Risk Framework
BMI	Body mass index
CFR	Code of Federal Regulations
CI	Confidence interval
C-SSRS	Columbia-Suicide Severity Rating Scale
DSMB	Data and Safety Monitoring Board
EAE	Experimental autoimmune encephalomyelitis
ECGs	Electrocardiograms
ER	Endoplasmic reticulum
FDA	Food and Drug Administration
GI	gastrointestinal
HR	Hazard ratio
IND	Investigational new drug
ITT	Intention to treat
LS	Least squares
LOCF	Last observation carried forward
MedDRA	Medical Dictionary for Regulatory Activities
mITT	Modified intention to treat
MMRM	Mixed Model for Repeated measures
NDA	New drug application
NEALS	Northeast ALS Consortium
OLP	Open-label phase
PAV	Permanent assisted ventilation
PB	Phenylbutyrate
PD	Pharmacodynamic
PET	Positron emission tomography

PK	Pharmacokinetic
pNF-H	Plasma Neurofilament heavy chain
PPN	Percent of predicted normal
PT	Preferred term
QD	Once daily
RA	Randomized to active (AMX0035) in the Randomized phase and continued into the open-label phase
RCP	Randomized controlled phase
RP	Randomized to placebo in the Randomized phase and continued into the open-label phase
SAP	Statistical analysis plan
SD	Standard deviation
SE	Standard error
SOC / SOC	Standard of care / System Organ Class
SVC	Slow vital capacity
TSPO	Translocator protein
TURSO	Taurursodiol

1. Executive Summary

1.1. Product Introduction

AMX0035 Powder for Oral Suspension is a fixed-dose combination of sodium phenylbutyrate (PB) and ursodoxicoltaurine (taurursodiol or TUDCA). The drug product is a ~10-gram powder-filled sachet containing 3 grams of sodium phenylbutyrate (PB) and 1 gram of taurursodiol. The contents of the sachet are mixed with 8 ounces of water and administered orally or via a feeding tube.

The proposed indication for AMX0035 is for the treatment of amyotrophic lateral sclerosis (ALS).

Sodium phenylbutyrate is approved in the United States (US) as BUPHENYL® for adjunctive therapy in the chronic management of patients with urea cycle disorders at total daily doses of 9.9-13g/m^2 given 3-6 times daily. Glycerol phenylbutyrate, a prodrug for phenylbutyrate is approved as RAVICTI® in management of urea cycle disorders at a total daily dose of up to 19g given 3 times daily (TID). Taurursodiol is a bile acid found in large amounts in the bile of bears and used in traditional Chinese medicine for various conditions. It is not approved in the US, and it approved in Italy as Tudcabil® and China and Turkey as Taurolite® for the treatment of disorders of bile production. Ursodiol (UDCA), a metabolite of TUDCA, is approved in US for treatment of primary biliary cirrhosis at total daily doses of 13-15 mg/kg given 3-4 times daily.

AMX0035 is proposed to be administered prior to a meal according to the following regimen:
Run-in Period:
The recommended starting dose of AMX0035 is 1 sachet once daily (OD) for 1-21 day.
Maintenance Dose:
The recommended maintenance dose of AMX0035 is 1 sachet twice daily, morning and evening (BID).
To improve overall acceptance and reduce bitter aftertaste, AMX0035 may be followed by a (b) (4), meal, or snack.

1.2. Conclusions on the Substantial Evidence of Effectiveness

The Applicant has provided data from a single study along with data intended to serve as confirmatory evidence. There are notable limitations and weaknesses to this data that are described throughout this review. However, given the serious and life-threatening nature of ALS and the substantial unmet need, it is appropriate to exercise regulatory flexibility in considering these results. FDA's regulations allow for FDA to exercise regulatory flexibility in

applying the statutory standards for establishing the safety and effectiveness of new therapies intended to treat persons with life-threatening and severely debilitating illnesses, especially where no satisfactory alternative therapy exists. Likewise, FDA's draft the Draft December 2019 Guidance for Industry: "Demonstrating Substantial Evidence for Effectiveness for Drugs and Biological Products," cites circumstances (e.g., severity and rarity of the disease and unmet medical need) that allows for flexibility in the degree of certainty in supporting a conclusion of the establishment of substantial evidence of effectiveness. Although the results of the analysis presented by the Applicant as well as the additional analyses conducted by the FDA do not persuasively demonstrate effectiveness from a statistical perspective, the clinical evidence may be considered sufficient to support approval. ALS is a life-threatening and severe disease with unmet medical need, and it is appropriate to exercise regulatory flexibility when considering the persuasiveness of the effectiveness data to establish substantial evidence of effectiveness in this case.

Please refer to Section 7.4 'Integrated Assessment of Effectiveness' of this review for a detailed discussion on the assessment of overall evidence of effectiveness of AMX0035 that leads to this recommendation.

1.3. Benefit-Risk Assessment

Benefit-Risk Integrated Assessment

AMX0035 Powder for Oral Suspension, a fixed combination of 3g Sodium Phenylbutyrate (PB) and 1g ursodoxicoltaurine (taurursodiol or TUDCA) is proposed for the treatment of amyotrophic lateral sclerosis (ALS). Phenylbutyrate is proposed by the Applicant to ameliorate endoplasmic reticulum stress through upregulation of chaperone proteins. Taurursodiol is proposed to ameliorate mitochondrial stress by reducing mitochondrial permeability and increasing the apoptotic threshold of the cell. It is postulated by the Applicant that a synergistic effect of the two compounds can reduce neuronal death by simultaneous inhibition of endoplasmic reticulum and mitochondrial stress.

ALS is a rapidly progressive neurodegenerative disease characterized by the death of motor neurons that results in increasing muscle weakness and impairment of functional abilities and death. Respiratory failure is the leading cause of death.

There are two approved therapies for ALS. Riluzole was approved in 1995 and showed a 2-3 month increase in survival but no benefit on muscle and neurological function. Edaravone was approved in 2017 and, showed a 2.5-point difference in favor of treatment on a 48-point ALS Functional Rating Scale-Revised (ALSFRS-R) at Week 24 (p=0.0013).

The effectiveness of AMX0035 was evaluated in a single, adequate, and well-controlled Study AMX3500 (CENTAUR) of 24 weeks duration in 137 ALS patients with a 2:1 randomization to AMX0035 or placebo. This study was followed by an open-label extension (AMX3500OLE) of up to 132 weeks duration, in 90 out the 137 subjects who completed the controlled phase of the study. The study evaluated a single dose regimen that included an initial treatment cycle of 1 sachet daily for 3 weeks followed by a dosing regimen of 2 sachets daily.

The Applicant reports a statistically significant mean treatment difference of 2.32 points with a modest p-value of 0.034 in favor of AMX0035 on the primary endpoint, ALSFRS-R rate of decline, between the treatment arm and placebo in the mITT population (subjects who receive at least one dose of study medication and have at least one post-baseline total ALSFRS-R score). There are several statistical concerns regarding this analysis including the linearity assumption and that the analysis ignored deaths that decrease its persuasiveness and its ability to serve as a single study to determine efficacy. FDA recommends a combined analysis of function and survival, such as the Joint Rank analysis, if there are

deaths in the study. In the double-blind treatment period in the ITT there were 5 deaths in the mITT and 7 deaths in the ITT population (subjects who receive at least one dose of study medication). FDA's joint rank analysis, with multiple imputation based on a missing-at-random assumption for handling missing data of ALSFRS-R and death has p-value of 0.063 for the mITT population, and a p-value of 0.079 for the ITT population. Additionally, there was weak support from secondary endpoints in the study. Of the three prespecified secondary endpoints, the Total ATLIS and SVC numerically favored AMX0035 and the biomarker, pNF-H, favored placebo. A post hoc analysis of time to death, based on vital status data collected on 136 of the 137 patients originally randomized in Study AMX3500, suggested a nominally significant survival benefit, p = 0.0475. However, time to death alone was not a prespecified key objective of the study.

There are no significant safety concerns with AMX0035. There were no differences in fatal and serious adverse events between AMX0035 and placebo. Most of these adverse events were manifestations and complications of the underlying ALS. The number of subjects that discontinued treatment due to Treatment Emergent Adverse Events (TEAEs) was higher in the AMX0035 treatment group (20.2%) compared to placebo group (10.2%) in the controlled phase of the study. These differences were largely due to higher incidences of diarrhea, abdominal pain, nausea and dysgeusia in the AMX0035 arm. The common TEAEs occurring in >5% of AMX3500 treated patients and >1% difference from placebo belonged to the gastrointestinal SOC (including diarrhea, abdominal pain, nausea, dyspnea, salivary hypersecretion). Others common TEAES included dizziness, disease progression, respiratory tract infection and fatigue, and dyspnea.

Although the results of the analysis presented by the sponsor as well as the additional analyses conducted by the FDA do not persuasively demonstrate effectiveness from a statistical perspective, the clinical evidence suggests that AMX0035 may be effective for ALS. Overall, the approval of AMX0035 in ALS, a life-threatening and severe disease with unmet medical need, is warranted given the regulatory flexibility that can be applied when considering the persuasiveness of the effectiveness data to establish substantial evidence of effectiveness.

Benefit-Risk Dimensions

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of Condition	• Amyotrophic Lateral Sclerosis (ALS) is a rapidly progressive neurodegenerative disease characterized by the death of motor neurons that results in complete paralysis and death.	ALS is a rapidly progressive neurodegenerative disease. Respiratory failure is the leading cause of death.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	• 85-90% of the ALS cases are sporadic while the remaining 10-15% of cases is inherited. • ALS patients typically first present with dysarthria, fasciculations, weakness in a limb, or some combination thereof. Within a year of diagnosis, most patients suffer from significant and increasing morbidities and impaired ability to function independently, including losing the ability to swallow, speak, or ambulate independently. • Approximately 50% of patients with ALS die within 3 years from the onset of symptoms and approximately 90% die within 5 years. The remaining patients survive between 5-10 years from symptom onset. Median survival is approximately 27-41 months from symptom-onset. • Death is generally due to complications (i.e., respiratory infections) arising from diaphragmatic failure.	
Current Treatment Options	• There are two approved therapies for ALS: • Riluzole (Rilutek™): Riluzole improved early survival, but measures of muscle function and neurological function did not show benefit. • Edaravone (Radicava™): A treatment difference of 2.49 (p=0.0013) in favor of edavorone was observed on the change from baseline in the ALS Functional Rating Scale-Revised (ALSFRS-R) Total score.	Although, riluzole and edaravone have been approved in ALS, the disease is still progressive. Therefore, there remains a significant unmet need for the treatment of ALS.
Benefit	• The Applicant reports a statistically significant mean treatment difference of 2.32 points with a modest p-value of 0.034 in favor of AMX0035 on the primary endpoint, ALSFRS-R rate of decline, between the treatment arm and placebo in the mITT population. There were several statistical concerns regarding this analysis. • FDA recommends Joint Rank analysis if there are deaths in the study. FDA Joint Rank analysis with multiple imputation based on a missing-at-random	The effectiveness data from CENTAUR and its open label extension is suggestive of a potential treatment benefit with AMX0035 that is not statistically persuasive. Application of regulatory flexibility allows for concluding substantial effectiveness with uncertainty given the unmet need in a severe and life-

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	assumption for handling missing data of ALSFRS-R and death has p-value of 0.063 for the mITT population, and a p-value of 0.079 for the ITT population. • Additionally, there was lack of support from secondary endpoints in the study, although Total ATLIS and SVC numerically favored AMX0035. The secondary biomarker endpoint, pNF-H, favored placebo. • A post hoc analysis of time to death, based on vital status data collected on 136 of the 137 patients originally randomized in Study AMX3500, suggested a nominally significant survival benefit, p = 0.0475. Note that time to death alone was not prespecified as a key endpoint and its nominal p-value is not highly persuasive.	threatening disease with an observed mild safety profile.
Risk	• There were no differences in fatal and serious adverse events between AMX0035 and placebo. Most of these adverse events were manifestations and complications of ALS. • Subjects that discontinued with treatment due to Treatment Emergent Adverse Event (TEAE) was higher in the AMX0035 treatment group (20.2%) compared to placebo group (10.2%) in the controlled phase of the study. These differences were largely due to higher incidences of diarrhea, abdominal pain, nausea and dysgeusia in the AMX0035 arm. • The common TEAEs occurring in >5% of AMX3500 treated patients and >1% difference from placebo belonged to the gastrointestinal SOC (including diarrhea, abdominal pain, nausea, dyspnea, salivary hypersecretion). Others common TEAES included dizziness, disease progression, respiratory tract infection and fatigue, and dyspnea. • There were no differences in laboratory abnormalities or vital signs between AMX0035 and placebo treated subjects.	Diarrhea, abdominal pain, nausea, dysgeusia and dizziness appear to be the most common TEAEs.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Risk and Risk Management	• There were no safety signals of concern that would warrant additional risk management beyond that recommended in the product labeling for AMX0035.	Risk management can be achieved through the recommendations in the product labeling and routine postmarketing surveillance.

1.4. Patient Experience Data

Patient Experience Data Relevant to this Application (check all that apply)

□	The patient experience data that was submitted as part of the application include:	Section where discussed, if applicable
	□ Clinical outcome assessment (COA) data, such as	[e.g., Sec 6.1 Study endpoints]
	□ Patient reported outcome (PRO)	
	□ Observer reported outcome (ObsRO)	
	□ Clinician reported outcome (ClinRO)	
	□ Performance outcome (PerfO)	
	□ Qualitative studies (e.g., individual patient/caregiver interviews, focus group interviews, expert interviews, Delphi Panel, etc.)	
	□ Patient-focused drug development or other stakeholder meeting summary reports	[e.g., Sec 2.1 Analysis of Condition]
	□ Observational survey studies designed to capture patient experience data	
	□ Natural history studies	
	□ Patient preference studies (e.g., submitted studies or scientific publications)	
	□ Other: (Please specify)	
□	Patient experience data that were not submitted in the application, but were considered in this review:	
	□ Input informed from participation in meetings with patient stakeholders	
	□ Patient-focused drug development or other stakeholder meeting summary reports	[e.g., Current Treatment Options]
	□ Observational survey studies designed to capture patient experience data	
	□ Other: (Please specify)	
X	Patient experience data was not submitted as part of this application.	

2. Therapeutic Context

2.1. Analysis of Condition

Amyotrophic Lateral Sclerosis (ALS) is a rapidly progressive neurodegenerative disease characterized by the death of motor neurons that results in complete paralysis and death. ALS generally strikes people between 40 and 60 years of age (median age 55). The majority of cases (85-90%) are sporadic, having no clear risk factors or family history, while the remaining 10-15% of cases are inherited. Of these familial ALS cases (FALS), about 10-25% (i.e., 1-3% of the total ALS cases) result from a specific genetic defect that leads to a mutation in the gene responsible for the SOD-1, most inheriting as an autosomal dominant trait (D90A is an exception). Significant covariates of survival are age at onset, site of onset, delay from first symptom to diagnosis, and rate of change in respiratory function and functional rating scales.

Etiology: Although the precise etiology of ALS is unknown, it is characterized by nerve cell death and inflammation. Together, these pathologic processes are thought to be key drivers of the progressive loss of motor neurons. Recent research has highlighted endoplasmic reticulum (ER) stress and mitochondrial stress as pathways of neuronal death and neuroinflammatory processes.[1,2]

Diagnosis: El Escorial World Federation of Neurology Criteria is used for the diagnosis of ALS. ALS diagnosis 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

[1] Manfredi G, Kawamata H. Mitochondria and endoplasmic reticulum crosstalk in amyotrophic lateral sclerosis. Neurobiol Dis. 2016; 90:35-42. ePub 2015 Aug 15. Review.

[2] Area-Gomez E, de Groof A, Bonilla E, et al. A key role for MAM in mediating mitochondrial dysfunction in Alzheimer disease. Cell Death Dis. 2018;9(3):335. Published 2018 Feb 28. doi: 10.1038/s41419-017-0215-0

clinical and electrophysiological signs.

Diagnosis is staged as Clinically Definite, Probable, Probable-Laboratory supported and Possible ALS.

Clinical Features: ALS patients typically first present with dysarthria, fasciculations, weakness in a limb, or some combination thereof. Within a year of diagnosis, most patients suffer from significant and increasing morbidities and impaired ability to function independently, including losing the ability to swallow, speak, or ambulate independently.

Life Span: Approximately 50% of patients with ALS die within 3 years from the onset of symptoms and approximately 90% die within 5 years; the remaining survive between 5-10 years from symptom onset. Median survival is approximately 27-41 months from symptom-onset[3]. Death is generally due to complications (i.e., respiratory infections) arising from diaphragmatic failure.

Incidence: The *prevalence* is approximately 4-8 cases per 100,000 (approximately 12,000-24,000 patients in the US).

2.2. Analysis of Current Treatment Options

The approved therapies are summarized in Table 1.

Table 1: Summary of Approved Treatment

Product (s) Name	Relevant Indication	Year of Approval	Route and Frequency of Administration	Efficacy Information	Important Safety and Tolerability Issues	Other Comments (e.g., subpopulation not addressed
FDA Approved Treatments [Combine by Pharmacologic Class, if relevant]						
Riluzole (RILUTEK)	ALS	1995	Oral	2 Adequate well-controlled studies. Riluzole improved early survival in both studies, but measures of muscle function and neurological function did not		

[3] Labra J, Menon P, Byth K, et al. Rate of disease progression: a prognostic biomarker in ALS. J Neurol Neurosurg Psychiatry. 2016 Jun;87(6):628-32. doi: 10.1136/jnnp-2015-310998. Epub 2015 Jul 7.

				show benefit		
Edaravone (RADICAVA, RADICAVA ORS)	ALS	2017 (Oral form approved 2022)	IV and oral	Single Study The primary efficacy endpoint was a comparison of the change between treatment arms in the ALSFRS-R total scores from baseline to Week 24. A treatment difference of 2.49 (p=0.0013) in favor of edaravone was observed	Requires surgical implanted ports for drug administration	Oral form approved on May 12, 2022, through bioequivalence

3. Regulatory Background

3.1. U.S. Regulatory Actions and Marketing History

AMX0035 is a new molecular entity and is not currently marketed in the US.

3.2. Summary of Presubmission/Submission Regulatory Activity

A brief chronology of the regulatory activity with the applicant regarding efficacy and safety related discussions during the development of AMX0035 and additional important milestones is tabulated below. The regulatory interactions regarding other review disciplines will be addressed in the respective reviews (i.e., chemistry, and nonclinical).

Date	Summary of Regulatory Activity
3/21/16	PIND WRO meeting minutes • Division recommended a joint rank analysis of the ALSFRS-R change from baseline and mortality as the primary analysis (this is the standard approach for drug development for ALS and widely known and is described in FDA guidance; this was the first of multiple

	recommendations to the Applicant about this issue – see discussion below)
3/15/17	Initial IND IND submitted the Phase 2 CENTAUR study in ALS proposed as the IND-opening study
5/8/17	Orphan Designation granted
4/20/18	Fast Track request denied (study not designed to address an unmet need)
9/17/18	Fast Track request denied (study not designed to address an unmet need)
3/6/19	Advice on Statistical Analysis Plan Comment on CENTAUR SAP: "If there are deaths, then the joint rank analysis of function and survival should be the primary analysis."
3/12/20	Type C Meeting 3/12/20 to discuss CENTAUR results: • Division stated that change from baseline in the ALSFRS-R using a joint rank analysis method is the most appropriate analysis method for the primary endpoint • It was apparent, on face, that the results of the single trial were incapable of independently demonstrating substantial evidence of effectiveness • Division stated that the Applicant should urgently begin work on a protocol for a second efficacy study. • The Applicant proposed to conduct additional post hoc survival analyses of open-label unblinded data from the open-label extension to potentially provide additional data supportive of the above results. FDA stated that the Applicant could submit additional data for review and the Division would provide any relevant feedback, but that it was unlikely that these open-label data would be adequate to provide independent substantiation of effectiveness.
3/27/20	Request from Applicant for Breakthrough Designation was denied (does not show an improvement over the two approved therapies)
4/1/20	New SAP submitted for post hoc survival analysis of OLE, after study was unblinded
5/1/20	Topline results of post hoc survival analysis of OLE study with a cut off of February 29, 2020, was submitted in form of an additional meeting request • The Division and Biometrics Review Team reviewed the SAP and topline results and found the following deficiencies with the analyses: ○ The overall survival analysis is not nominally significant (p=0.064). In addition, type 1 error was not adequately controlled, as several of the secondary endpoints in the hierarchy for the double-blind phase failed (e.g., ATLIS Total p=0.112 and SVC p=0.076, as well as survival at Week 24 p=0.596).

	○ There were many discontinuations prior to the OLE (29% for placebo and 37% for drug), rendering the delayed-start analysis uninterpretable. ○ The SAP for the OLE survival analysis was submitted in April 2020, after the double-blind CENTAUR study was unblinded in November 2019. ○ The inclusion of tracheostomy in the definition of survival was unacceptable, as there is subjectivity and variability among patients and providers as to the time that a tracheostomy is placed (e.g., in anticipation for the need for permanent ventilation), and tracheostomies may also be placed for the management of secretions.
2/4/21	Type C Meeting • The Division reiterated the position that although the CENTAUR and CENTAUR-OLE data are encouraging, another randomized, placebo-controlled study would be necessary to support a marketing application. • The Applicant planned to conduct another Phase 3 study. "The Division acknowledged that the Applicant's planned Phase 3 study, A35-004, may meet the criteria for such a study, provided that the endpoints, including survival endpoints, were pre-specified and clinically meaningful. The Division discussed the possibility of the inclusion of an interim analysis at 24 weeks in the analysis plan that, if positive, may be able to provide the needed independent substantiation of effectiveness needed to support a future NDA.
4/28/21	Phase 3 Protocol A35-004 planned to be conducted in 600 ALS patients was submitted for Review
7/15/21	Pre-NDA meeting • FDA requested Applicant submit a pre-NDA meeting to discuss submission of the NDA for closer evaluation of the data and the published survival analysis • Division requested that the Applicant provide justification in the NDA submission that the available data satisfy the combination rule. • Regarding safety data from Study A35-004, the Division requested to submit to the NDA blinded reports of all serious adverse events (SAEs) and deaths, along with hyperlinks to the narratives, no later than the target date for the 120-day safety update. • Regarding efficacy data from Study A35-004, the Division requested (appropriately firewalled) primary endpoint descriptive results from all patients who have completed 24 weeks of study treatment by 4 months after submission of the application (this was further revised- see below).

8/3/21	Applicant's proposed timeline for providing CENTAUROLE data and supportive data from the Phase 3 Study A35-004 submitted to the Agency
8/10/21	Advice letter to Applicant: • The Division did not agree to the submission of interim report for CENTAUROLE with a cutoff date of (b) (4). The Division requested that a final CENTAUROLE study report with a cut-off date of March 2021 be submitted to the NDA • Regarding the proposal for providing unblinded data from Study A35-004 during the NDA review, the Division noted that Study A35-004 will be very early in its conduct at the time of the NDA submission and will only have data for a small portion (i.e., approximately 30 participants may have data for ≥12 weeks) of the total population of approximately 600 patients that is planned to be enrolled. Because there are potential risks to study integrity that could accompany any plans for unblinded assessment of data during the trial conduct and the small amount of data that will be available from that unblinding is unlikely to be informative, hence the Division did not request that the unblinded data from Study A35-004 be submitted during the NDA review.

3.3. Foreign Regulatory Actions and Marketing History

Sodium phenylbutyrate and taurursodiol was recently approved in Canada with the trade name Albrioza under the regulatory authority, "Notice of Compliance with Conditions (NOC/c)". This form of market authorization is granted to a product on the basis of promising evidence of clinical effectiveness. One of the conditions of the approval is the provision of data from an ongoing Phase 3 study (PHOENIX) trial ongoing. That study will enroll approximately 600 subjects at over 70 sites in the US and Europe, and it is expected to complete in late 2023 or early 2024 with results available shortly thereafter.

4. Significant Issues from Other Review Disciplines Pertinent to Clinical Conclusions on Efficacy and Safety

4.1. Office of Scientific Investigations (OSI)

No significant issues identified.

4.2. Product Quality

No significant issues identified.

4.3. Clinical Microbiology

Not applicable

4.4. Nonclinical Pharmacology/Toxicology

No significant issues identified.

4.5. Clinical Pharmacology

Please refer to clinical pharmacology review.

5. Sources of Clinical Data and Review Strategy

5.1. Table of Clinical Studies

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Table 2 Listing of Clinical Trials Relevant to this NDA/BLA

Trial Identity	Trial Design	Regimen/ schedule/ route	Study Endpoints	Treatment Duration/ Follow Up	No. of patients enrolled in each arm	Study Population
Controlled Clinical Study						
AMX3500 (US, 25 Northeast ALS Consortium (NEALS) centers) Primary Efficacy/Safety	Randomized, Double-Blind, Placebo-controlled	1 sachet BID orally (1 sachet = 1 g taurursodiol and 3 g sodium phenylbutyrate)	slope of progression with the ALSFRS-R	24 weeks	AMX0035= 89 Placebo = 48	ALS N=137
Uncontrolled Clinical Study						
AMX3500-OLE (US) **Long term Safety OL Extension of AMX3500**	Open-label extension	1 sachet BID orally (1 sachet = 1 g taurursodiol and 3 g sodium phenylbutyrate)	Long term Safety Assess rate of progression on the ALSFRS-R scale Determine the rate of key study events including tracheostomy, hospitalization, and death	Up to 132 weeks	AMX0035= 56 Placebo = 34	ALS N=90

5.2. Review Strategy

The evidence of clinical effectiveness of AMX0035 in the treatment of ALS is obtained from the controlled Study AMX3500 (CENTAUR). In addition, survival analyses were conducted using all data including Study AMX3500 (CENTAUR) and its open-label extension Study AMX3500OLE (CENTAUR OLE) to assess overall survival benefit from AMX0035. This is a combined review on the part of the Clinical and Biometrics Disciplines. The efficacy data were reviewed by both the clinical (Dr. Tandon) and statistics reviewers (Dr. Massie). However, all statistical analyses reported in this review to assess effectiveness of AMX0035 based on the primary and secondary endpoints were reviewed and confirmed by Dr. Massie. Dr. Tandon conducted the safety review. Dr. Li (Data Scientist) assisted with the safety assessment. Dr. Tandon performed the risk-benefit analysis in this review. Consult review was requested to the Division of Cardiology for the adequacy of the QTc assessments.

6. Review of Relevant Individual Trials Used to Support Efficacy

6.1. Study AMX3500 (CENTAUR)

Evaluation of the Safety, Tolerability, Efficacy and Activity of AMX0035, a Fixed Combination of Phenylbutyrate (PB) and Taurusodiol (TUDCA), for Treatment of Amyotrophic Lateral Sclerosis (ALS)
First Subject enrolled: 22 June 2017
Last subject last follow-up visit: 25 September 2019
Study Completion: 25 September 2019

6.1.1. Study Design

Overview and Objective

Primary:

- Assess safety and tolerability of AMX0035 over a 6-month period
- Measure the impact of AMX0035 using the slope of progression with the revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R).

Secondary:

- 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)
- Assess the impact of AMX0035 on biomarkers, including phosphorylated axonal neurofilament H subunit (pNF-H) levels and 18 kDa translocator protein (TSPO) uptake
- Assess the impact of AMX0035 on disease progression as measured by Slow Vital Capacity (SVC) decline, time to tracheostomy, and survival

- Develop concentration-response models of taurursodiol and PB at steady-state after administration of AMX0035 sachet twice-daily (BID); and
- Measure the impact of AMX0035 on survival.

Trial Design

AMX-3500 was a multicenter, randomized, placebo-controlled, double-blind, parallel group, 24-week study evaluating the safety, tolerability, efficacy, pharmacokinetics (PK), and biological activity of AMX0035.

Study visits occurred at Screening (to determine study eligibility), Baseline (Day 1, initiation of study medication administration), and Week 3 (day 21), Week 6 (day 42), Week 12 (day 84), Week 18 (Day 126), and Week 24 (Day 168). Phone calls were also conducted at Weeks 9, 15, 21 and 28 to administer the ALSFRS-R and assess for treatment compliance, adverse events (AEs), and changes in concomitant medications. For those patients who withdrew from the study early, or those eligible but not enrolling in the Open-Label Extension (OLE) study, a final follow-up telephone interview 28 days (+5 days) after the completion of dosing was conducted to administer the ALSFRS-R and to assess for AEs and changes in concomitant medications 4 weeks after the last dose of study medication. Any change from the above visit window was considered an out of window visit deviation.

For a subset of consenting and qualifying subjects (n=9), a magnetic resonance-positron emission tomography (MR-PET) sub-study was conducted to evaluate the potential treatment-related effects of study medication on TSPO uptake on MR-PET scan.

Dosing Regimen: AMX0035 in a single sachet was administered orally as **1 sachet once daily for the first 3 weeks followed by 1 sachet BID if tolerable after the first 3 weeks for the remainder of the 24-week treatment period**. Subjects were randomized in a 2:1 ratio to receive either AMX0035 or matching placebo.
A bittering agent was added to the placebo too to reduce risk of unblinding due to bitter taste of the drug.
Subjects were instructed to add contents of the sachet into a cup or other container and then add approximately 8 oz. (1 cup) of room temperature water and stir vigorously. Study medication was to be taken within 1 hour of mixing into water. To mask the bitter taste of the medication, subjects could use Listerine pocket strips or spray, have some food or drink milk.

Statistical Reviewer's Comment:
The randomization scheme was to be independently developed and was to indicate the treatment assignment and the subject numbers to be used by each site. The randomization scheme was to be managed by the manufacturer.

Population: Male or Female patients who were ≥18 and <80 years of age with a confirmed diagnosis of definite sporadic or familial ALS.

Key Inclusion Criteria:

- Male or female, aged 18 to 80 years of age
- Definite diagnosis of sporadic or familial ALS as defined by the World Federation of Neurology revised El Escorial criteria (i.e., clinical evidence alone by the presence of upper motor neuron [UMN], as well as lower motor neuron [LMN], signs of neurodegeneration in at least 3 of 4 regions [i.e., brainstem (bulbar cranial motor neurons), cervical, thoracic, and lumbosacral spinal cord (anterior horn motor neurons)] of the central nervous system [CNS])
- ≤ 18 months since first ALS symptom onset
- SVC >60% of predicted value for gender, height, and age at the Screening Visit
- 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 were permitted in the study.
- Adequate contraception requirements for men and women described in the protocol.

MR-PET Substudy Inclusion Criteria:
In addition to the above this subset was also required to have:

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

Clinical Reviewer's Comment:
Since edaravone was not approved at the time of initiation of study, the requirements on use of edaravone were added in a subsequent protocol amendment.

Key Exclusion Criteria:

- Presence of tracheostomy
- Exposure to PB, taurursodiol, or ursodiol within 3 months prior to the Screening visit or planning to use these medications during the course of the study
- History of known allergy to PB or bile salts
- Abnormal liver function defined as aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) >3 times the upper limit of normal
- Renal insufficiency as defined by estimated glomerular filtration rate (eGFR) <60 mL/min/1.73m^2.
- Poorly controlled arterial hypertension (systolic blood pressure [SBP] >160 mmHg or diastolic blood pressure [DBP] >100 mmHg) at the Screening visit
- History of Class III/IV heart failure
- History of cholecystectomy

- 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.
- Severe pancreatic or intestinal disorders that may alter the enterohepatic circulation and absorption of taurursodiol, including biliary infections, pancreatitis, and ileal resection
- Clinically significant unstable medical condition
- Exposure to investigational small molecules with 30 days and monoclonal antibodies with 90 days of screening
- Exposure to disallowed medications

MR-PET Sub-study Exclusion Criteria:
In addition to the above this subset was also required to not have:

- Exposure to immunomodulatory medications within 30 days of the Screening Visit
- Any contraindication to undergo magnetic resonance imaging (MRI) studies

Study Endpoints

Primary

- Rate of decline in ALSFRS-R questionnaire (slope) at Screening, baseline, and every 3 weeks
    - ALSFRS-R has a maximum score of 48 points broken into 4 domains (bulbar, fine motor, gross motor and breathing) comprising of 3 questions each rated on an ordinal scale of 0-4. Higher scores indicate better performance. The domains are given 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 equivalency of phone versus in-person testing, and the equivalency of study subject versus caregiver responses for ALSFRS-R have been established. The ALSFRS-R could therefore also be given to the study subject over the phone. All ALSFRS-R evaluators had to be NEALS certified.

Clinical Reviewer's Comment:
ALSFRS-R, is thought to be a clinically relevant measure of functional change in ALS and was the basis for the approval of edaravone. The analysis approach is discussed later in this review.

Secondary (shown in hierarchical order)

- Rate of decline in isometric muscle strength as measured by Accurate Test of Limb Isometric Strength (ATLIS) at screening, baseline, and every 6 weeks
    - The ATLIS device measures isometric strength using a fixed load cell and a wireless dynamometer with standard positions. 12 muscle groups for the Upper and Lower Extremity include: right and left ankle dorsiflexion, knee extension, grip strength, elbow extension and flexion and knee flexion are averaged for the analysis. Two attempts of each maneuver were performed during every assessment, adding a third attempt if the first 2 differed by more than 15%. The highest score of all trials at a time point were to be used for analysis. Raw values were standardized to percent predicted normal (PPN) strength based on sex, age, weight, and height (See Appendix for Coefficients and intercepts for ATLIS Standardization)[4]
    - For each of the 12 muscle groups, a standardized ATLIS score was calculated by dividing the maximum observed score for each subject and visit combination by the predicted score. If a subject had no motion in a limb and could thus not be tested, the subject's observed score was recorded as 0 (translating to a standardized score of 0 as well). If a subject had motion in a limb but was unable to complete the testing for some other reason, these data were considered missing. A score of 0 divided by the predicted score was still to be zero, so the zeros were to be considered "standardized ATLIS scores."
    - The "Upper Extremity ATLIS" score was obtained by averaging the 6 standardized upper muscle groups (left grip, right grip, left elbow flexion, right elbow flexion, left elbow extension, right elbow extension). The average score was calculated only if at least 4 of the 6 items were observed.
    - The "Lower Extremity ATLIS" score was obtained by averaging the 6 standardized lower muscle groups (left knee extension, right knee extension, left knee flexion, right knee flexion, left ankle dorsiflexion, right ankle dorsiflexion). The average score was calculated only if at least 4 of the 6 items were observed.
    - The "Total ATLIS" score was obtained by averaging the Upper and Lower ATLIS scores; both Upper and Lower ATLIS scores were required to make this calculation.
- Biomarker of neuronal health: Neurofilament heavy chain (pNF-H) plasma levels at baseline and every 6 weeks.

[4] Andres, P. et al. Developing normalized strength scores for neuromuscular research. Muscle and Nerve. 2013

  - Neurofilaments will be used as a potential marker 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
  - (Note: Neurofilament light (NFL) was an exploratory endpoint)
- Respiratory function as measured by slow vital capacity (SVC) decline at screening, baseline, and every 6 weeks
  - The vital capacity (VC) was determined using the upright SVC method
  - Three VC trials were required for each testing session, however up to 5 trials may have been performed if the variability between the highest and second highest VC was 10% or greater for the first 3 trials. Only the 3 best trials were recorded on the eCRF
- Overall rates of survival, defined as:
  - Death: the protocol allowed for monitoring of death after a patient had been terminated from the trial prior to completion of the planned 24 weeks of follow-up
    - Date of death was collected for patients during the course of the trial. In addition, the protocol allowed for monitoring of death after a patient had been terminated from the trial prior to completion of the planned 24 weeks of follow-up, and information on whether a subject had died could 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/*)
  - Tracheostomy (irrespective of reason for tracheostomy whether respiratory distress or control of mucus secretion)
  - Permanent assisted ventilation (PAV) defined as more than 22 hours daily of non-invasive mechanical ventilation for more than 1 week (7 days). The date of onset of PAV was the first day of the 7 days

- Time to hospitalization (e.g., for placement of feeding tube, infusion port for edaravone, or related to a SAE) and the duration of hospitalization for SAEs
- The concentration-response model of PB and taurursodiol at steady-state
- As part of a MR-PET sub-study, TSPO uptake on MR-PET scans was also evaluated at baseline and between Weeks 12 to 21 to assess neuroinflammation. Due to small sample size this data was not to be analyzed but rather presented in a listing.

Statistical Reviewer's Comment:
The SAP does not address experiment-wise control of type I error other than stating that the secondary endpoints are listed in hierarchical order. Although the upper ATLIS reached nominal significance the Total ATLIS was listed first among the secondary endpoints, and it was not significant. Therefore, the Upper ATLIS result is not significant after accounting for multiplicity because the higher priority Total ATLIS failed to achieve statistical significance according to the plan for multiplicity adjustment.

Clinical Reviewer's Comment:
ATLIS is a new device that was specifically designed to alleviate the reproducibility concerns that exist for prior strength measurements such as handheld dynamometry.

The ability of Neurofilament heavy chain (pNF-H) to assess effectiveness of drugs is not established at this time, and the clinical significance of a change in pNF-H is unclear. In general, it may be hypothesized that a therapy that shows benefit in the treatment of ALS would also decrease pNF-H levels, if the drug had an effect on the neurodegeneration of the disease.

SVC is an appropriate outcome measure of respiratory function in patients with ALS.

In the protocol, overall survival was a composite endpoint defined as death, tracheostomy and PAV (as death equivalent), however the survival analysis included separate analyses for death only as well as death and death equivalent. A well-defined assessment of permanent-assisted ventilation is important to include in studies to assist in the interpretation and analysis of survival; however, inclusion of tracheostomy in overall survival would not be acceptable as there is considerable variability in the timing of tracheostomy placement in ALS (e.g., it may be placed for secretion management or in anticipation of future respiratory failure). Similar concerns for subjectivity in clinical practice apply for hospitalizations as well.

Statistical Analysis Plan (Dr. Tristan Massie)

Final SAP Dated 9 October, 2019 (version 1.0 with Amendment 1 was dated 1 August, 2018)
First subject enrolled: 22 June, 2017
Last subject Last Follow-up Visit: 25 September, 2019

Sample Size Consideration:
The Applicant found in the Pooled Resource Open-Access ALS Clinical Trial Database (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. PRO-ACT includes longitudinal data pooled from Phase 2 and 3 ALS clinical trials. 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 followed over 6 months would provide 80% power to detect a 30% treatment effect when tested at a two-sided alpha of 0.1. The Applicant added covariates which may improve the model's ability to fit this population and may have higher power.

Statistical analysis and data tabulation were to be performed using the following subject populations unless specified otherwise:

1. **Safety Population**: The safety population included all subjects who received at least one dose of study medication. Subjects in this population were to be analyzed based on the actual treatment they received.
2. **Modified Intent-to-Treat (mITT) Population**: The mITT population was to 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 were to be analyzed based on the treatment assigned.
3. **Per Protocol (PP) Population**: The Per-Protocol (PP) population included all mITT subjects who took the assigned study medication per the study protocol and did not have any major protocol deviations which excluded them from the PP analysis.

Visit Consideration:
Visit windowing was to 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 visits, were to 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) was to be categorized as a follow-up visit. Follow-up visits were not to be included in efficacy modeling but were to 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 were to be averaged, and a worst-case approach was to 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-ups results available. Summaries of follow-up results were to be interpreted accordingly.

Efficacy Analyses:
All continuous primary, secondary, and exploratory efficacy measures were to use the same statistical model (ALSFRS-R, ATLIS lower, ATLIS upper, ATLIS total, SVC, pNF-H and four individual ALSFRS-R domains) and were to 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) interacting with time were to be included in the analysis. Time was to be a quantitative measure in the primary analysis, with day 0 being the baseline/randomization visit. Time for subsequent visits was to be the number of days since randomization. All post-baseline visits were to be included in the efficacy analysis, even if they were categorized as the same nominal visit. This means that post-baseline unscheduled visits or telephone calls were to be included in the model.

The primary efficacy endpoint is the slope of change in the ALSFRS-R over time. The Applicant reports that 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, they believe it is acceptable that in-office and telephone interview records be included in the

same analysis.[5] Efficacy data from the follow-up telephone interview was not to be included since subjects were off study drug at this assessment. Any pre-treatment record(s) that is (are) not the baseline record was not to be included in the analysis. Efficacy results from the MR-PET Visit 1 were also not to be included in the analyses because these measurements were done at a different facility and rated by different monitors.

The Applicant reports that historical analyses have shown that del-FS is a strong predictor of future progression since ALS has a linear disease progression.[6] 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. 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 were to include del-FS. Additional analyses performed on study subjects showed that decline since symptom onset was a significant predictor not only for ALSFRS-R (del-FS), but also for other efficacy outcomes like ATLIS. For this reason, each model was to include a del- score based on the outcome variable and the same formula was to 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 for the outcome, as for SVC, the observed maximum score across all active and placebo subjects was to be used in the derivation for "Maximum Efficacy Score." Thus, all efficacy models were to 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, in the Applicant's opinion, will not affect 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:

$$Yi,t = \mu + ui + (\beta 0 + bi) \times t + \beta 1 \times zi \times t + \beta 2 \times Agei \times t + \beta 3 \times DelFSi \times t + \beta 4 \times DelYi \times t + \varepsilon i,t$$

[5] Kaufmann P, Levy G, Montes J, et al; QALS study group. Excellent inter-rater, intra-rater, and telephone-administered reliability of the ALSFRS-R in a multicenter clinical trial. Amyotroph Lateral Scler. 2007;8(1):42-6.
[6] Karanevich AG, Statland JM, Gajewski BJ, et al. Using an onset-anchored Bayesian hierarchical model to improve predictions for amyotrophic lateral sclerosis disease progression. BMC Med Res Methodol. 2018;18(1)19. doi: 10.1186/s12874-018-0479-9.

- *i* represents the ith 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
- *Yi,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
- *ui* is the random intercept for each subject and has an unspecified bivariate normal distribution
- *bi* 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
- *DelFSi 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
- *DelYi 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
- $\beta 0$ is the estimated slope for time
- $\beta 1$ is the estimated slope for treatment
- $\beta 2$ is the estimated slope for age at baseline (years)
- $\beta 3$ is the estimated slope for del-FS
- $\beta 4$ is the estimated slope for del-efficacy (corresponding to *Yi,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, $\beta 2$ is the expected increase in the efficacy outcome for a one-year increase in age over 1 week. The Applicant reports that historical and pre-SAP analyses have shown ALS to be a disease with linear progression over time. However, the Applicant acknowledges that 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 was to be modified to include quadratic terms for time. If the quadratic terms for time are insignificant (p-values >0.10) then linearity was to be assumed, and the linear primary model was to be used for analysis. If at least one of the interaction terms was significant (p-value<0.10), then the quadratic version of the primary model was to be used for the analysis. P-values for the quadratic terms in the model were to be presented. If at least one of the quadratic terms was significant then the summary statistics described in the subsequent paragraph were to be presented and the same statistics were to be presented for the linear primary model.

The difference in active treatment and placebo slope was to 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 were to 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 were also to 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 were to 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 were all to be reported and to accompany the estimates from the MMRM outlined in this section.

Statistical Reviewer's Comment:
The final SAP does not provide the details for the quadratic model. A "Note to File" document clarifying the terms in the quadratic model was made on 21 November 2019 (the date of unblinding was reportedly 26 November 2019).

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.

21 November 2019 Clarification Note
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.

Survival Analyses

Survival analyses were to 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) were to be included in this analysis. Events which occurred after 28 weeks were to be addressed in the extension analysis. In addition, a combined survival analysis was to be performed where any one of the 3 events was to be considered a failure, and the time for the first occurrence of any of the 3 events was to be analyzed.

This combined survival analysis was to 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) were to be included in the analysis.

Statistical Reviewer's Comment:
The Applicant applied a cutoff for death events of exactly 24 weeks after baseline in their reported results.

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:

$h(t) = ho\ (t) \times \exp\ (b1x1 + b2x2 + b3x3)$

- t represents the survival time
- h(t) is the hazard function determined by a set of covariates (x1, x2 and x3);
- the coefficients (b1, b2 and b3) measure the impact (i.e., the effect size) of the covariates
- x1 represents treatment (active or placebo)
- x2 represents del-FS
- x3 represents age at baseline
- the term ho(t) is called the baseline hazard. It corresponds to the value of the hazard function
- if all x values are equal to zero.

In order to be conservative, the likelihood-ratio test was selected as the primary test statistic to determine whether or not the treatment had a significant impact on the hazard.

<u>Interaction with Important Concomitant Medications</u>

Two medications of interest could be taken during the clinical trial: edaravone (Radicava) and riluzole. Efficacy outcomes were to be analyzed by comparing efficacy scores over time between treatment groups while accounting for time on concomitant medications of interest. The main efficacy model was to be used and a term to account for time on concomitant medication were to 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 a "hockey stick" trajectory where the subjects' ALSFRS 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 was to 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

was to 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. The estimated slope and p-value for the interaction involving time on concomitant medication and treatment over time were to 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 was to be concluded that there is a significant interaction between time on concomitant medication and treatment over time.

Sensitivity Analyses for Missing at Random (MAR) Assumption

A MMRM, using multiple imputation from the control arm to complete assessments missing after discontinuation of study drug was to 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 the Applicant believes it provides a lower bound on efficacy, again under the MAR assumption that time of stopping study medication depends only on past history and covariates.

Statistical Reviewer's Comment:

Since deaths are ignored in the above sensitivity analysis, it does not necessarily provide the asserted lower bound on efficacy.

Left Censoring for Intercurrent Event of Death and Death Equivalent Events

The primary analysis, linear or quadratic depending on the results, was to 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 or death equivalent events, were to be assumed 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 were to be carried out using PROC NLMIXED. The starting values for the fixed variables were to be the point estimates from the primary analysis. All variance parameters were to 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

$$\frac{1}{\sqrt{2\pi\sigma_e^2}} \exp\left(-\frac{(Y_{i,t} - \theta_{i,t})^2}{2\sigma_e^2}\right)$$

And the likelihood for censored outcomes will be

$$\Phi\left(\frac{Y_{i,t}-\theta_{i,t}}{\sigma_e}\right)$$

Statistical Reviewer's Comments:

The following comment was provided to the Applicant on the January 25, 2019, SAP before unblinding: "You need to specify the estimand and how to handle intercurrent events such as death or starting other treatments which if ignored could bias the primary linear mixed model. If there are deaths, then the joint rank analysis of function and survival should be the primary analysis. You need to prespecify how to objectively assess linearity and specify a backup analysis in case linearity does not hold. You need to specify sensitivity analyses for missing data. Your definition of modified intent-to-treat population, that you designated as primary, seems to include patients who are treated but have no post-baseline efficacy assessments. These patients are normally excluded under the assumption that there will be few of them. Please clarify how randomized patients with no post-baseline efficacy assessments will be handled in the primary analysis."

Clinical Reviewer's Comment:
The primary analysis did not use a combined analysis of the ALSFRS-R and survival, such as the joint rank. There were seven deaths in the study, which is not insignificant, and which make a joint rank analysis critical to understand the study results, as the occurrence of death creates functional data that are missing not at random, or the anomalous result that death appears to be a better outcome than a lower ALSFRS-R score. Missing data as a result of death are not missing at random, and therefore, are not appropriate to impute data after death. The Applicant was informed of the need to use a joint rank analysis in a PIND meeting in 2016, before the conduct of the CENTAUR study. This was also communicated in email advice on the SAP in 2019. Joint rank-analyses were later conducted as a post-hoc analysis.

The analyses of secondary endpoints also followed the primary slope model and therefore have the same concerns as the primary analysis, i.e., a combined analysis of function and survival should be applicable to these endpoints too.

Key Post-hoc Analyses:

- The first post-hoc sensitivity model was a post hoc joint rank model in the efficacy (mITT) population that incorporated all survival events into the analysis of function (ALSFRS-R), providing adjusted estimates that accounted for potential bias due to subject death. The model ranked subjects by time to death (deaths prior to Day 168) and then by change in ALSFRS-R total score. This ranked score was then analyzed as the

outcome of an analysis of covariance model that included the same covariates as the primary model but replaced the covariates with ranked covariates.

- The primary analysis for all continuous outcomes was a random-slope, linear mixed model (adjusted for age and pre-baseline ALSFRS-R slope) that assumed a shared baseline between active and placebo groups. A change-from-baseline analysis that did not make this shared baseline assumption, but did assume linearity over time, was performed post hoc for all continuous outcomes in the mITT population.

Protocol Amendments

The protocol was amended 7 times. The following change could impact the efficacy results if an imbalance is observed between treatment arms that would favor the treatment group:

- In version 3.0, concomitant administration of edaravone was allowed following its approval in the US.

Other protocol changes would not impact efficacy results.

6.1.2. Study Results

Compliance with Good Clinical Practices

The study was conducted in compliance with the International Council for Harmonization (ICH) Guideline on Good Clinical Practice (GCP) and Title 21 Part 56 of the United States (US) Code of Federal Regulations (CFR) relating to Institutional Review Boards (IRBs) and the principles of the 18th World Medical Assembly (Helsinki 1964) World Medical Assemblies, Declaration of Helsinki.

Financial Disclosure (See Appendix)

Patient Disposition

A total of 177 subjects with ALS were screened for study participation, and of these, 137 were enrolled, randomized, and received treatment with study medication (AMX0035 [89 subjects] or placebo [48 subjects]).

Of the 137 subjects in the Safety Population, **105 (76.6%) completed the 24-week double-blind study**. Ninety-seven of the 105 subjects completed the study on drug, and 8 subjects (7 on drug and 1 one on placebo) completed the 24-week double-blind period, but discontinued study medication early. The remaining 32 (23.4%) subjects prematurely discontinued from the study. The discontinuations were balanced across treatment groups with 10 (20.8%) and 22 (24.7%) in the placebo and AMX0035 group, respectively (p=0.67).

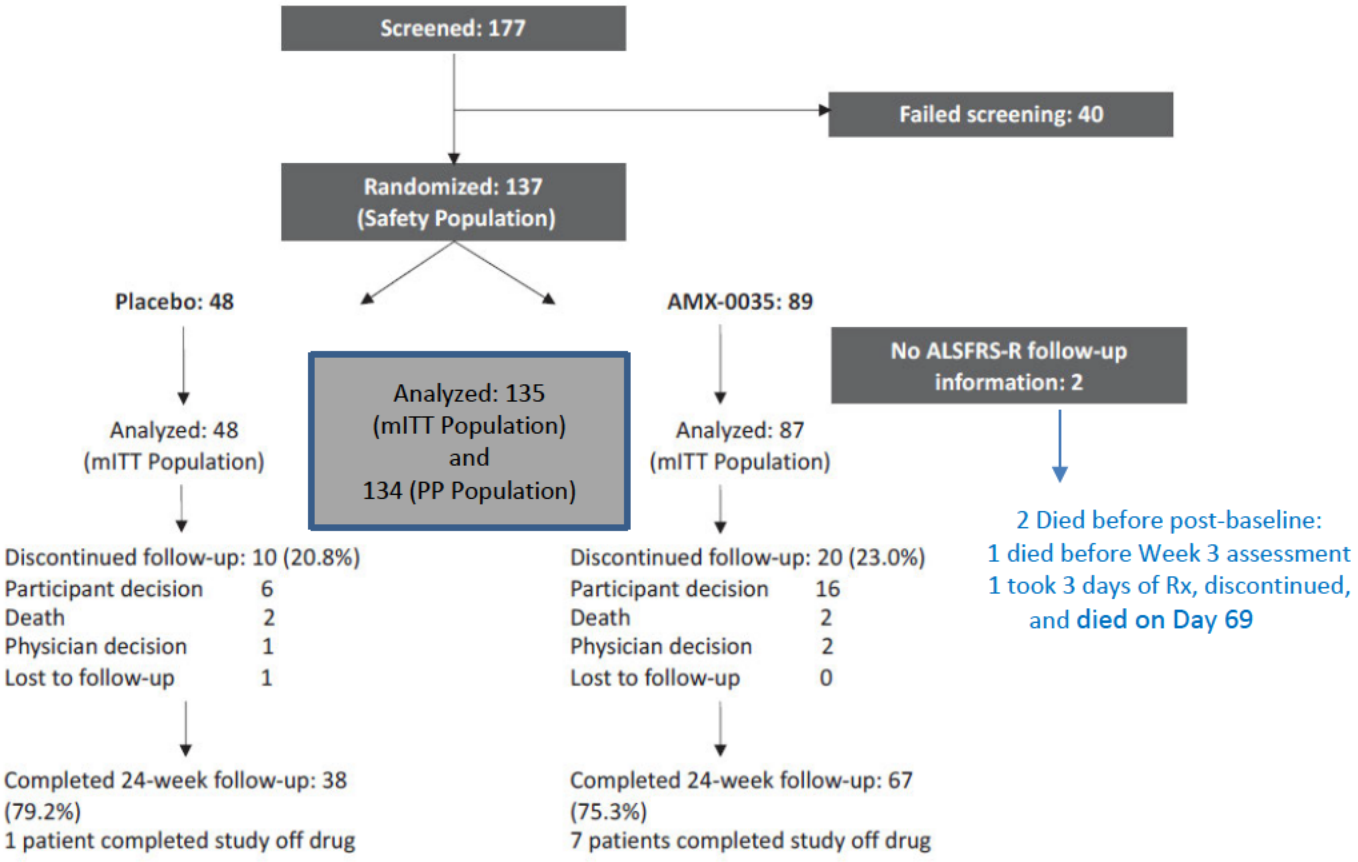
The overall subject disposition in the mITT population is given in Table 3. This Table excludes the 2 out of 137 subjects that did not have a post-baseline efficacy assessment (both of whom received drug and discontinued early).

Table 3: Overall Subject Disposition of mITT Population

	Placebo+SOC (N=48) N (%)	AMX0035+SOC (N=87) N (%)	Overall (N=135) N (%)
Total number discontinued	10 (20.8)	20 (23.0)	30 (22.2)
Total number completed 24 weeks	38 (79.2)	67 (77.0)	105 (77.8)
Completed on study medication	37 (77.1)	60 (69.0)	97 (71.9)
Discontinued medication prior to completion	1 (2.1)	7 (8.0)	8 (5.9)

The flow chart on subject disposition is given in Figure 1:

Figure 1 Subject Disposition



Source: CSR N216660

Table 4 Reasons for Discontinuation (mITT Population)

	Placebo+SOC (N=48) N (%)	AMX0035+SOC (N=87) N (%)	Overall (N=135) N (%)
Total Discontinuations	10 (28.8)	20 (23.0)	30 (22.2)
Patient Decision	**6 (12.5)**	**16 (18.4)**	**22 (16.3)**
Adverse event	3 (6.3)	10 (11.5)	13 (9.6)
Disease Progression	2 (4.2)	5 (5.7)	7 (5.2)
Termination of Participation	1(2.1)	1 (1.1)	2 (1.5)
Death*	**2 (4.2)**	**2 (2.3)**	**4 (3.0)**
Died	1 (2.1)	2 (2.3)	3 (2.2)
Death Equivalent	1 (2.1)	0	1 (0.7)
Physician Decision	**1 (2.1)**	**2 (2.3)**	**3 (2.2)**
Termination of Participation by Investigator	1 (2.1)	2 (2.3)	3 (2.2)
Lost to follow-up	**1 (2.1)**	**0**	**1 (2.1)**

*Note: Death equivalent is defined as requiring tracheostomy or permanent assisted ventilation. Some deaths were recorded after patient withdrawal from trial and are not accounted in reason for discontinuation.
There were 7 deaths during the 24-week study period (5 on AMX0035 and 2 on placebo). 3 of the 7 patients discontinued study participation prior to the death, therefore death is not listed in the reason for discontinuation only 4 are listed in the Table above. A total of 2 discontinued for disease progression (AMX0035 and Placebo) and 1 for AE (AMX0035).

Table 5 provides details of subjects that died. In addition, Subject (b) (6) met death equivalent defined as requiring tracheostomy or PAV.

Table 5 Details of Subjects that Died During Study AMX3500

Subject ID	Treatment	Study Discontinuation By subject	Withdrawal day	Death day
AMX3500-(b) (6)	AMX0035	Disease progression	77	86
AMX3500-	AMX0035			145
AMX3500-	AMX0035			153
AMX3500- *	AMX0035	Adverse Event	33	69
AMX3500- *	AMX0035			22
AMX3500-	Placebo	Disease progression	27	29
AMX3500-	Placebo			152

5 of these patients were on either Riluzole or edavorone
*not included in mITT population because of no post-baseline efficacy assessment

In addition to these 7 subjects that died with the 24-week study period, 8 other subjects died after the study duration of 24 weeks. These 8 subjects had discontinued from the study and died later without enrolling in the extension study (these subjects were on AMX0035 from 0-18 weeks).

Statistical Reviewer's Comment:
Amongst the non-completers that died there were 2 subjects on placebo and 3 subjects on AMX0035 in the mITT population (in the ITT population there were 2 additional AMX0035 deaths).
*There were **missing 24-week ALSFRS-R data**:*

- *In the placebo group: 46/48 survived the double-blind phase: Of these 8/46 (**17.4%**) have missing Week 24 ALSFRS-data (38 non-missing)*
- *In the AMX0035 group: 84/89 survived the double-blind phase: Of these 15/84 (**17.9**%) have missing Week 24 ALSFRS-data (69 non-missing)*

*In addition, there was a **problem that happened with the implementation of the randomization** early in the trial. Apparently, the first 17 patients were all assigned to drug and the next 9 were then assigned to placebo (reportedly due to drug production/ shipping problems amounting to unavailability of required double-blind placebo detailed in [\\CDSESUB1\evsprod\nda216660\0001\m5\53-clin-stud-rep\535-rep-effic-safety-stud\als\5351-stud-rep-contr\amx3500\randomization-scheme-codes.pdf](\\CDSESUB1\evsprod\nda216660\0001\m5\53-clin-stud-rep\535-rep-effic-safety-stud\als\5351-stud-rep-contr\amx3500\randomization-scheme-codes.pdf)*

(b) (4), *the **unblinded study statistician who was on the DSMB,** detected this issue during preparation for the **November 7, 2017 DSMB** meeting and proceeded to work with* (b) (4), *the drug distributor for the study, to understand why this had happened. Upon detecting this,* (b) (4), *consulted with* (b) (4) *and decided to move a group of placebo kit numbers forward to restore the intended 2:1 allocation ratio of active to placebo kits and thereby balance the two groups in the early epoch of the study. Moving placebo kits forward resulted in gaps nearer to the end of the pick list and could have resulted in an issue in which placebo kits would not be available to ship if not corrected. As such, **in February of 2019**,* (b) (4), *in collaboration with* (b) (4) *and* (b) (4), *made **another** slight revision to the pick list. This change was implemented in advance of any (further) shipping issue occurring.*

The implications of this randomization error are discussed under the ALSFRS-R results section of this review.

Clinical Reviewer's comment:
On January 17, 2020, (b) (4) *brought to the attention of the team that he had made this shipping correction. An erratum was issued on **26 January 2020**, stating that upon review, 18 patients received AMX0035 and the number 17 in the report should be corrected to 18, for a total of 27 deviations (26 mITT).*

The overall summary of drug exposure is summarized in Table 6.

Table 6 Summary of Drug Exposure (mITT population)

Parameter	Controlled Phase (N=135)		
	Placebo (N=48)	AMX0035 (N=87)	Overall (N=135)
Duration of Exposure (Weeks)			
Mean (SD)	21.5 (5.8)	19.7 (7.9)	20.6 (7.02)
Median	23.9	23.9	23.9
Min, Max	1.0, 25.9	0.6, 31.6	0.6, 31.6
Drug Compliance (%)			
Mean (SD)	90.2 (15.7)	90.9 (17.3)	90.7 (16.7)
Median	95.6	96.1	96
Min, Max	7.7, 100.3	16.7, 166.7	7.7, 166.7
Duration of exposure (categories) n(%)			
0 to ≤3weeks	1 (2.1)	6 (6.9)	7 (5.2)
>3 to ≤12 weeks	4 (8.3)	11 (12.6)	16 (11.8)
>12 to ≤18 weeks	3 (6.2)	4 (4.6)	7 (5.2)
>18 to ≤21 weeks	2 (4.2)	5 (5.7)	7 (5.2)
>21 to ≤ 24 weeks	17 (35.4)	22 (25.3)	39 (28.9)
>24 to ≤27 weeks	21 (43.8)	36 (41.4)	57 (42.2)
>27 to ≤33 weeks	0	3 (3.4)	3 (2.2)
Number of Subjects (n [%])			
Increased Dose to 2 Sachets	45 (93.8)	79 (90.8)	124 (91.9)
Did Not Increase Dose to 2 Sachets	3 (6.3)	8 (9.2)	11 (8.1)

Protocol Violations/Deviations

The percentage of subjects with protocol deviations was similar between treatment groups:

- 82 (92.1%) of subjects in the AMX0035-treatment group had a total of 279 documented deviations
- 43 (89.6%) subjects in the placebo group had a total of 138 documented deviations

The majority of protocol deviations were classified as minor.

Improper documentation of Informed Consent Form (ICF), visit deviation and Study drug not being dispensed per protocol, including dosing compliance were the major deviations. A list of major protocol deviations other than ICF are summarized below:

Table 7 Major Protocol Deviations

Deviation Category	Placebo+SOC (N=48) N (ID)	AMX0035+SOC (N=87) N (ID)
Study Drug Dispense	3	6
Dosing Compliance	9	11
Eligibility Criteria (details below)	1	3
Had a systolic blood pressure of 175 mmHg at the Screening visit; systolic blood pressure was required to be ≤160 mmHg.	1 (# (b) (6))	-
Did not meet inclusion criteria #3 (Is the subject's date of ALS symptom onset <18 months prior to Screening Visit)		1 (# (b) (6))
Did not have clinical signs of UMN and LMN degeneration in at least 3 of 4 regions (i.e., brainstem, cervical, thoracic, and lumbosacral spinal cord), however clinical signs of UMN and LMN degeneration were found in bulbar, cervical or lumbar regions and other symptoms were consistent with a diagnosis of definite ALS. This subject however is classified as probable ALS according to correct interpretation of the revised El Escorial criteria.		1 (# (b) (6) 1 (#

ID=Subject ID

Clinical Reviewer's Comment:
In addition to the major deviations noted above, the Applicant notes 49 deviations that were considered minor and were not recorded in the EDC system. These deviations were related to study drug not dispensed per protocol and dosing compliance related. These mainly occurred when compliance was <80%. Subject stopped taking the drug due to gastrointestinal adverse events, bad taste of the drug, threw the empty sachets or did not return unused drug, so compliance could not be accounted for. A total of 38 subjects were affected by dosing compliance or dispensing error in the study. The impact of these deviations during certain periods of the study is unclear as the missing drug accountability data is not clear.

Table of Demographic Characteristics

There were no imbalances in terms of age at enrollment, gender, and race between the treatment arms. There were greater number of subjects that were ≥ 65 years of age in the AMX0035 arm compared to the placebo arm (28.1 vs 14.5%) as shown in Table 8.

Table 8 Demographic Characteristics (ITT)

Demographic Parameters	Treatment Group			
	Placebo+SOC (N=48) n (%)	AMX0035+SOC (N=89) n (%)	Total (N=137) n (%)	P-value vs placebo
Sex				
Male	32 (66.7)	61 (68.5)	93 (67.9)	0.823
Female	16 (33.3)	28 (31.5)	44 (32.1)	
Age				
Mean years (SD)	57.3 (7.6)	57.9 (10.6)	57.7 (9.6)	0.715
Median (years)	57.5	60	59	
Min, max (years)	36, 79	31, 79	31, 79	
Age Group				
< 65 years	41 (85.5)	64 (71.9)	105 (76.6)	0.075
≥ 65 years	7 (14.5)	25 (28.1)	32 (23.4)	
≥ 75 years	1 (2.1)	3 (3.4)	4 (2.9)	
Race				
White	46 (95.8)	84 (94.4)	130 (94.9)	0.907
Black or African American	1 (2.1)	2 (2.2)	3 (2.2)	
Asian	1 (2.1)	2 (2.2)	3 (2.2)	
Other	0	1 (1.1)	1 (0.7)	
Ethnicity				
Hispanic or Latino	1 (2.1)	6 (6.7)	7 (5.1)	
Not Hispanic or Latino	47 (97.9)	83 (93.3)	130 (94.9)	
Region				
United States	48 (100)	89 (100)	137 (100)	

Other Baseline Characteristics (e.g., disease characteristics, important concomitant drugs) (ITT population)

There were 10% more subjects in the placebo group compared to the AMX0035 group had limb-onset ALS, and 10% more in the AMX0035 group with brain onset ALS, suggesting more severe subjects in the AMX0035 group. A total of 9% more subjects in the AMX0035 group did not have a family history of ALS, and about 5% more in the placebo group that did have a family history of ALS. Without information on the specific genetic mutations causing the familial ALS, the impact of this difference is unclear. The baseline total ATLIS score was better by 3 points in favor of AMX0035 group. There were imbalances in concomitant riluzole and edavarone use, but this was greater in the placebo arm at baseline, and not in favor of the AMX0035 group (Table 9).

Table 9 Other Baseline Characteristics (ITT Population)

Baseline Disease Parameters	Treatment Group		
	Placebo+SOC (N=48)	AMX0035+SOC (N=89)	Total (N=137)
Time Since onset of ALS Symptoms (Months)			
Mean (SD)	13.6 (3.6)	13.5 (3.8)	13.5 (3.8)
Median	13.3	13.9	13.7
Time Since onset of ALS Diagnosis (Months)			
Mean (SD)	6.3 (3.2)	5.9 (3.3)	6 (3.2)
Median	6	5.3	5.7
ALS Onset Location n (%)			
Brain Stem	10 (20.8%)	26 (29.2%)	36 (26.3%)
Limb	38 (79.2%)	61 (68.5%)	99 (72.3%)
Respiratory System	0	1 (1.1)	1 (0.7)
Multiple	0	1 (1.1)	1 (0.7)
Family History of ALS n (%)			
Yes	7 (14.6%)	9 (10.1%)	16 (11.7%)
No	38 (79.2%)	78 (87.6%)	116(84.7%)
Unknown	3 (6.3%)	2 (2.2%)	5 (3.6%)
DEL-FS			
Mean (SD)	0.93 (0.60)	0.96 (0.42)	0.94 (0.49)
Median	0.76	0.88	0.85
ALSFRS-R			
Mean (SD)	36.7 (5.1)	35.7 (5.8)	36 (5.5)
ATLIS			
Mean (SD)	53.9 (20.9)	56.8 (20.0)	55.78 (20.4)
SVC%p			
Mean (SD)	83.9 (15.9)	82.7 (19)	83.3 (17.2)
Use of Riluzole or Edaravone			
Yes	42 (87.5%)	64 (71.9%)	106 (77.3%)
Use of Riluzole			
Yes	37 (77.1%)	60 (67.4%)	97 (71%)
Use of Edavarone			
Yes	24 (50%)	23 (25.8%)	47 (34.3%)

Statistical Reviewer's Comment:
A sensitivity analysis suggested that the randomization error described earlier doesn't seem to have affected the balance in an obvious way, except it cannot be ruled out that the unblinded statistician's altering the randomization may have had an impact for balance in use of edaravone and riluzole (it was 82 vs. 80% for the placebo and AMX0035 group, respectively, for the preplanned randomization schedule, and 88% vs. 72% for the placebo and AMX0035 group,

respectively, for the assigned treatments).

Clinical reviewer's Comment:
I explored if any of the disease baseline characteristics and its overall range in treatment groups could bias the efficacy outcome in any way in both the placebo-controlled and the extension phase of the study. Looking into the prognostic factors amongst the baseline characteristics of subjects, it appeared that family history of ALS and very high Del-FS at baseline were likely to be more predictive of disease progression than baseline ALSFRS-R score or ALS onset location in the open-label extension study.

Treatment Compliance, Concomitant Medications, and Rescue Medication Use

Treatment Compliance
Treatment compliance was monitored by counting returned and unused sachets during study visits (Weeks 6, 12, 18, and 24). Non-compliance by subjects was defined as taking less than 80% or more than 125% of the planned doses of study medication as determined by sachet counts. A total of 19 (13.9%) subjects received <80% of planned doses overall (12 [13.5%] receiving AMX0035 and 7 [14.6%] receiving placebo). There was no difference in compliance between treatment groups.

A total of 136 of the 137 enrolled subjects began the study on the dose of 1 sachet of study medication (i.e., AMX0035 or placebo); one subject (Subject (b) (6) in the AMX0035 group) was initiated on 2 sachets in error. This was reported as a major protocol deviation. A total of 79 of the 89 (88.8%) in the AMX0035 group and 45 of the 48 (93.8%) in the placebo group of enrolled subjects received 2 sachets after 3 weeks of the study.

Clinical Reviewer's Comment:
A few subjects had not returned their unused sachets in certain periods during the study, so accurate estimate of compliance is not clear. It is unclear if these were accounted for in the DA.xpt dataset (drug accountability dataset).

Concomitant Medications
Antacids containing aluminum hydroxide or smectite (aluminum oxide) were prohibited within 2 hours of study drug administration as they inhibit absorption of taurursodiol.

In addition, the following medications were prohibited:

- HDAC inhibitors, including valproate, vorinostat (Zolinza), romidepsin, chidamide, panobinostat, lithium, butyrate, and suramin
- Probenecid
- Bile acid sequestrants, including cholestyramine and cholestyramine light; Questran and Questran Light; Welchol; colestid and colestid flavored; or Prevalite

Concomitant medications administered in ≥15% of subjects overall include: riluzole and edavarone (ALS), dextromethorphan (cough), quinidine (antiarrhythmic), paracetamol (pain), Vitamin D, Coenzyme Q10 (antioxidant), baclofen (muscle relaxant), ibuprofen and acetylsalicylic acid (pain and inflammation), Vitamin B12.

Clinical Reviewer's Comment:
Table 10 shows that there are imbalances at baseline in the standard of care, however these imbalances are not in favor of AMX0035 arm, as more patients on placebo were receiving riluzole or edaravone at baseline. The protocol required stable does of riluzole in the study but edavorone could be started, dosing changed, or stopped during the trial. There was an imbalance in more subjects in the AMX0035 arm starting edaravone after initiating the study (4% in placebo vs 12% in AMX0035). In addition, 3.4% of subjects started riluzole after study entry in the AMX0035 arm. ***Overall, 14 (16%) subjects in the AMX0035 group started ALS medication after entry into the study vs only 2 (4%) in the placebo group.*** *This may lead to bias in the comparison.*

Table 10 Standard of Care Prior to and Concomitant to the Study

Standard of Care	Placebo (n=48) N (%)	AMX0035 (N=89) N (%)	Overall (137) N (%)
Use of Riluzole or Edavarone prior to entry			
Riluzole	37 (77%)	60 (67%)	97 (71%)
Edavorone	24 (50%)	23 (26%)	47 (34%)
Use of Riluzole or Edavarone after entry into the study			
Riluzole	0 (0%)	3 (3.4%)	3 (2.2%)
Edavorone	2 (4%)	11 (12%)	13 (9.5%)
Use of Riluzole or Edavarone concomitantly			
Riluzole	37 (77%)	63 (71%)	100 (73%)
Edavorone	26 (54%)	34 (38%)	60 (44%)

Statistical Reviewer's Comment:
Post-baseline starting of edaravone or riluzole is slightly imbalanced for the revised randomization groups (i.e., using as-treated for the first 27 subjects) and also less imbalanced if the preplanned randomization schedule is imputed (16% vs. 4% as compared to 13% vs. 11%).

Efficacy Results – Primary Endpoint

Data
\\CDSESUB1\evsprod\NDA216660\0001\m5\datasets\amx3500\analysis\adam\datasets\adqs.xpt;

The primary efficacy endpoint for the study was the rate (slope) of decline in capability and independence in 12 functional activities relevant to ALS as measured by total ALSFRS-R questionnaire scores assessed using a shared-baseline, mixed-effects model in the mITT population (excluding 2 drug deaths who had no post-baseline ALSFRS-R's but were treated).

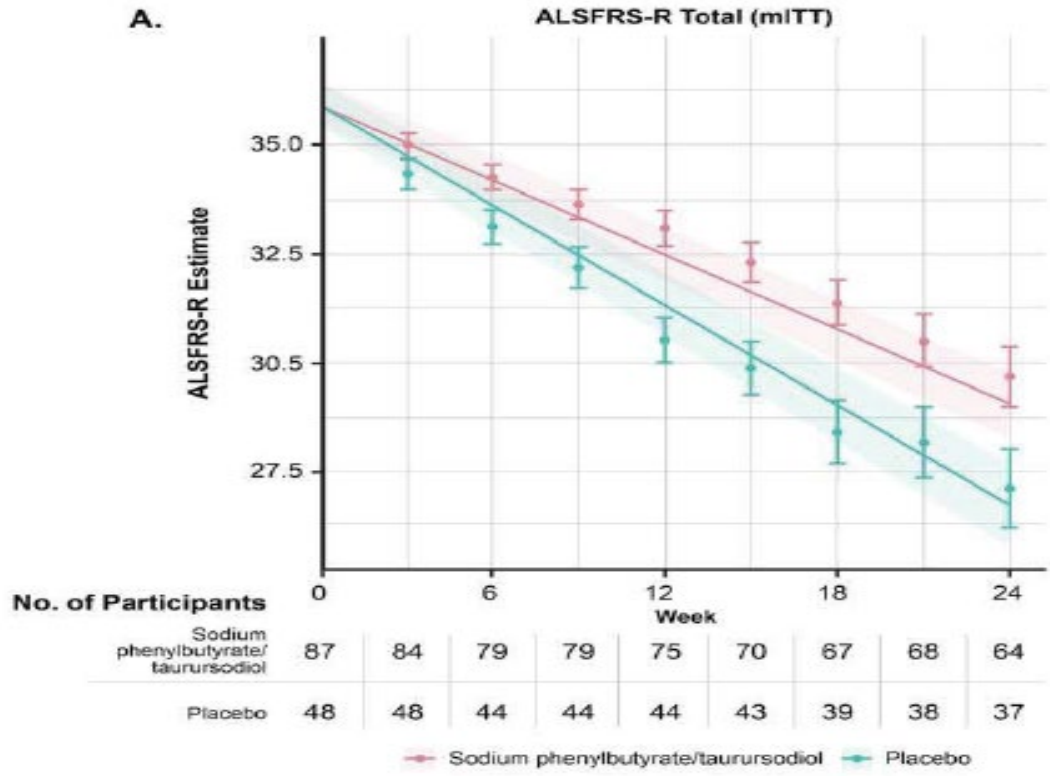
AMX0035 met the prospectively defined primary endpoint and demonstrated a statistically significant slowing of disease progression as measured by the slope of the ALSFRS-R total score compared to placebo **(p=0.0340)** based on this model. The AMX0035 treatment group had an estimated LS mean ALSFRS-R Total score (LS mean=29.06) that was 2.32 points higher at Week 24 compared to the placebo group (LS mean=26.73). The **Applicant estimated a treatment group difference at Week 24 of 2.32 (S.E.=1.09) points** on ALSFRS-R based on their prespecified mixed effects shared baseline slope model (Table 11 and Figure 2).

Table 11 ALSFRS-R Total Score at Week 24: mITT Population (N=135): Applicant's Analysis

Endpoint Time Point	Estimate (SE)		Difference			
	Placebo (N=48)	AMX0035 (N=87)	Week 24 Difference (SE)	95% CI	p-value	Weeks Function Retained (%)
ALSFRS-R Total						
Week 24	26.73 (0.975)	29.06 (0.781)	2.32 (1.094)	0.18, 4.47	0.0340	6.1 (33.9%)

Source: Study AMX3500 CSR page 88

Figure 2 Estimated Rate of Decline in ALSFRS-R Total Score Over 24 Weeks (Applicant's Primary Analysis)



Source: Study AMX3500 CSR Page 89

Statistical Reviewer's Analyses and Comments on Applicant's Primary Analyses:

Concerns on linearity assumption:

The Applicant's primary analysis assumes linearity of ALSFRS-R over time. The linearity assumption forces the treatment difference to grow over time, which could be unrealistic, and in fact, conflicts with the reviewer's mean-per-visit MMRM estimates in this respect (see below). The Applicant's November 21, 2019, document clarifying the quadratic model to check the primary model's assumption of linearity of ALSFRS-R over time indicates that a random effect for the quadratic term would be included and that the quadratic model would be used if any of the quadratic terms were significant at 0.10, but the Applicant did not follow this plan in the report. Applicant Table 14.2.2.3 (page 410 of Section 14 tables and figures (Table 12)) clearly shows the p-value for the quadratic weeks *Del-FS total term p=0.0001, but this is for a post-hoc quadratic model substituted by the Applicant in place of the prespecified quadratic model. The clinical study report states no significant quadratic effects were observed. There were, in fact, no significant quadratic effects for the prespecified quadratic model (note that this same term is very close to the p=0.10 cutoff with p=0.1016). Although the Applicant's

substitution of a post-hoc quadratic model in the report may be a source of confusion, the bottom line is that the Applicant's prespecified quadratic model and significance level for deciding between quadratic and linear models supports the linear model.

Table 12 Applicant's Quadratic Efficacy Analysis of ALSFRS-R

Effect/Time Point	Estimate (SE)	95% CI	p-value
Intercept	35.9455 (0.49336)	34.9698, 36.9213	<0.0001
Quadratic Weeks	0.0005 (0.00095)	-0.0013, 0.0024	0.5765
Quadratic Weeks*Age	-0.0001 (0.00010)	-0.0003, 0.0001	0.4041
Quadratic Weeks*Del-FS Total	0.0076 (0.00195)	0.0037, 0.0114	0.0001
Weeks	-0.3448 (0.03168)	-0.4070, -0.2826	<0.0001
Weeks*Age	0.0007 (0.00327)	-0.0057, 0.0071	0.8314
Weeks*Del-FS Total	-0.4272 (0.06400)	-0.5528, -0.3016	<0.0001
Weeks*Treatment	0.0950 (0.04580)	0.0051, 0.1849	0.0385

Source: Table 14.2.2.3 CSR page 410

Time Point	Estimate (SE)		Difference			
	Placebo+SOC (N=48)	AMX0035+SOC (N=87)	Estimate (SE)	95% CI	p-value	Weeks Function
ALSFRS-R Total						
Week 24	26.83 (0.977)	29.11 (0.780)	2.28 (1.099)	0.12, 4.44	0.0385	6.6 (37.8%)

Source: Study AMX3500 CSR Page 94

The **Applicant reported a difference of 2.28 (S.E.=1.10**) based on this quadratic model for which the quadratic terms are not allowed to vary by treatment group, **p=0.0385**. The model corresponding to that Applicant table has no random effect for the quadratic term, which conflicts with the inclusion of such a term in the model specification in the prespecified "note to file" clarifying the details of the quadratic model. Neither of these two quadratic models allows the quadratic effect to vary by treatment group.

The quadratic model implemented as in the Applicant's analysis statistical analysis plan document (Nov 2019 analysis plan note/addendum clarifying the quadratic model) produces an estimated **Week 24 treatment difference of 1.68 (S.E.=1.06, p=0.1134).**

With the addition of a random effect for quadratic term, and a difference between treatments in the quadratic term, this reviewer found that the quadratic alternative model estimates a **Week 24 difference of 1.97 (S.E.=1.06, p=0.0648)** which is considerably smaller than the linear model, and the quadratic interaction with treatment term is nominally significant (p=0.0060), suggesting it should be included. Note that the prespecified quadratic model assumed that the quadratic weeks effect was the same for both treatment groups, but the p-value for this varying by treatment arm is 0.0052, hence this analysis adds the treatment*quadratic weeks interaction term. Note that although this quadratic model implies possibly different curvature between groups, unequal curvature is also possible with the traditional mean-per-visit MMRM,

since the visit means are allowed to vary freely by treatment group. The quadratic analyses are summarized in Table 13.

Table 13 Quadratic Model Analyses

Quadratic Model Details		Week 24 LS Mean Difference	Std Error	p-value
w/out quadratic weeks random effect (Applicant)	and no treatment * quadratic weeks interaction	2.28	1.10	0.0385
w/quadratic weeks random effect (SAP specified)	but no treatment * quadratic weeks interaction (SAP specified)	1.68	1.06	0.1134
w/quadratic weeks random effect	and treatment * quadratic weeks interaction	1.97	1.06	0.0644

Source: Statistical Reviewer's Analyses

Concerns on "shared baseline":
The primary analysis model also uses a "shared baseline" in the model. The "shared baseline" term is not a common term but seems to mean that the baseline assessment is modeled as part of the dependent variable data in the primary analysis, and it assumes no treatment group difference at time=0. Randomization should support this lack of difference at time 0, but there are 4 patients with negative times for the baseline visit, and in such cases the model would imply a treatment difference prior to time 0, which does not make sense. Although randomization typically produces balance in variables at baseline across groups, the baseline ALSFRS-R was 1 point higher for placebo than for AMX (not a significant difference but a numerical difference).

As shown in row 2 of Table 13, the primary result does not seem robust to the exclusion of baseline data as a (repeated) assessment of the dependent variable for the analysis. In particular, when this statistical reviewer **excluded baseline data** as a repeated measure (at time 0) of the primary efficacy measure in the analysis, a **p-value of 0.0622** was obtained for the estimated slope difference.

Table 14 Impact of Including Baseline Visit as One of Repeated Measures (Visits) in Model of Primary Efficacy Dependent Variable

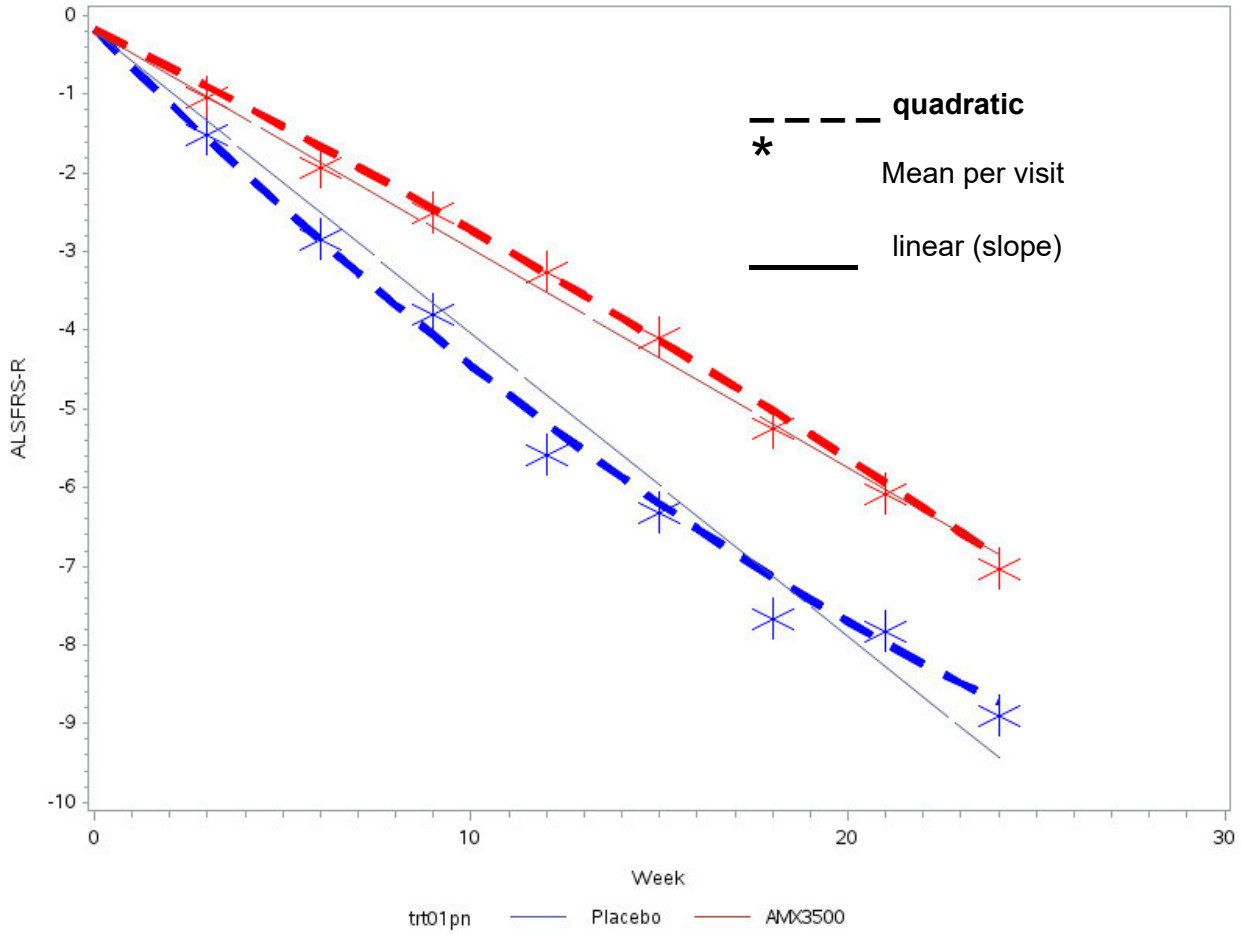
N Analysis patients*	N Analysis records	Est Slope Diff (Drug-Placebo)	Std. Error of Slope	Week 24 Diff	p-value
135 (Applicant)	1075 (including baseline)	0.097	0.046	2.32 (1.09)	0.0340
135 (Reviewer)	940 (Excluding baseline)	0.089	0.047	2.13 (1.14)	0.0622

*Applicant's analysis Excludes 2 AMX3500 deaths classified as non-mITT population
Source: Statistical Reviewer Analysis

Concerns on primary model data fit:
Furthermore, the traditional separate mean by visit MMRM analysis, as well as quadratic model (with random quadratic weeks effect as prespecified, but also allowing quadratic weeks to vary by treatment group) for ALSFRS-R over time, suggest that the primary linear model is also biased at Week 24, with the slope model suggesting greater benefit than the other models.

Figure 3 shows LS Mean ALSFRS-R by Visit from various analyses. MMRM by Visit point estimates are marked by the * symbol. The linear fit and quadratic fit (allowing quadratic weeks to vary by treatment group) are also shown in Figure 3. Because the blue slope model line extends below the * symbol at Week 24 and the dashed blue quadratic curve, it is clear that the linear slope model predicts the highest group difference at Week 24. Having a bigger Week 24 treatment difference than two competing models, one of which (MMRM) more directly estimates the Week 24 difference, i.e, without imposing assumptions about the functional form between visits which could potentially allow earlier visits data to have undue influence on the Week 24 difference, suggests bias of the linear slope model.

Figure 3 Comparison of Models of ALSFRS-R over the First 24 Weeks (mITT)

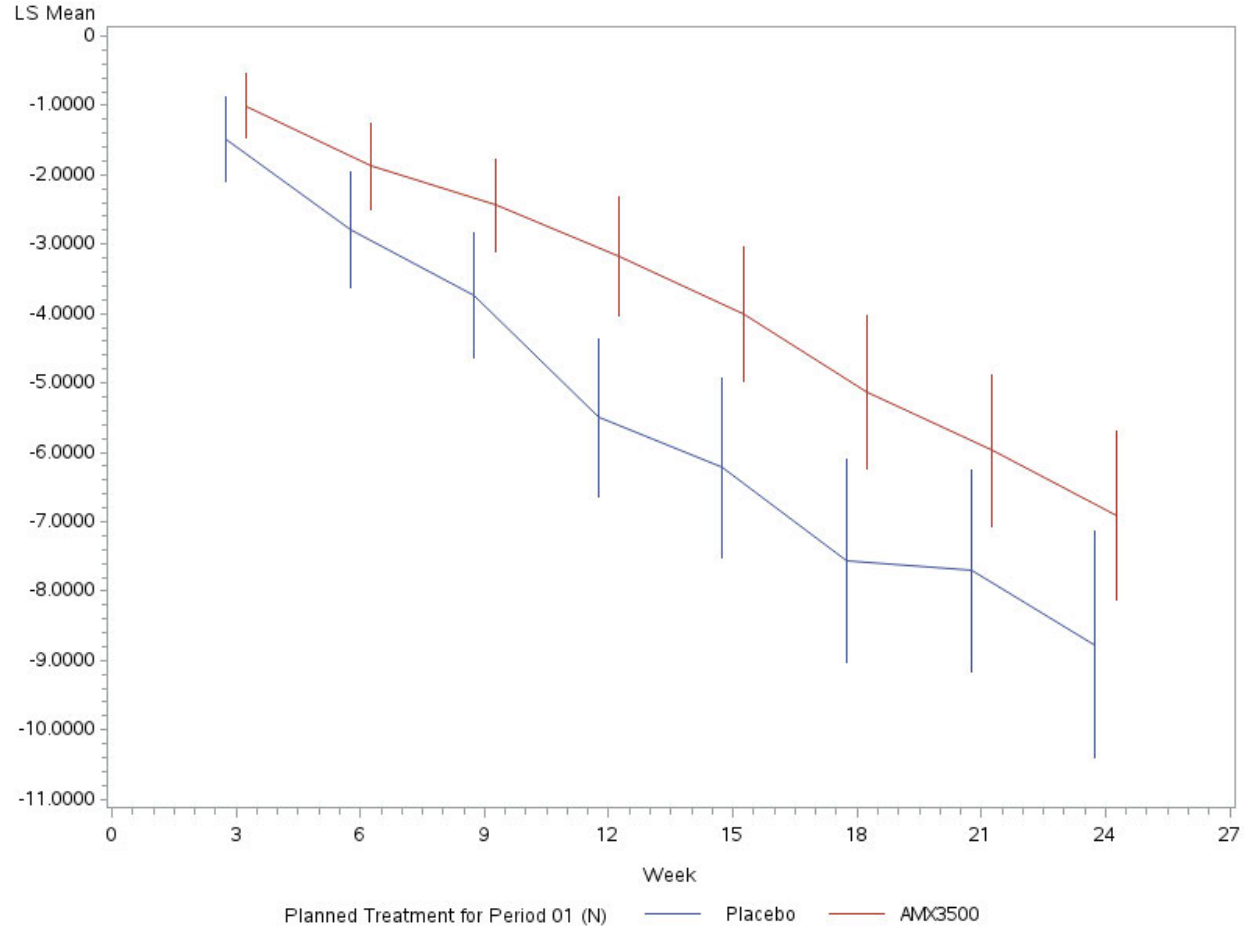


Note: Results based on change from baseline and excluding baseline visit from dependent variable

Source: Statistical Reviewer Analysis

Figure 4 shows the LS Mean ALSFRS-R estimates by group across Visits with (2 Standard Error bars) derived from a commonly used separate mean by visit mixed model for repeated measures analysis. Based on this analysis there appears to be a slight trend towards less group difference at the end (Week 24). Figure 4, as well as the exploratory by-visit p-values for the group difference, illustrate this trend. The **Week 24 difference is estimated as only 1.86 and the associated p-value is 0.0749** (> 0.05) based on this more traditional analysis than the analysis provided by the Applicant.

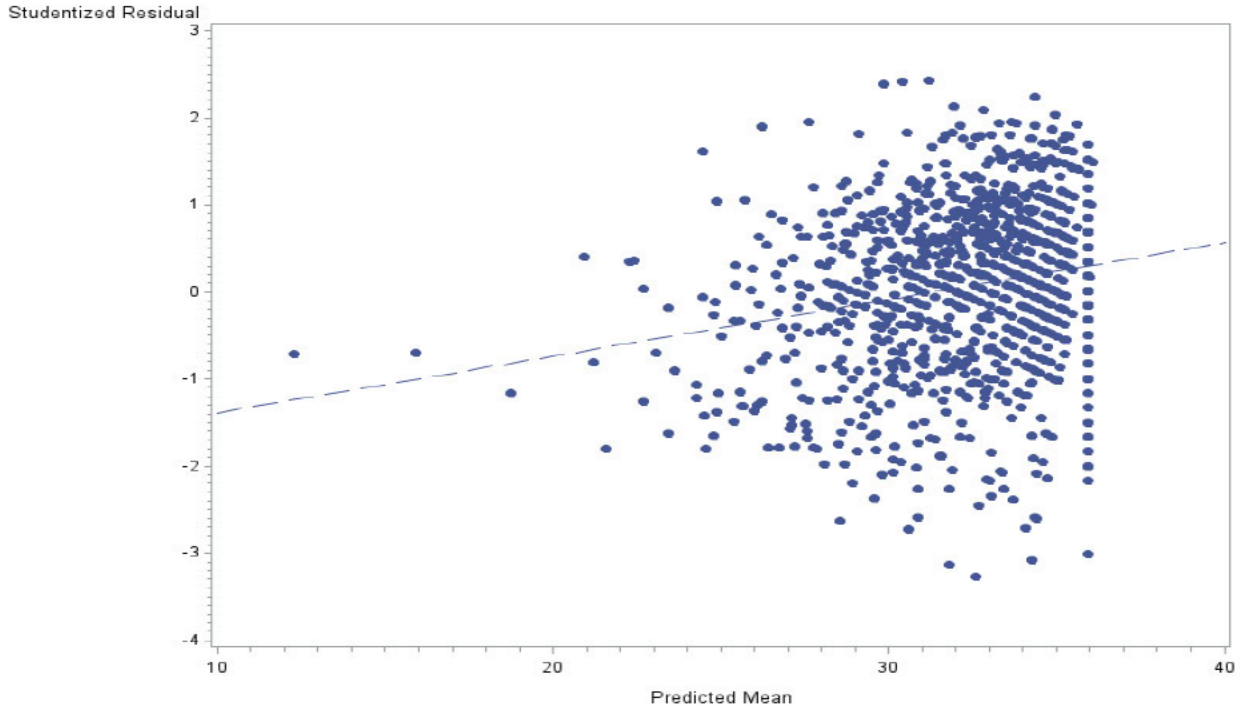
Figure 4 Traditional Separate Mean-by-Visit MMRM Analysis with 95% Confidence Interval of Estimates of Visits



Source: Statistical Reviewer Analysis

Figure 5 shows a residual plot, studentized/standardized residual (difference between actual value and predicted value) on the y-axis vs. predicted value by the model on the x-axis. This is a common way to assess the adequacy of the primary model fit. If the model fits well then there should be no pattern in the figure other than a random spread around the horizontal line at y=0. However, on this plot, there appears to be an increasing trend from left to right as indicated by the superimposed simple linear regression line. This suggests a lack-of-fit problem with the primary analysis (shared baseline mixed effects slope) model.

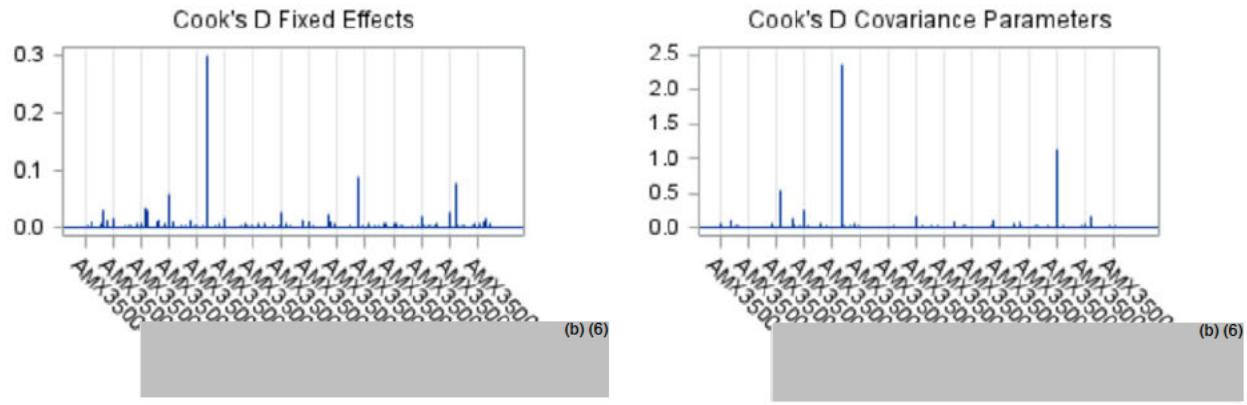
Figure 5 Residual versus Predicted ALSFRS-R Plot for Primary Model (mITT)



Source: Statistical Reviewer Analysis

Cook's D statistic, another measure of model fit, and influential observation is shown for fixed effects on the left panel, and for covariance parameters in the shared baseline mixed effects slope model on the right panel in Figure 6. We can observe from higher bars in the figure that some patients have high influence on the final model fit.

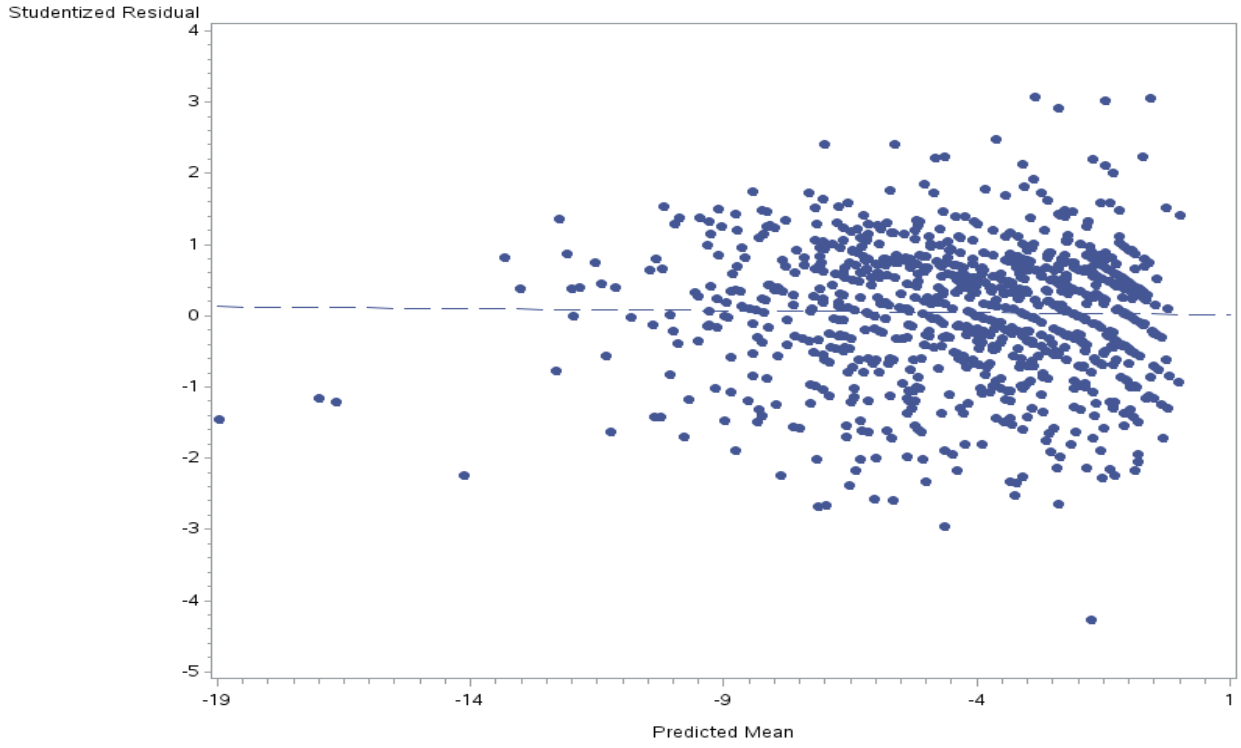
Figure 6 Model Diagnostics for the Primary Model



Source: Statistical Reviewer Analysis

The corresponding residual plot for the MMRM model (Figure 7) shows a better random scatter around the horizontal line at 0 and less of a pattern than the slope model's residual plot, suggesting that the MMRM model is a better fit to the data. Additionally, the Akaike's information criterion (AIC), which is often used to select between two competing models, also suggests that the MMRM is a better fit to the data. This criterion accounts for differences in the number of parameters in the respective models, with smaller being better [slope model AIC=4428.6 vs. MMRM AIC=4234.7].

Figure 7 Plot of Studentized Residual vs. Predicted value for the MMRM model of ALSFRS-R (mITT)



Source: Statistical Reviewer Analysis
Note: Dependent variable in this MMRM is change from baseline

Table 15 summarizes the results for the treatment difference in Week 24 ALSFRS-R for the Applicant's primary analysis, the shared baseline mixed effects slope model, and for the statistical reviewer's more traditional mean-per-Visit MMRM model. Note that the reviewer's traditional MMRM used exactly the same post-baseline ALSFRS-R data as the primary slope model (using continuous weeks from baseline as a covariate, rather than a visit window category variable). The reviewer also notes that the analysis plan had discussed windowing of

data but provided no comprehensive windowing algorithm or details on the corresponding analysis model.

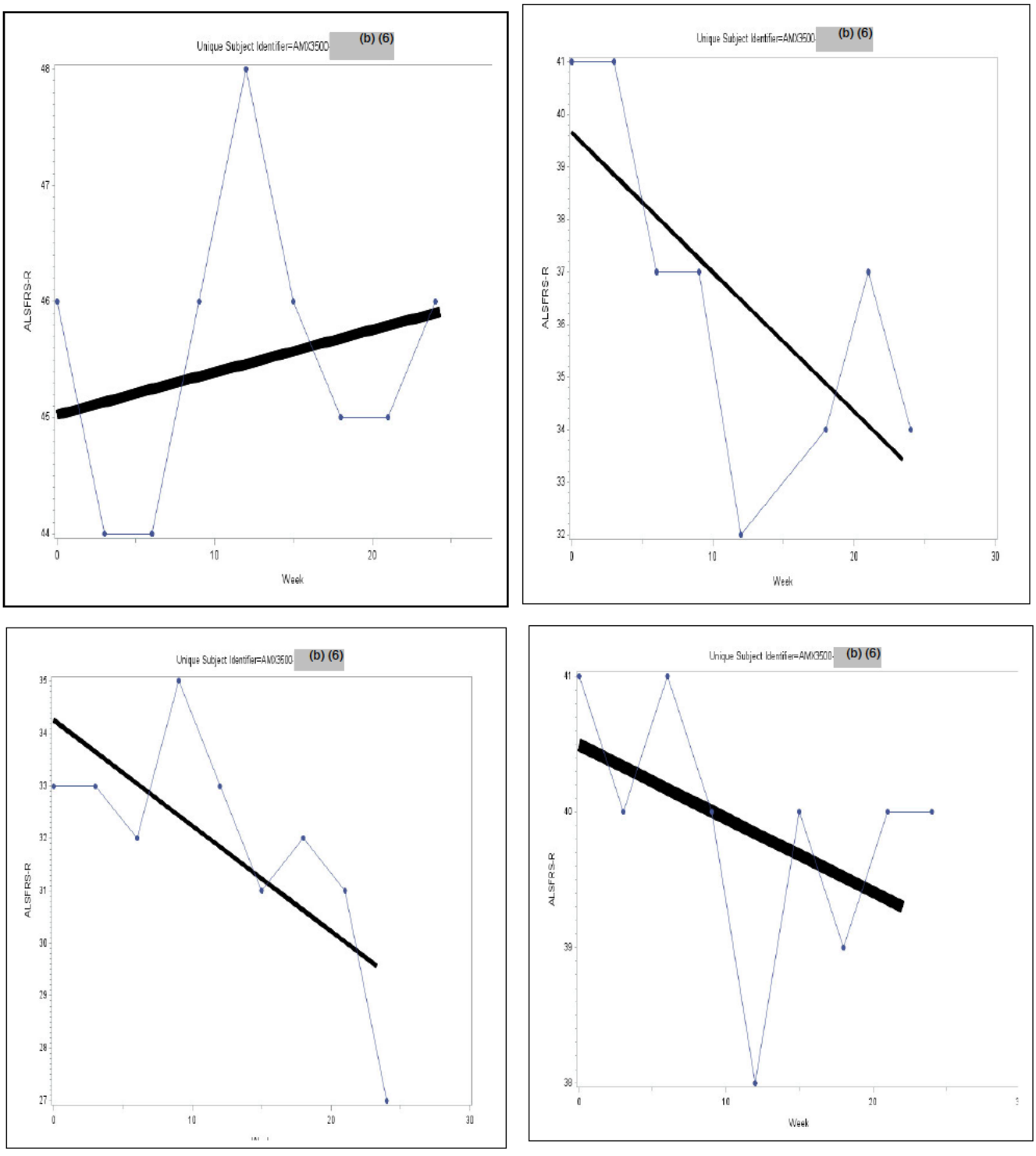
Table 15 Comparison of Applicant's Slope Model and Reviewer's MMRM Model (mITT)

Model	Estimate (SE)		Difference		
	Placebo (N=48)	AMX0035 (N=87)	Week 24 Difference (SE)	95% CI	p-value
ALSFRS-R Total at Week 24					
Shared baseline Slope model (Applicant)	26.73 (0.975)	29.06 (0.781)	2.32 (1.094)	0.18, 4.47	0.0340
MMRM (Reviewer) Change from Baseline	-8.77 (0.83)	-6.91 (0.62)	1.86 (1.04)	-0.19, 3.91	0.0749

Note: The MMRM is adjusted for delfs*Visit, and age*Visit, Visit, Treatment, and Treatment*Visit. Due to the inclusion of the baseline as a covariate it excludes the baseline assessment data from the dependent variable which is change from baseline for this analysis

A few selected examples of the slope model predicted ALSFRS-R values compared to actual ALSFRS-R data for individual patients are shown below to further illustrate the questionability of the primary analysis model's assumption of the linearity of ALSFRS-R over time (Figure 8).

Figure 8 Illustration of deviation from linearity of ALSFRS-R in selected individuals



Source: Statistical Reviewer Analysis

Statistical Reviewer's comment: *To the reviewer, it seems that the slope model does not fit these data well across all visits, all of which contribute to the slope difference estimate. The more objective extended model-based likelihood ratio test against linearity (adding quadratic and testing their significance, i.e., any difference from 0 for these parameters) supports these observations.*

Therefore, the Applicant's reported primary analysis is potentially biased optimistically. The Applicant's primary slope analysis does not handle deaths appropriately, which may also have caused bias, e.g., it may predict some retaining function after death and additional decline after death for individual patients who died.

Concerns on implications of randomization error:
As discussed earlier in the review under subject disposition (6.1.2), a randomization error occurred at the start of the study where the first 18 patients all received AMX0035 (13% of total sample size) and 9 received placebo [Total 27 (26 mITT)].

A strict ITT analysis, on which the validity of the statistical analysis and its interpretation relies, would require analysis according to the actual randomization. The assignments for the first 27 patients differ from the randomization because the first 18 patients were all given drug due to lack of adequate supplies of placebo, and in an effort to correct this, the next 9 patients were all given placebo.

The Applicant has reported results for the as-assigned treatment groups rather than the usual as-randomized/ITT treatment groups. They conducted a sensitivity analysis excluding the first 27 patients, **but this may be insufficient because the appendix to the Applicant's study report suggests that further changes to the randomization were made in 2019**. **Excluding the first 27 patients (26 mITT) the estimated slope was 0.105 (S.E.=0.051) p=0.042, with a corresponding Week 24 treatment difference estimate of 2.52. However, the randomization error is still a concern because it cannot be undone and it's a considerable proportion of the sample size for this single study.**

Post hoc Joint rank Analysis of ALSFRS-R

As stated in the regulatory history on the development program for AMX0035, FDA had many prior interactions with the Applicant. The Division recommended to perform a combined analysis of survival and function, such as the joint rank analysis of the ALSFRS-R change from baseline and mortality, as the primary analysis starting at the Pre-IND meeting on March 21, 2016, and at the Type C meeting on March 12, 2020, after the submission of topline results of Study AMX0053 to the Division. Upon the request of the Division, the Applicant performed a post hoc joint rank analysis since the Applicant had not planned to do so, despite the Division's

recommendation since 2016 and the recommendation in the Guidance for Industry, *Amyotrophic Lateral Sclerosis: Developing Drugs for Treatment*.

The Applicant performed a joint rank post hoc analysis by ranking subjects first by time to death, then by change from baseline in ALSFRS-R, in order to account for both time to death and change in ALSFRS-R. A regression was performed on this ranked outcome with treatment, ranked age, and ranked del-FS as terms in the regression. The p-value for treatment was used as the p-value for this analysis. The ranked version of age and del-FS were used for consistency with the outcome; however, this ranking of covariates was not necessary or prespecified, and produces slightly more favorable results than models of the joint rank in which these covariates are not ranked.

Based on the **Applicant's analysis**, the randomized group LS Mean Rank sums are 72.93 (3.92) and 59.07 (5.29) with an estimated difference of **13.85 (6.61), p=0.0381** (Table 16).

Table 16 Applicant's Joint Rank Analysis of ALSFRS-R (mITT)

	Placebo+SOC	AMX0035+SOC	Difference	p-value
Joint Rank Analysis	59.07 (5.29)	72.93 (3.92)	13.85 (6.61)	0.0381

Source: Study AMX3500 CSR page 90

Variable	Mean (SE)	t Statistic	p-value
Intercept	103.92 (8.68)	11.97	<.0001
Ranked Age	-0.14 (0.08)	-1.66	0.0987
Ranked Del-FS	-0.32 (0.08)	-3.94	0.0001
Treatment	-13.85 (6.61)	-2.10	0.0381

Source: Table 14.2.29.2 Section 14 of CSR

Statistical Reviewer's Analysis and Comments on Applicant's Joint rank Analyses:

Concerns in handling of missing data (MAR):
Only one hundred seven (107) of 130 survivors had a Week 24 ALSFRS-R assessment (23 missing the 24-week data included 8/46 (17.4%) randomized to placebo and 15/84 (17.9%) randomized to AMX0035). The Applicant used **Last Observation Carried Forward (LOCF) for missing data** in the joint rank analysis. Using LOCF for missing data in the joint rank forces comparing the Week 3 assessments for 5 patients (who dropped out with only Week 3 ALSFRS-R) with the Week 24 assessments when comparing them to the 107 completers. Likewise, 2, 4, 4, 4, 3, and 1

patient(s) require a comparison of their last assessment at Week 6, 9, 12, 15, 18, and 21, respectively, with the Week 24 assessment for the 107 completers. This is not a fair comparison and is likely to cause bias, especially in a degenerative disease for which scores tend to worsen over time.

Using instead the **Last Common Visit (LCV) approach for missing data,** the statistical reviewer found a joint rank difference of **12.23 (S.E.=6.64) p=0.0679** for ranked covariates. In this missing data handling method, each pair of patients are ranked and in case of two surviving patients, if there is missing data at Week 24 then the last post-baseline visit at which both have non-missing ALSFRS-R is used to rank the two patients. A patient's individual joint rank is determined by comparing them to all other patients in this way.

Alternatively, using 50 **Multiple Imputations for missing data** (to predict Week 24 for those missing and then compare the observed or predicted (where applicable) Week 24 ALSFRS-R with Week 24 ALSFRS-R for all patients and do this multiple times to account for uncertainty in the imputations) for handling missing data, the estimated week 24 difference in rank sums was **12.58 [SE 6.76], p=0.0626.**

Statistical Reviewer's comment: *The statistical reviewer believes that only the joint rank with Multiple Imputation (MI) for missing data is valid under missing at random and purely estimates the Week 24 treatment group difference. The LOCF and Last Common Visit missing data handling methods for the joint rank estimate a weighted combination of the treatment group difference at various visits, depending on the observed missing data pattern. Thus, these are only valid under missing completely at random. Therefore, the statistical reviewer believes that the joint rank with multiple imputation for the handling of missing data is the more appropriate joint rank analysis, although it should be noted that the multiple imputation analysis could be biased if the missing data is missing not at random. Note that the Applicant did not prespecify a joint rank analysis and the reviewer does not believe that the prespecified sensitivity analysis with left censoring of ALSFRS-R for deaths is appropriate (see below for further discussion).*

Table 17 shows multiple joint rank analyses varied by handling of missing ALSFRS-R data in survivors, and population (mITT or ITT).

Table 17 Joint Rank Analyses of ALSFRS-R and Death by Different Missing Data Handling Methods and Different Analysis Population (mITT or ITT)

Analysis specifications	Missing Data Handling	LS Mean Difference	Std Error of Difference	p-value
mITT (Applicant)	Last Observation Carried Forward	13.85	6.61	0.0381
mITT	Last Common Visit without missing data	12.23	6.64	0.0679
mITT	50 Multiple Imputation for Week 24 ALSFRS-R	12.58	6.76	0.0626
ITT	Last Observation Carried Forward	12.85	6.67	0.0563
ITT	Last Common Visit without missing data	11.20	6.71	0.0975
ITT (Reviewer's Recommended)	50 Multiple Imputation for Week 24 ALSFRS-R	12.00	6.82	0.0785

Source Statistical Reviewer Analyses
Note: Ranking of covariates in the Joint Rank Analysis was not prespecified and p-values without this ranking of covariates (i.e., using unranked version of age and del-FS covariates) were slightly larger for all of these analyses

Sensitivity Analyses

Concerns on left censoring of ALSFRS-R for deaths:

Due to the risk that death or intercurrent events could bias results, a left-censored model was conducted by the Applicant as a prespecified sensitivity analysis.

Table 18 Applicant's Analysis of ALSFRS-R with Left Censoring for Deaths

Endpoint Time Point	Estimate (SE)		Difference			
	Placebo+SOC (N=48)	AMX0035+SOC (N=87)	Estimate (SE)	95% CI	p-value	Weeks Function
ALSFRS-R Total						
Week 24	26.66 (0.966)	28.99 (0.775)	2.33	0.18, 4.47	0.0335	6.0 (33.6%)

Source: AMX3500 CSR Page 94

The Applicant did not prespecify a joint rank analysis and the reviewer does not believe that the sensitivity analysis with left censoring of ALSFRS-R for deaths is appropriate. This analysis appears to assume censored values are only no better than the worst of all observed values for a given patient. Therefore, a death could have a better mean and a non-zero probability of a better outcome than a surviving subject. The Applicant's implementation of left censoring doesn't include a record for the likelihood or probability at Week 24 in cases for which the patient died prior to the Week 21 Visit (it only creates a left censored record for the next visit after death). However, it is known in case of death that ALSFRS-R would be worse than the last observed ALSFRS-R at Week 24. The left censoring analysis implies uncertainty in the Week 24 ALSFRS-R value for deaths, but actually it is known to be 0 in cases of death after the event.

Statistical Reviewer's Comment: *The statistical reviewer considers the Applicant's prespecified analysis handling deaths in the analysis of ALSFRS-R biased and inappropriate.*

Concerns on ALSFRS-R analysis adjusted for concomitant medication use:
The Applicant's other sensitivity analysis evaluating the impact of post-baseline starting of ALS medications does not account for the fact that these concomitant ALS medication events are post-baseline events and, thus, likely not occurring at random throughout the study population. Therefore, the events may confound the test for treatment effect regardless of this analysis (which assumes those who started these medications post-baseline are representative of those who did not). In fact, the OLE SAP (01 November 2019) states on page 32 that "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". This clearly suggests awareness of a limitation of their own model for the double-blind period that attempted to correct for post-baseline use of Edaravone and Riluzole (see Table 19).

There was a higher proportion starting Edaravone or Riluzole post-baseline in the drug arm (14/89 [15.7%] ITT) compared to the placebo arm (2/48 [4.2%] ITT).

Table 19 Applicant's analysis of ALSFRS-R with time dependent covariates for Concomitant Medication Use

Endpoint Time Point	Estimate (SE)		Difference			
	Placebo+SOC (N=48)	AMX0035+SOC (N=87)	Estimate (SE)	95% CI	p-value	Weeks Function Retained (%)
ALSFRS-R Total Edaravone Use						
Week 24	26.77 (0.994)	28.92 (0.798)	2.15 (1.122)	-0.05, 4.35	0.0559	5.6 (30.8%)
ALSFRS-R Total Riluzole Use						
Week 24	26.66 (0.969)	28.99 (0.778)	2.34 (1.094)	0.19, 4.48	0.0330	6.1 (33.8%)
ALSFRS-R Total Edaravone and Riluzole Use						
Week 24	26.66 (0.989)	28.92 (0.802)	2.26 (1.117)	0.07, 4.45	0.0433	5.9 (32.3%)

Source: AMX3500 CSR Page 95

The primary analysis did not censor ALSFRS-R data collected after post-baseline starting of ALS concomitant medications.

However, these concomitant ALS treatment intercurrent events are difficult to correct for, and yet the primary analysis inclusion of data after these intercurrent events could have confounded the test for treatment effect. Simply excluding data after the events doesn't fix the problem either, because it changes the balance of the follow-up time and it also assumes that the patients with these events are a representative subset of those who did not, which is not likely, considering that they needed additional treatment. In fact, this last point is what creates the difficulty of modeling away the intercurrent event problem. The Applicant's sensitivity analysis related to concomitant medications relies on strong and unverifiable assumptions, e.g., linearity together with a simple change in slope after starting concomitant medication, which is unverifiable due to being post-baseline and within a non-random subset of subjects. Therefore, the Applicant's sensitivity analysis related to concomitant medication use does not resolve the potentially confounding issue of a higher proportion of the drug arm starting edaravone or riluzole post-baseline.

Statistical Reviewer's Sensitivity Analysis

A reviewer sensitivity analysis **excluding the shortest dosed non-mITT death** (#subject (b) (6)) and with 50 multiple imputations found estimated group mean rank sum differences of **12.12 (S.E.=6.78), p=0.0726** (for ranked covariates). In the corresponding analysis with LOCF instead of multiple imputations this difference was 13.43 (SE=6.63), p=0.0447 for ranked covariates, (Table 20).

Table 20 Sensitivity Analysis excluding shortest dosed non-mITT death (#subject (b) (6))

Analysis specifications	Ranking of Covariates	LS Mean Difference	Std Error of Difference	p-value
50 MI	Ranked	12.12	6.78	0.0726
	Unranked	19.2	14.4	0.1818
LOCF	Ranked	13.43	6.63	0.0447
	Unranked	21.8	13.1	0.0978

Source Statistical Reviewer Analyses

Post-hoc ALSFRS-R Analysis using Change from Baseline

After the Applicant's primary results were presented to the Division of Neurology, the Division also requested the Applicant to perform a change from baseline analysis since in the Applicant's primary model, the non-baseline subtracted ALSFRS-R was the dependent variable rather than the usual ALSFRS-R change from baseline (baseline subtracted ALSFRS-R at each visit). The Applicant's change from baseline analysis is again a slope model rather than the usual separate mean by visit mixed model for repeated measures (MMRM), but compared to their primary analysis model it excludes the baseline data from the dependent variable (included instead as a covariate).

When using the change from baseline model, the Applicant reports a between group difference on the ALSFRS-R of 2.92 points (p=0.010) in the mITT population (Table 21).

Table 21 Applicant's Change from Baseline Analysis (mITT)

Endpoint Time Point	Estimate (SE)		Difference		
	Placebo+SOC (N=48)	AMX0035+SOC (N=87)	Estimate (SE)	95% CI	p-value
ALSFRS-R Total Change from Baseline at Week 24					
Week 24	-9.62 (0.913)	-6.70 (0.682)	2.92 (1.134)	0.70, 5.15	0.0101

Source: Study AMX3500 CSR page 90

Model Effect	Estimate (SE)	95% CI	p-value
Baseline Score	-0.0036 (0.00368)	-0.0108, 0.0037	0.3325
Weeks (slope)	-0.3347 (0.02521)	-0.3841, -0.2852	<0.0001
Weeks*Age	-0.0017 (0.00244)	-0.0065, 0.0031	0.4947
Weeks*Del-FS Total	-0.2225 (0.04802)	-0.3167, -0.1282	<0.0001
Weeks*Treatment (group difference in slope)	0.1218 (0.04727)	0.0290, 0.2146	0.0101

Source Table 14.2.18.3 CSR page 1079

Statistical Reviewer's Comment:
The questions raised above about the linearity of the ALSFRS-R over time for the observed data would apply to, and be a limitation to, this change from baseline slope analysis as well, in addition to this being a post-hoc analysis.

ALSFRS-R analysis and concomitant medication use (Dr. Tristan Massie)

The protocol inclusion criteria stipulated that patient taking riluzole during the trial needed to be on a stable dose of riluzole for the duration of the study. In contrast, edaravone was approved near the start of the trial, on May 5, 2017, which may have presented ethical challenges for preventing trial patients to use it, and therefore edaravone changes were permitted in the study:

Forty-two 42/48 (87.5%) placebo and 64/89 (71.9%) AMX were using either edaravone or riluzole at baseline.

- More subjects on placebo arm 40/48 (83.3%) vs. 56/89 (62.9%) were using edaravone or riluzole at baseline **and didn't start another concomitant ALS medication post-baseline (2 placebo and 14 AMX not counted here in numerator because while they were on one at baseline, they started the other post-baseline**).
- More subjects on AMX0035 arm started edaravone or riluzole post baseline (intercurrent events):
  - 2/42 [5%] of those on placebo who ever took (2/48 [4.2%] ITT)

- 14/70 [20%] of those on AMX0035 who ever took (14/89 [15.7%] ITT)
- This excess of ALS treatment intercurrent events in AMX0035 arm could have confounded the test for treatment effect since ALSFRS-R was relatively balanced at baseline despite more placebo patients using riluzole at baseline (any riluzole dosing was supposed to be fixed at baseline).
- In addition, post-baseline starting of edaravone or riluzole is slightly imbalanced for the revised randomization groups and also less imbalanced for the preplanned randomization schedule (16% vs. 4% as compared to 13% vs. 11%).

Table 22 Cross classification of Baseline vs. Post-Baseline starting of Riluzole and/or Edaravone

Table of TRT01PN by bothpriorst									
	Bothpriorst a N indicates started one post-baseline Y indicates on one at baseline								
TRT01PN(Planned Treatment for Period 01 (N))	Never either	N	Y	N	NY	Y	YN	YY	Total
1	6 12.50	0 0.00	16 33.33	0 0.00	2 4.17	5 10.42	0 0.00	19 39.58	48
2	19 21.35	2 2.25	34 38.20	4 4.49	7 7.87	3 3.37	1 1.12	19 21.35	89
Total	25	2	50	4	9	8	1	38	137

–

In summary, a higher proportion who were randomized to the AMX0035 group started edaravone or riluzole after randomization and study drug initiation.

These intercurrent events could confound the test for treatment effect as more on AMX0035 started edaravone after baseline, and data after starting these medications were not censored in the primary analysis. This presents further uncertainty in the primary results, making them less reliable, because there is no way to correct for these intercurrent events after the fact unless they occur in a balanced way across groups (which they did not) and across covariates within groups, but this is very unlikely to be the case. More subjects starting edaravone in the

AMX0035 arm after baseline could partially or fully explain a random high apparent difference in ALSFRS-R between treatment groups at Week 24.

Regardless of this concomitant medication imbalance, the Week 24 difference based on the primary slope analysis is not supported by the reviewer's more traditional Mean by Visit mixed model for repeated measures analysis (Table 15) which doesn't assume linearity of ALSFRS-R over time and uses exactly the same post-baseline ALSFRS-R data.

Table 23 Concomitant Edaravone Use and Timing of Use

Table of Treatment Group by Timing of Edaravone Use					
		Start of Concomitant Edaravone			
		Never	Post-Baseline	Before-Baseline	Total
Placebo	N	22	2	24	48
	%	45.83	4.17	50.00	
AMX0035	N	55	11	23	89
	%	61.80	12.36	25.84	
Total	N	77	13	47	137
	%	56.20	9.49	34.31	100

In addition, there were also 0 on placebo and 3 on AMX0035 that started riluzole post baseline (1 on edaravone at baseline and 2 not on edaravone at baseline). One of these had a missing start day of the month. Depending on the unknown start day this could be a pre-baseline start but based on the known month of starting would still not satisfy the protocol requirement to be on a stable dose of riluzole 30 days prior to screening, thus this was counted as a post-baseline start of riluzole.

Table 24 examines the proportions starting concomitant ALS medications (edaravone or riluzole) post-baseline in those who could have started one or the other post-baseline, i.e., those not on both at baseline (19 placebo [39.6%] and 19 [21.4%] AMX were on both at baseline).

Table 24 Post Baseline ALS Medication Start Among Those who could have started Riluzole or Edaravone post-baseline: (not on both at baseline)

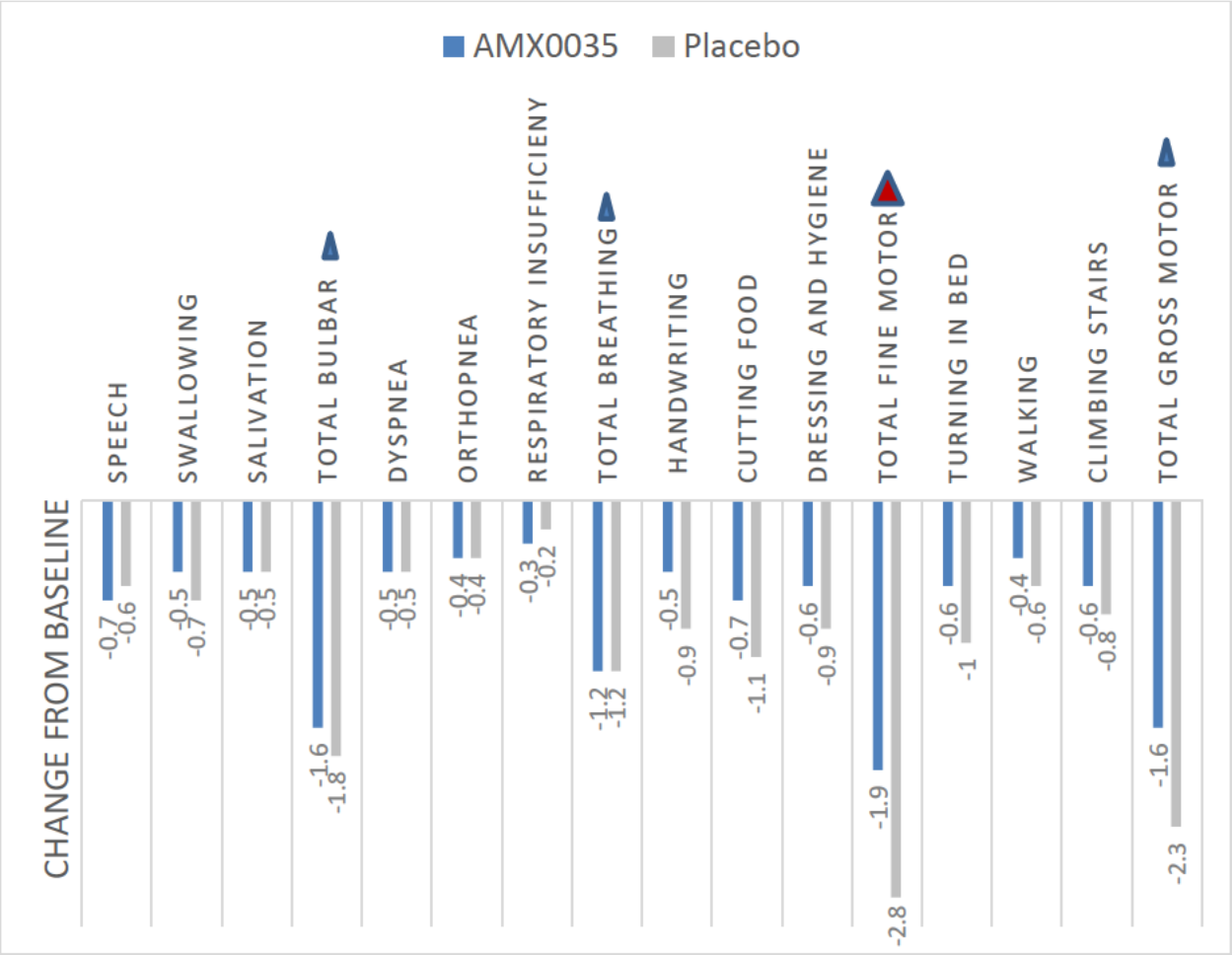
		Post Baseline ALS Medication Start		
		No	**Yes**	**Total**
Placebo	N	27	2	29
	%	93.10	6.9	
AMX0035	N	56	14	70
	%	80.00	20.00	
Total	N	83	16	99
	%	83.8	16.16	100

Note: Likelihood ratio test of association between use and treatment group p=0.0847

Clinical Reviewer's Comment:
In addition to all statistical concerns mentioned above including the start of edaravone post randomization, there were other review issues that decrease the persuasiveness of the study results.
The reviewer notes that the impact of unblinding due to the bitter taste of the drug and gastrointestinal AEs is unknown. Subjects were asked not to report the bitter taste as an AE. However, both patient and investigator were asked to guess the treatment to which the subject was assigned. For the investigator predictions, there were 49% correct predictions in the active arm and 40% correct predictions for placebo arm. A total of 44% of subjects on treatment guessed that they were on active drug and 63% of subjects on placebo guessed that they were on placebo. There were 12-14 % of missing data in either group of responses.

*I also reviewed the change from baseline (raw statistics) in the 4 subscale scores of the ALSFRS-R (i.e., bulbar, fine motor, gross motor, and breathing). Treatment differences in change from baseline in most components ranged from 0-0.4 points (*Figure 9*). The largest treatment difference was seen for the fine motor (Upper) domain of the ALSFRS-R (1.9 points). The Applicant also reports the largest treatment difference for the fine motor domain of the ALSFRS-R, with an estimated LS means treatment difference of 1.04 points. The other domains showed treatment differences of 0.36-0.52 based on the slope analysis. Only Fine Motor Domain was nominally positive in the Applicant's analysis.*

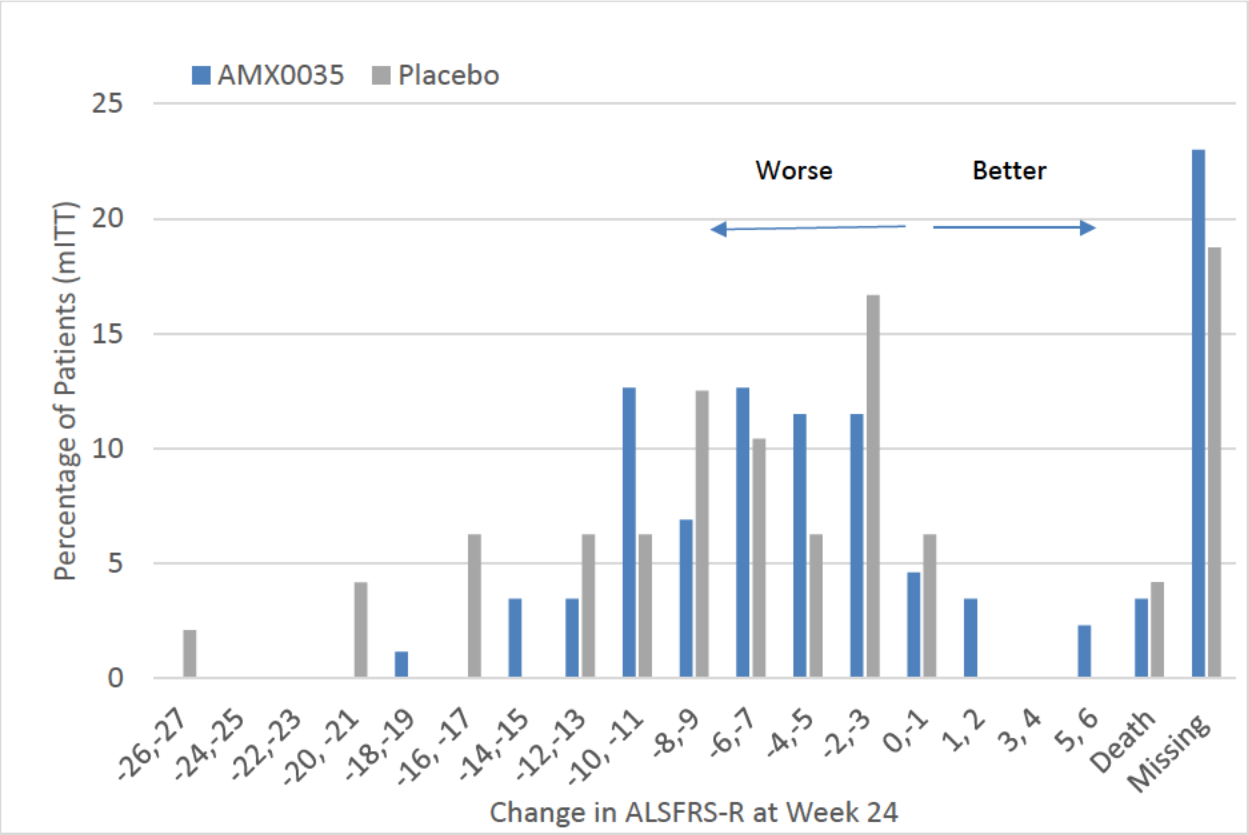
Figure 9 ALSFRS-R Individual components and Total Domains



Source: Clinical Reviewer Analyses

In addition, the distribution of change from baseline to Week 24 in ALSFRS-R scores is shown in the following Figure 10. There were 5 subjects in the AMX0035 arm that showed an increase in ALSFRS-R, 3 subjects in the placebo arm that showed a decline of >20 points in the ALSFRS-R total score, one of which had the highest rate of decline in ALSFRS-R of 3.1 at baseline. There were 23% missing data in the AMX0035 group.

Figure 10 Distribution of change from baseline to Week 24 in Total ALSFRS-R scores (mITT)



Source: Clinical Reviewer Analyses

Data Quality and Integrity

There were difficulties reconciling some SDTM datasets with the ADaM datasets, some of which were addressed by the Applicant. There were some missing exposure data as the patient was lost to follow up. Due to unavailability of CRFs these could not be confirmed.

Efficacy Results – Secondary and other relevant endpoints

Rate of Decline in Isometric Muscle Strength as Measured by ATLIS

For the first secondary endpoint, Total ATLIS, the absolute mean percent predicted normal strength for the AMX0035 treatment group at Week 24 was 2.82 percentage points higher than the placebo group (p=0.1129, (Table 25). Additional exploratory analyses were conducted on the components of Total ATLIS scores including Upper and Lower ATLIS. On the Upper ATLIS, the absolute mean percent predicted normal strength for the AMX0035 treatment group at Week 24 was 4.27 percentage points higher than the placebo group (nominal p=0.0420). On the

Lower ATLIS, the absolute mean percent predicted normal strength for the AMX0035 treatment group at Week 24 was 2.09 percentage points higher than the placebo group (nominal p=0.3424).

Table 25 Applicant's Analysis of ATLIS (Percent of Normal Strength) at Week 24: mITT Population (N=135)

Time Point	Estimate (SE)		Difference			
	Placebo	AMX0035	Estimate (SE)	95% CI	p-value	Weeks Function Retained (%)
Total ATLIS						
N	47	84				
Week 24	36.26 (2.224)	39.08 (1.990)	2.82 (1.774)	-0.67,6.31	0.1129	3.5(16.9%)
Upper ATLIS						
N	47	85				
Week 24	32.36 (2.590)	36.63 (2.316)	4.27(2.089)	0.16, 8.38	0.0420	4.9(25.4%)
Lower ATLIS						
N	48	85				
Week 24	39.09 (2.664)	41.17 (2.371)	2.09 (2.195)	-2.23,6.41	0.3424	2.7(12.7%)

Source: Study AMX3500 CSR, Page 91

Statistical Reviewer's Comment:
For the secondary endpoint designated first after the primary in the hierarchy, ATLIS; there are actually 3 possible analyses for ATLIS, i.e., for upper score, lower score and total score, and the analysis plan was not clear on the prioritization among these which creates a multiplicity issue. ***The Applicant reported a slope difference for ATLIS Upper of 0.1778 (S.E.=0.08705) p=0.0420.*** *The statistical reviewer assigned 2 more records to Week 24 for the drug group than the Applicant which resulted in an estimated slope of 0.1720 (S.E.=0.0866), p=0.0479. This was based on 541 records from 132 patients (48 placebo and 84 AMX0035) [the Applicant reports 47 placebo and 85 AMX0035 in the study report, page 323 of section-14 Tables and Figures].*

The reviewer estimated a Week 24 difference in ATLIS upper ***scores based on a traditional MMRM*** *(repeated measures analysis with separate mean by visit rather than assuming a linear trend across visits and excluding baseline from the dependent variable* ***and with the Applicant's covariates of delFS and delATLIS, rather than baseline ATLIS upper, and their interactions with visit)) of 3.09(S.E.= 2.13), p=0.****1483 [based on 123 subjects and 406 post-baseline ATLIS records].*

Missing data was higher for ATLIS than ALSFRS-R with 31/48 (64.6%) in placebo arm and 55/87 (63.2% or 61.8% considering 2 ITT/non-mITT deaths) in AMX3500 arm having Week 24 assessments.

Clinical Reviewer's Comment:
In the Applicant's reported sensitivity analysis conducted after removing the affected 25 patients did continue to show a nominally positive benefit on ALSFRS-R slope analysis but did not demonstrate any significant benefits on any of the secondary endpoints, including Upper ATLIS, demonstrating that there may have been some effect of the randomization error on the study results for Upper ATLIS.

There were some imbalances in the ATLIS score as baseline that favored the AMX0035 arm by 2.9 points that appeared to be driven by Upper ATLIS. However, the relevance of this to overall results remains unclear.

Rate of Decline in Phosphorylated Neurofilament Heavy and Light (pNF-H and pNF-L) Levels

Plasma pNF-H levels were second in the hierarchy of secondary endpoints. Applicant's analysis of the results for the biomarker, pNF-H, did not indicate any treatment-related difference between groups (Table 26). The mean rate of change in the plasma pNF-H concentration was 3.58 pg/mL per month with AMX0035 and -2.34 pg/mL per month with placebo (p=0.2601).

Additionally, analysis of the results for the exploratory endpoint biomarker pNF-L (not a secondary endpoint) also did not indicate any treatment-related difference between groups. The mean rate of change in the plasma NF-l concentration was 1.45 pg/mL per month with AMX0035 and 2.04 pg/mL per month with placebo (p=0.5021).

Table 26 Applicant's Analysis of Neurofilament biomarkers at Week 24 (mITT population)

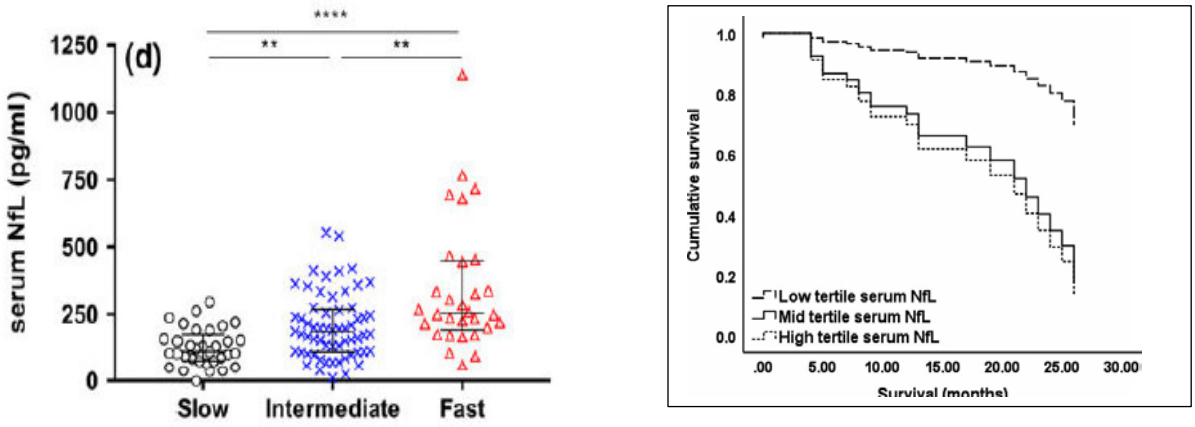
Time Point	Estimate (SE)		Difference		
	Placebo+SOC (N=47)	AMX0035+SOC (N=84)	Estimate (SE)	95% CI	p-value
pNF-H					
Week 24	374.25 (38.81)	406.95 (35.82)	32.7 (28.98)	-24.33.89.75	0.260
Change per month	-2.34 (4.18)	3.58 (3.19)	5.93 (5.25)	-4.41, 16.26	
pNF-L					
Week 24	101.08 (5.85)	97.85 (5.26)	-3.23 (4.8)	-12.71. 6.24	0.502
Change per month	2.04 (0.70)	1.45 (0.52)	-0.59 (0.87)	-2.3, 1.13	

Source: CSR Section 14 Table 14.2.1.13 and Table 14.2.1.23

Clinical Reviewer's comment:
Neurofilaments (NF) were used as mechanistic measure of neuronal death. Numerous studies have shown that NF levels are increased in patients with ALS, not only in CSF, but also in serum or plasma. As NFs are produced by neurons, the serum/plasma levels are 10-fold lower compared to CSF levels. In the current study only NF plasma levels were measured. As marker of neuronal injury, it is anticipated that neuroprotective treatments would result in lower NF levels, however the plasma pNF-H concentrations favored the placebo group in the CENTAUR study.

Gille et. al[7]. found a significant correlation in Serum NF-L levels in patients with ALS stratified according to their disease progression rate in slow (black circles), intermediate (blue crosses) and fast progressors (red triangles). The authors found that based on a Kaplan–Meier analysis, the survival curve of patients with ALS belonging to the different tertiles of serum NF-L levels was significantly different. The serum NF-L levels were stratified as low tertile (<115 pg/ml), mid-tertile (115 pg/ml ≤ x ≤ 235 pg/ml) and high tertile (>235 pg/ml).

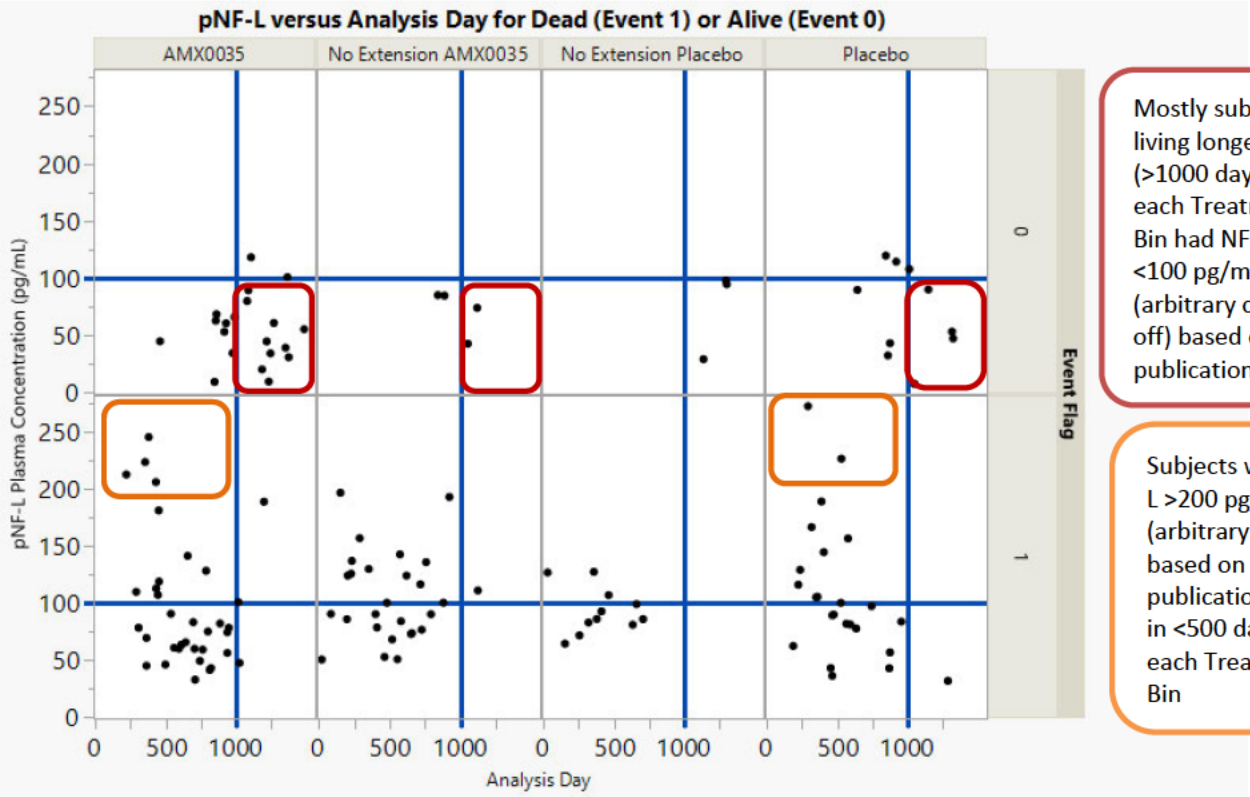
[7] Gille et al (2018) Serum neurofilament light chain levels as a marker of upper motor neuron degeneration in patients with amyotrophic lateral sclerosis; Neuropathology and Applied Neurobiology, doi: 10.1111/nan.12511



Source: Gille et al. 2018

Based on this finding I explored the NF-L levels at baseline in patients that died and those that were alive at the March 1 cuff off analysis for death in patients on AMX0035, on placebo that transitioned to AMX0035 in the open label extension and those that did not enter the open label extension (4 treatment Bins) as shown below in Figure 10. Event "1" on the Y2-axis indicates subjects that died and Event "0" on the Y-2 axis indicate subjects that were alive at the cutoff date of March 1, 2021. 'Analysis day' on the X-axis in the Figure is the day that the event (vital status) was recorded as dead or alive. NF-L levels are plotted on the Y-axis. I arbitrarily chose a cut of baseline NF-L levels of 100 pg/ml (horizontal blue lines) close to the cutoff of 115 pg/ml chosen in the above publication. Gille et al characterized these in the slow tertile. All subject alive for >1000 days were in this group and could be slow progressors. However, there is an overlap in time to death in subjects with NF-L <100, as also seen in published studies. The Figure also shows that most subjects with NF-L >100 to<200 pg/mL (mid tertile) died in <1000 days (vertical blue lines at 1000 days) in each Treatment Bin. All subjects with NF-L >200 pg/mL (probable high tertile) died in <500 days. There are other factors also that should be considered independent predictors of survival such as site of onset, FVC, time from symptom onset, rate of decline in ALSFRS-R etc.

Figure 11 Relationship between pNF-L levels and survival status



Note: Placebo group here has received AMX0035 in OLE (i.e. delayed start)
Source: Clinical reviewer analysis

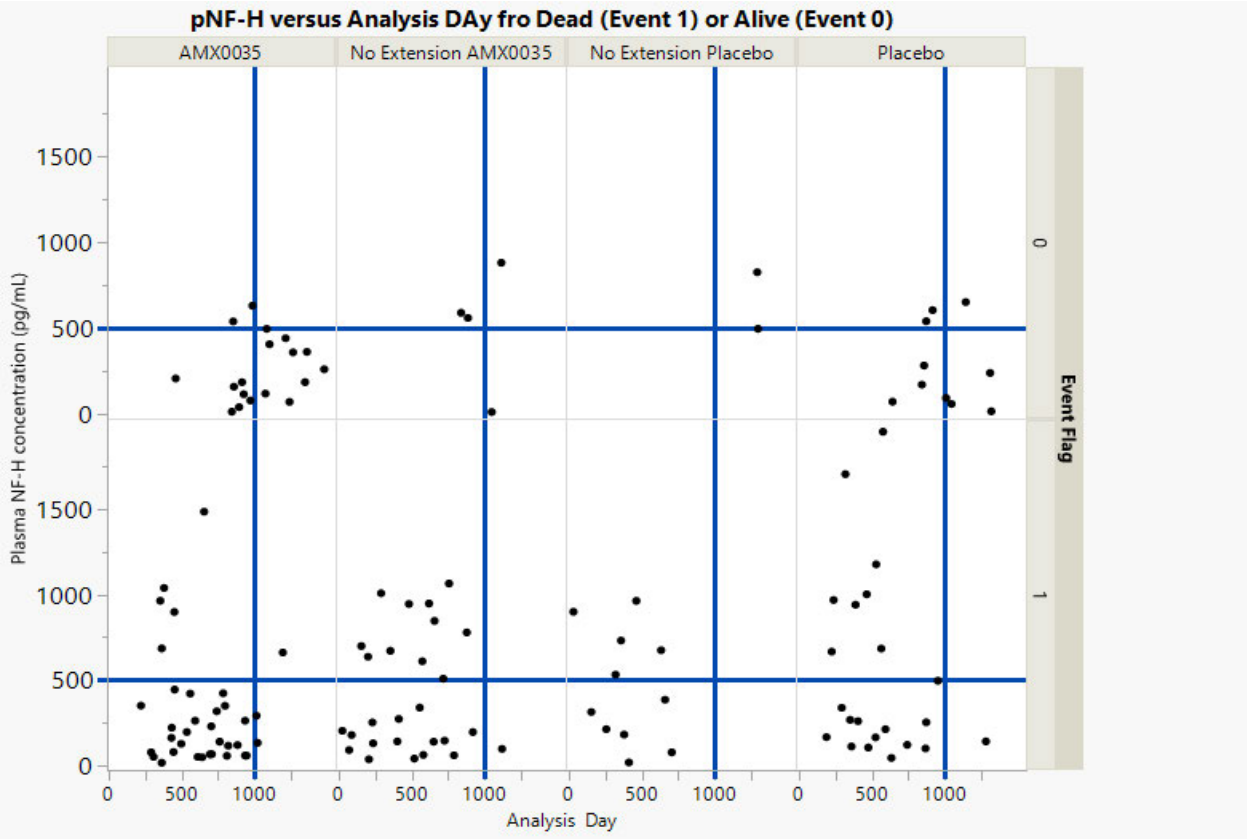
There are no clinical trials that have shown a survival benefit with reduction in NF levels. However, prediction of survival of ALS patients with higher NF levels are unfavorable. In the CENTAUR study there was no difference between treatment and placebo in either NF-H or NF-L levels post-treatment. Overall, it is evident that subjects with higher baseline NF-L levels have poorer prognosis irrespective of duration on AMX0035. The mean (SD) baseline levels of pNF-L were slightly higher in the placebo group [95.7 (49.4), range 7, 273] compared to AMX0035 arm [88.9 (49.6), range 9, 246].

Similar trends were also seen with baseline pNF-H levels exploring levels with a cut off of 500 pg/ml (horizontal blue lines) and survival (Figure 11). Low levels have been reported in subjects with long survival. Most subjects with higher baseline pNF-H had a reported event of death. The mean (SD) baseline levels of pNF-H were again higher in the placebo group [462.6 (432.9), range 14, 1955] compared to AMX0035 arm [345.7 (322.1), range 10, 1487].

There is again considerable overlap in time to death in subjects with pNF-H levels <500 pg/mL as there could be many independent predictors of survival, however subjects with much higher pNF-H levels mostly are in the category of event=1 and shorter survival time.

The sensitivity and specificity for ALS is thought to be better for pNF-H than for pNF-L[8] in published studies comparing both neurofilament subunits. It is unclear which of these NFs have better predictive value. Since the study included ALS patients early in the disease (<18 months since onset, SVC>60%), very high levels of NFs were not seen in this study.

Figure 12 Relationship Between pNF-H Levels and Survival Status



Source: Clinical reviewer analysis

Rate of Decline in Respiratory Function as Measured by SVC

[8] Poesen et al; Diagnostic and prognostic performance of neurofilaments in ALS; Frontiers in Neurology, January 20189, Volume 9, article 1167

SVC volumes were standardized to predicted percent normalized value (PPN) based on age, sex, and height (Knudson 1983[9]). Average of the PPN is compared between the AMX0035 and placebo groups. Average of SVC % Predicted was 5.11 points higher in the AMX0035 group at Week 24 compared to placebo, although the p-value was not significant (p=0.0763) (Table 27).

Table 27 Applicant's Analysis of SVC at Week 24

Time Point	Estimate (SE)		Difference			
	Placebo+SOC (N=48)	AMX0035+SOC (N=87)	Estimate (SE)	95% CI	p-value	Weeks Function Retained (%)
SVC % Predicted						
Week 24	61.06 (2.812)	66.17 (2.327)	5.11 (2.872)	-0.54, 10.76	0.0763	5.5 (29.8%)

Source: Clinical Study Report, Page 92

Statistical Reviewer Analysis and Comment:
The Week 24 treatment group difference in SVC was also not significantly different based on the ***more traditional separate mean by visit MMRM (with the Applicant's covariates of delFS and delSVC, rather than baseline SVC, and their interactions with visit): 5.42 (S.E.= 2.82), p=0.0570*** *[452 post-baseline SVC assessments between Week 6 and 24 from N=130 of the randomized patients].*

Survival Analysis

Single and combined survival analyses were performed using the Cox proportional hazards model with covariates of del-FS and age at baseline for the outcomes of death, death equivalent, and hospitalization (death equivalent was defined as time to death, PAV, or tracheostomy). Note that PAV only and tracheostomy only were not analyzed as there was only 1 event of each in a singular placebo patient (both occurred in the same placebo subject). As shown in Table 28, while all of the analyses (except death alone) directionally favored AMX0035, none were statistically significant.

[9] Knudson RJ, Lebowitz MD, Holberg CJ, et al. Changes in the normal maximal expiratory flow volume curve with growth and aging. Am Rev Respir Dis. 1983;127:725-34.

Table 28 Applicant's Summary of Survival Analyses at Week 24 (mITT)

Categorical Outcome	Estimated Percentage of Event (SE)		Hazard Ratio: Active vs. Placebo (95% CI)	P-Value
	AMX0035	Placebo		
Death, Death Equivalent, or Hospitalization	19.2 (4.20)	31.0 (6.78)	0.575 (0.290, 1.152)	0.1122
Death or Death Equivalent	2.8 (1.69)	4.4 (3.02)	0.632 (0.110, 3.924)	0.5960
Hospitalization	17.4 (4.07)	27.7 (6.50)	0.590 (0.286, 1.234)	0.1530
Death Events Only	2.6 (1.65)	2.6 (2.28)	1.016 (0.151, 9.753)	0.9873

Source: Clinical Study Report, Page 92

Statistical Reviewer's Comment:
Two ITT deaths in the treatment group are not accounted for in these survival analyses; therefore, clearly none of the survival analyses for the 24 Week double-blind treatment period suggest a significant treatment group difference.

Duration of Hospitalization

There was no significant difference between treatment groups in the duration of hospitalization (Mean 1.4 days in the placebo group compared with 1.2 days in the active group, p=0.709)

TSPO Uptake as Measured by MR-PET Scan in Sub-study
A sub-study was conducted in consenting and qualifying subjects to evaluate TSPO uptake on MR-PET scans performed. Of the 137 subjects enrolled in AMX3500 study, 9 qualified for and enrolled in the MR-PET sub-study. Considering the limited number of subjects enrolled and evaluated in this sub-study, no formal analyses were performed for data collected.

Dose/Dose Response

Only one dose was evaluated in Study AMX0035.

6.2 AMX3500OLE

6.2.1 Study Design

Overview and Objective

The primary objective of the study was to assess the long-term safety of oral (or feeding tube) administration of AMX0035 via sachet (3 g phenylbutyrate [PB] and 1 g taurursodiol) twice daily (BID).

The secondary objectives of the study were to measure:

1. Rate of progression on the Amyotrophic Lateral Sclerosis Functional Rating Scale – Revised (ALSFRS-R) scale
2. The rate of key study events including tracheostomy, hospitalization, and death
3. Accurate Test of Limb Isometric Strength (ATLIS) rate of progression
4. Rate of progression of slow vital capacity (SVC)

Trial Design

AMX-3500-OLE was a multicenter, OLE in which all subjects received active treatment (AMX0035). There was to be a new formulation of the investigational product for the open label extension that had been optimized for better taste. The total treatment duration in the OLE was up to 132 weeks, and this review includes data through the last subject's last visit date of March 1, 2021, which includes data through at least Week 24 of the OLE (Week 48 overall) for all subjects and includes death data through a final cutoff date of March 1, 2021. Subjects were permitted to continue on a stable dose of riluzole or edaravone during the OLE.

A total of 90 eligible subjects enrolled into the extension and received AMX0035 orally (or via feeding tube) administered as 2 sachets per day taken as one in the morning and one in the evening (1 sachet BID regimen). To maintain the study blind, subjects initiated dosing in the OLE using the same regimen they were on at the end of the double-blind period (i.e., subjects who switched from placebo to AMX0035 were not titrated using a starting dose of 1 sachet daily for the first 3 weeks).

Clinical Reviewer's Comment:
The Applicant notes in the Clinical Study Report that during the OLE, the investigators, evaluators, and subjects remained blinded to the randomized treatment assigned at the beginning of the double-blind main study. However, the protocol (including all amendments) does not state if the blind would be maintained in the OLE, what steps would be taken to maintain the blind, or which study personnel (e.g., patients, investigators, etc.) would remain blinded.

Key Inclusion Criteria:

- Completion of all visits in the randomized, double-blind AMX3500 main study. Subjects who received tracheostomy or permanent assisted ventilation (PAV) while in the randomized, double-blind trial could elect to enroll in the OLE so long as they completed all visits in the main study.
- Must enroll in the OLE within 28 days of the Week 24 visit of the main study

Key Exclusion Criteria:

- Discontinued study drug prematurely in the double-blind phase of the study for reasons other than tracheostomy or PAV
- Exposure to or anticipated requirement for any disallowed medication
- Any ongoing AEs that in the opinion of the Site Investigator are clear contraindications to the study drug
- Unstable cardiac or other life-threatening disease emergent during the randomized, double-blind study.
- Any other medical condition that will place the subject on risk

Study Endpoints

Safety was the primary endpoint for the OLE; all efficacy endpoints were considered secondary.

Efficacy of AMX0035 was assessed by the ALSFRS-R questionnaire, isometric muscle strength as measured by ATLIS, and respiratory function as measured by SVC, all assessed as rate of decline or progression (slope of decline). Clinic visits occurred at Screening/Baseline, Week 6, 12, 24, 36, 52, 68, 84, 100, 116 and 132.

The rate of key study events including tracheostomy, hospitalization, and death, was initially last of all secondary endpoints in the initial protocol but second in the hierarchical testing of secondary endpoints (November 2019 SAP). The overall survival endpoint was defined as death, tracheostomy (irrespective of reason for tracheostomy whether respiratory distress or control of mucus secretion) or PAV. PAV was defined as more than 22 hours daily of non-invasive mechanical ventilation for more than 1 week (7 days). The date of onset of PAV was the first day of the 7 days.

Date of death was collected for subjects throughout the course of the trial. In addition, the protocol allowed for monitoring of death after a subject had been terminated from the trial and information on whether a subject had died could 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 Statistical Analysis Plan

(http://ssdmf.info/). A professional firm, Omnitrace, was contracted to conduct such a search with cutoff dates of February 29, 2020, July 20, 2020, and March 1, 2021, to determine survival status of all subjects.

Statistical Analysis Plan

All efficacy analyses were performed using data from the baseline of the randomized, double-blind, placebo-controlled main study through Week 24 of the OLE or Week 48 overall. Week 24 of the OLE was chosen to match the study duration of 24-weeks in the main study. One exception to this was the efficacy analysis of time to death, which used cutoff dates of July 20, 2020, and March 1, 2021. ALSFRS-R, ATLIS, and SVC were analyzed by the same slope model as the controlled phase of the study.

The OLE efficacy analyses have 2 study arms defined below. This description also includes subjects that did not enter the OLE (as used in the overall survival analyses discussed in this review):

- **"RA Group"**: Those randomized to active (AMX0035) in the main 24-week randomized study
- **"RP Group"**: Those randomized to placebo in the main 24-week randomized study

The following terminology is used in this review to describe the treatment groups in the open label extension AMX3500OLE where all subjects received active treatment. This description of this group only includes subjects that entered the OLE Phase.

- **"PA Group"** refers to the group of subjects randomized to placebo in the controlled or main phase and who switched to AMX0035 upon enrollment in the OLE (i.e., Placebo to Active)

- **"AA Group"** refers to the group of subjects randomized to AMX0035 in the main phase and who stayed on AMX0035 upon enrollment in the OLE (i.e., Active to Active)

November 1, 2019 SAP (Dr. Tristan Massie)

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

Survival Analyses

Survival analyses were to 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 was to be continued out to the end of the OLE study observation, and the analysis was also to 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 days after the main study ended (24 weeks + 28 days) were to be included, since events which occurred within 28 days were to be addressed in the main study analysis. An additional analysis including 2 treatment arms was also to 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 were to 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 was to be performed for each of the 3 survival outcomes listed above and the combined analysis of "Death or Equivalent." This analysis was to 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. The analysis was to be repeated for the OLE population (OLE mITT and OLE PP) using as baseline the first day of treatment in the OLE.

March 27, 2020 SAP for Survival Analysis (Submitted April 1, 2020)

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.

Masking
The authors of the complementary SAP for survival stated therein that they had not had any access to the raw study data, analysis datasets, or the study database. Furthermore, although the subjects who entered the OLE were all receiving active treatment, the authors were masked to the originally assigned treatment groups in the main study and had no access to any individual-level treatment group data. The SAP authors were to continue to remain masked until the document was finalized. The SAP specified that baseline age, baseline ALSFRS-R, and del-FS, the rate of disease progression prior to entering the study, were to be the covariates in the survival analysis.

Statistical Reviewer's Comment:
Compared to the prespecified SAP, the supplementary SAP for survival added baseline ALSFRS-R as a covariate and this may have been influenced by the initial survival analysis of the OLE. This first survival analysis of the OLE data was presented at the March 12, 2020 Type C meeting at which time the Applicant reported that the p-value for time to death was smaller when baseline ALSFRS-R was added as a covariate.

Main analysis: Death
The main analysis was to compare time to death between the two treatment regimens. The date of death was to be initially recorded on the CRF. **The vital status sweep may have captured additional deaths and associated death dates through the cut-off date of February 29, 2020**.

Statistical Reviewer's Comment:
There were additional vital status sweeps after February 29, 2020 to which the same comment would apply correspondingly.

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 were to 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 was to 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 was to be used as the definitive date if it occurred before February 29, 2020.
- The OLE snapshot may capture death dates that are later than February 29, 2020. In these cases, subjects were to 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 were to 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 was to be estimated overall using the Kaplan-Meier method. The hazard ratio of death comparing the Original Active group (AA) to the Original Placebo group (PA) was to 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 **was to 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. When the sample size is large, the likelihood ratio test generally 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 would 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: $H_o b = 0$ 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 are 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, this test statistic is χ^2 with 1 degree of freedom.

The analysis was to 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.

Supportive analysis: Death or death equivalent
A supportive analysis was to compare time to death or death equivalent between the two treatment regimens. Death and death equivalent events were to be tabulated by treatment regimens. As mentioned in the SAP, the data for death equivalent events were to be recorded in the locked CENTAUR database and an interim snapshot of the OLE's database. The OLE database may have still been undergoing data collection at the time of the data snapshot for analysis.

For death equivalent events:

- Tracheostomy: analyses were to 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 were to apply:

- The vital status sweep may capture additional deaths for those who were previously recorded as alive or lost to follow-up.
- The analysis was to 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 was to be used as the definitive date.
- The OLE snapshot may have captured death dates or death equivalent dates that are later than February 29, 2020. In these cases, subjects were to 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 was to assume that these subjects did not have a death equivalent event in this period and was to 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, were to 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 were to 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 was to 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 was to 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 was to use the likelihood ratio test as described above.

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

Sensitivity analyses
Sensitivity analyses were to 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).

Additional exploratory analyses
Additional analyses were to explore the effect of concomitant riluzole, edaravone, or both on survival. Variables indicating the following may be explored descriptively and/or included as covariates in exploratory analyses for mortality:

- Edaravone use at baseline
- Riluzole use at baseline
- Edaravone and riluzole use at baseline

No analysis was planned to explore the relationship of post-baseline initiation of edaravone or riluzole on survival or combined survival.

The SAP further states that “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”.

Other analyses may explore how cumulative time on active treatment affects survival. These analyses were to include all patients who received active treatment starting from the time of exposure to active drug. Analyses may include plotting a single Kaplan-Meier curve for survival from time of exposure to active drug and examining for changes in trajectory over time; a Cox proportional hazard model with survival time as the outcome including prognostic variables as covariates; and potentially including a time-varying covariate of time on active drug since those who withdrew or were lost-to-follow up will not be on active drug.

Protocol Amendments

The OLE was added to the main study protocol in Version 3.0.
In Version 6.0, the OLE was extended from 52 weeks to a total of 132 weeks

6.2.2 Study Results

Financial Disclosure

Same as Controlled Study (See Appendix)

Patient Disposition

Ninety-seven (97) subjects who completed the AMX3500 double-blind main study on study medication were eligible for enrollment into the OLE. In addition, 1 subject who had a brief (approximately 1 week) drug disruption at the very end of the main study was also permitted to enter the OLE. Of these, 90 subjects continued into the OLE, 34 of whom had been originally randomized to placebo and 56 who had been originally randomized to active drug.

Source: Study AMX3500 OLE CSR, page 45

Only 2 subjects completed the study. The most common reasons for study discontinuation were subject discontinuation of trial participation and death (Table 29).

Table 29 Reasons for Discontinuation (Safety Population)

	PA (N=34) N (%)	AA (N=56) N (%)	Overall (N=90) N (%)
Total Discontinuations	34 (100)	54 (96.4)	88 (97.8)
Withdrawal by subject	18 (52.9)	33 (58.9)	51 (56.7)
Adverse event	1 (5.4)	3 (2.9)	4 (4.4)
Disease Progression	3 (8.8)	9 (16.1)	13 (13.3)
Termination of Participation by Subject	10 (29.4)	15 (26.8)	25 (27.8)
Withdrew Consent	3 (8.8)	6 (10.7)	9 (10)
Subject Transitioning to compassionate use protocol	1 (2.9)	0	1 (1.1)
Subject died	11 (32.4)	8 (14.3)	19 (21.1)
Termination of Participation by Investigator	1 (2.9)	3 (5.4)	4 (4.4)
Termination of Participation by Applicant	4 (11.8)	7 (12.5)	11 (12.2)
Lost to follow-up	0	3 (5.4)	3 (3.3)

Source: Adapted from Applicant's Table T14.1.1 Verified by Clinical Reviewer

Protocol Violations/Deviations

Most of the protocol violations were related to an Informed Consent violation, dosing compliance, and visit outside the window. These protocol violations do not impact the survival analyses.

Table of Demographic Characteristics

Demographic characteristics were balanced for those entering the OLE phase (Table 30 and Table 31)

Table 30 Demographic Characteristics (Safety Population)

Demographic Parameters	Treatment Group		
	PA (N=34) n (%)	AA (N=56) n (%)	Total (N=90) n (%)
Sex			
Male	24 (70.6)	43 (76.8)	67 (74.4)
Female	10 (29.4)	13 (23.2)	23 (25.6)
Age at OLE Enrollment			
Mean years (SD)	58.5 (7.4)	57.8 (10.2)	58.0 (9.2)
Median (years)	59.0	60.0	59.0
Race			
White	33 (97.1)	52 (92.9)	85 (94.4)
Black or African American	0	1 (1.8)	1 (1.1)
Asian	1 (2.9)	2 (3.6)	3 (3.3)
Other	0	1 (1.8)	1 (1.1)
Ethnicity			
Hispanic or Latino	1 (2.9)	4 (7.1)	5 (5.6)
Not Hispanic or Latino	33 (97.1)	52 (92.9)	85 (94.4)

Source: Clinical and Statistics Reviewer Analysis

Other Baseline Characteristics (e.g., disease characteristics, important concomitant drugs)

Table 31 Other Key Baseline Characteristics

Demographic Parameters	Treatment Group		
	PA (N=34) n (%)	PP (N=56) n (%)	Total (N=90) n (%)
Time Since onset of ALS Symptoms (Months)			
Mean (SD)	18.9 (3.8)	19.3 (4.32)	19.2 (4.11)
Median	18.8	20.4	19.1
Time Since onset of ALS Diagnosis (Months)			
Mean (SD)	11.8 (3.5)	11.7 (3.8)	11.7 (3.6)
Median	11.1	10.8	11.0
Family History of ALS			
Yes	4 (11.8)	4 (7.1)	8 (8.9)
Unknown	2 (5.9)		
DEL-FS			
Mean (SD)	1.05 (0.73)	0.95 (0.47)	0.98 (0.58)
Median	0.82	0.90	0.89
ALSFRS-R Total Score			
Mean (SD)	30.0 (9.16)	30.3 (8.47)	30.1 (8.69)
Median	32.5	32.0	32.0
Use of Edavarone at or prior to entry			
Yes	16 (47.1)	27 (48.2)	43 (47.8)
Use of Riluzole at or prior to entry			
Yes	28 (82.4)	42 (75)	70 (77.8)
Use of either Riluzole or Edavarone prior to entry			
Yes	30 (88.2)	46 (82.1)	76 (84.4)

Note: Analysis from baseline of OLE Source: Clinical and Statistics Reviewer Analysis

Treatment Compliance, Concomitant Medications, and Rescue Medication Use

Compliance was lower in the OLE phase compared to controlled phase; however, it was similar between treatment groups (Table 32). The number of drug administration distributions was also similar between treatment groups.

Table 32 Compliance in the OLE Phase.

	Treatment Group		
	PA (N=34) n (%)	AA (N=56) n (%)	Total (N=90) n (%)
Study Drug Compliance (%)			
N	33	55	88
Mean (SD)	85.2 (19.0)	86.8 (23.1)	86.2 (21.6)
Median	90.6	93.3	92.4

Source: AMX3500-OLE CSR Table 7, page 56

Efficacy Results - Primary Endpoint

The primary endpoint of the OLE was safety, and all efficacy endpoints were secondary. These analyses only included patients enrolled in the OLE. Additionally, the applicant conducted a number of survival analyses based on death alone and also with death equivalents (e.g., tracheostomy and hospitalizations). The determination of death was based on a vital status search that included patients from AMX0035 who enrolled in the OLE and follow-up of those who did not enroll in the OLE.

Data Quality and Integrity

No CRFs were available for some sites and or subjects. Many subjects discontinued the study. The clinical care in these subjects after discontinuation is unknown.

Efficacy Results

Rate of progression on the ALSFRS-R Total Score at Week 24 of OLE

The Applicant assigned the rate of progression on the ALSFRS-R total score at Week 24 of OLE (Week 48 overall) as the first secondary endpoint in the hierarchical order for secondary endpoints. The Applicant reported an extended slope treatment difference of 4.23 points in total ALSFRS-R scores (p=0.0239)

Clinical Reviewer's Comment
There was significant attrition of patients due to patient discontinuations from the study within the 24 weeks of OLE (48 weeks overall), rendering the extended slope analysis uninterpretable. In addition, all the statistical concerns of linearity assumption associated with the slope analyses of CENTAUR Study also apply to the extended slope analyses from CENTAUR OLE Study. Out of 34 enrolled in the OLE in the RP group, only 19 remained at Week 48, and out of the 56 enrolled in the RA group, only 36 had Week 48 data on ALSFRS-R (i.e 55/135 mITT subjects remained at Week 48). Furthermore, Dr. Massie confirms that by Week 48 (Day 336) there are 10 (21%)

placebo and 13 (15%) drug death events which are ignored in this analysis, thus it is likely biased. With such a high proportion of deaths and missing data the Applicant's OLE ALSFRS-R slope analysis to Week 48 is not an interpretable analysis (ignores deaths and very high proportion of possibly informative missing data).

Therefore, the Applicant's extended slope analysis results are inconclusive of treatment benefit due to high proportion of missing data at week 48. Similar analyses have been reported for ATLIS and SVC as 3rd and 4th secondary endpoints, where even fewer subjects have data on ATLIS and SVC at Week 48. These analyses have limited interpretability and hence are not reported in this review.

In addition, the role of potential unblinding due to the bitter taste and/or acute gastrointestinal symptoms of treatment remains unclear, especially in patients who were switching from placebo to active drug in the OLE.

***Survival Analyses from Double-Blind Through OLE Phase (*Dr. Tristan Massie)**

Non-participation in the extension study was moderately high and slightly higher for those originally randomized to drug: 29% in the placebo group and 37% in the AMX0035 group did not participate in the extension study.

There is a multiplicity of survival analyses for the OLE due to the initial analysis prior to any vital status sweep, followed by several vital status sweeps (various censoring dates of February 29, 2020, July 20, 2020, and March 1, 2021). The Applicant first reported analyses of OLE survival endpoints in a Type C meeting on March 12, 2020, based on a **25 September 2019 cutoff (log rank reported as p=0.0621** for death or death equivalent and, also **with an additional covariate of baseline ALSFRS-R, p=0.0380** in the mITT population. The survival sweeps were done as a further check on ongoing survival data. An effect on survival was not expected since it was second in the hierarchy of secondary endpoints after ALSFRS-R progression and lower in the order for the double-blind period. The p-value for time to death is not very small and not persuasive. The p-values seem to be trending higher with the final vital status sweep of March 1, 2021, having a p-value of 0.0518 for Overall Survival in the ITT population (based on the supplementary SAP specified likelihood ratio test for survival comparison).

The Applicant reported a possible treatment effect on Survival endpoints in the Open Label Extension as shown for several data cutoffs in Table 33.

Table 33 Applicant's Analysis: Key Study Events: Death, Tracheostomy, and First Hospitalization

	July 20, 2020 Data Cutoff				March 1, 2021 Data Cutoff			
	Median Survival Estimate (Months)				Median Survival Estimate (Months)			
Population and Outcome	RA+SOC	RP+SOC	Hazard Ratio [95%CI]	P-Value	RA+SOC	RP+SOC	Hazard Ratio [95%CI]	P-Value
mITT	N=87	N=48	-	-	N=87	N=48	-	-
Time to First Hospitalization, Death, or Death Equivalent (Original SAP)	14.8	9.8	0.532 [0.349, 0.811]	0.0034	14.8	10.0	0.615 [0.408, 0.925]	0.0196
Time to First Hospitalization	Not Reached	14.1	0.564 [0.335, 0.949]	0.0311	31.8	14.1	0.595 [0.355, 0.996]	0.0482
Time to Death	25.8	18.9	0.540 [0.330, 0.884]	0.0143	23.5	18.7	0.619 [0.399, 0.951]	0.0324
Time to Death or Death Equivalent	25.8	18.5	0.514 [0.315, 0.837]	0.0074	23.5	17.9	0.597 [0.387, 0.923]	0.0203
ITT	N=89	N=48	-	-	N=89	N=48	-	-
Time to death (From Supplemental SAP)	25.8	18.9	0.567 [0.348, 0.923]	0.0226	23.5	18.7	0.644 [0.416, 0.995]	0.0475
Time to First Hospitalization, Death, or Death Equivalent	14.8	10.0	0.548 [0.360, 0.833]	0.0049	14.8	10.0	0.631 [0.420, 0.940]	0.0264
Time to First Hospitalization	Not Reached	14.1	0.574 [0.342, 0.964]	0.0360	31.8	14.1	0.605 [0.362, 1.011]	0.0552
Time to Death or Death Equivalent	25.8	18.5	0.539 [0.333, 0.874]	0.0122	23.2	17.9	0.621 [0.403, 0.957]	0.0308

Abbreviations: CI = confidence interval; mITT = modified intent to treat population; RA = randomized to AMX3500 in the main phase, subjects who continued in

Source: Study AMX3500 OLECSR Page 61

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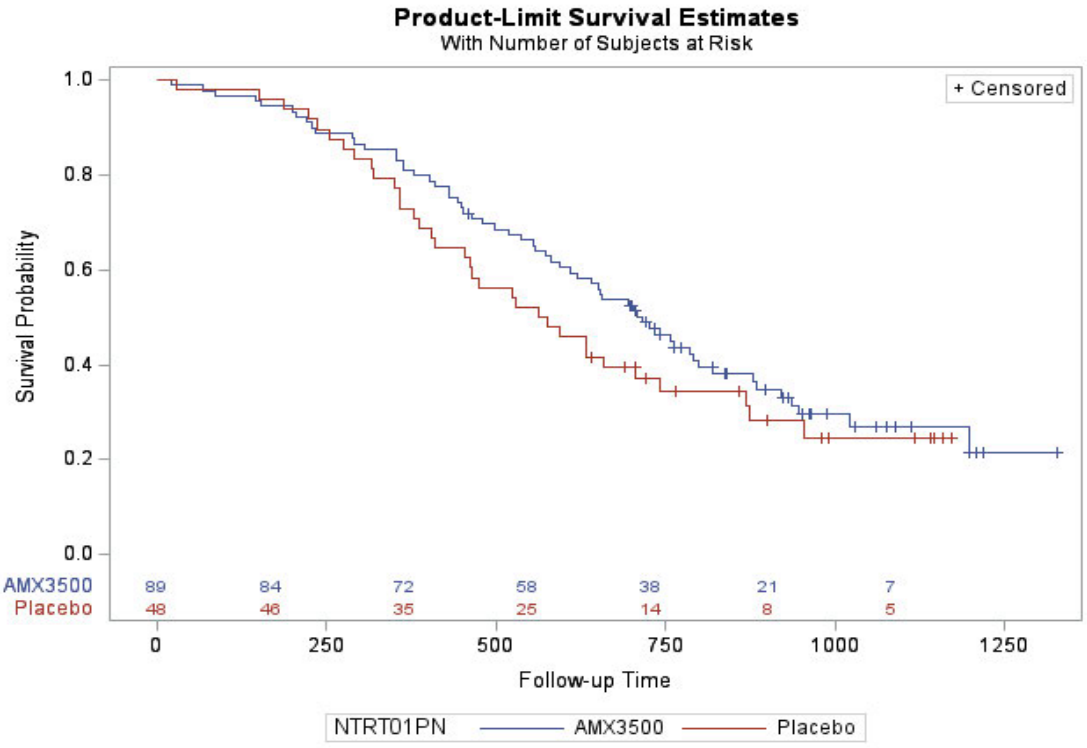
Statistical Reviewer's Comment

Time to death was not the primary survival endpoint specified in protocol; rather, the composite of death, tracheostomy, and permanent ventilation (>22 hours/day) was the prespecified primary survival endpoint. This is a multiplicity issue in addition to the elevation of the time to death survival endpoint, in general, compared to the protocol. The survival composite was listed 2nd among secondary endpoints. An analysis of the components including time to death was specified, but time to death was given no priority over the other two components or the composite itself, and time to death alone was not specifically listed among endpoints or objectives.

The Applicant is reporting the **Cox proportional hazards model** parameter tests in Table 32, which looks slightly better, e.g., **ITT p=0.0475 for Time to Death with March 1, 2021 cutoff**, and not the supplementary **SAP for survival specified Likelihood ratio test for survival** which looks

slightly worse**, p=0.0518**, for this analysis. For the mITT population using the March 1, 2021 cutoff, the Time to death p-value based on the Likelihood ratio test specified primary in SAP is p=0.0359.

Figure 13 Overall Survival-not Adjusted for Covariates (mITT: March 1, 2021 cutoff date)



Source: Statistical Reviewer Analysis

Without the baseline ALSFRS-R as a covariate, since it was not prespecified as such, but with the prespecified covariates of age and Del-FS, the hazard ratio for time to death in the ITT population with **March 1, 2021 cutoff is 0.635 with a 95% confidence interval of (0.411, 0.982) and the p-value is 0.0453**.

Given the unexpected time to death result, as evidenced by it being a non-prioritized secondary endpoint, and it coming from a less well- controlled, open-label extension study in which a significant proportion chose not to enter, it is important to assess the robustness of the Applicant's reported result.

The statistical reviewer found that the exclusion of an outlier patient (b) (6) increases the ITT time to death likelihood ratio p-value to 0.0558 (or even the treatment parameter test p-value reported as 0.0475 to 0.0510), with corresponding hazard ratio estimate of 0.651, 95% C.I. of

[0.423,1.002]. For the mITT population, the p-value increases to 0.0395 without this patient. This AMX0035 patient had del-FS (1.91) and age (73) both above the 95th percentiles, the worst observed baseline ALSFRS-R (18), and lived to at least day 953 with only about 500 days on drug, i.e., was censored at this length of follow-up. This subject has the biggest likelihood displacement as well as the biggest LMAX (eigenvector element), two statistical measures of the influence of individual observations on the overall result.

Another patient, (b) (6), a AMX0035 subject who started edaravone post-baseline and had 518 days (38 weeks according to Applicant) of treatment and died at day 1197, has a big impact on the treatment difference estimate such that exclusion of this patient results in a p-value of 0.0693 (HR=0.6681 with 95% C.I. of [0.43, 1.03]). The mITT time to death analysis likelihood ratio p-value increases to 0.0524 HR=0.64 [0.42, 1.00] without the latter patient (Table 34).

Table 34 Implications of Single Outliers

Exploratory exclusion of single subject	Hazard Ratio	95% C.I.	p-value	Comment
(b) (6)	0.6509	(0.4230,1.0018)	0.0558	Had treatment for 500 days, lived to Day 953
(b) (6)	0.6681	(0.43, 1.03)	0.0693	Started edaravone post baseline, had treatment 518 days, and died on Day 1197

Source: Statistical Reviewer Analysis

The point of these exploratory exclusions is to assess the robustness of the survival (time to death) result and it seems that the significance can be overturned by a single patient which means it is not very robust and not statistically persuasive.

Table 35 shows the impact of including additional deaths with event dates beyond the March 1, 2021, cutoff and one unclear case treated as a survivor by the Applicant but having a record of a funeral.

Table 35 Impact of Handling of Some Additional Unaccounted Death in the Survival Analysis

Data Cutoff	Total N	Hazard Ratio	95% C.I.	p-value	Additional Death Handling
03/01/2021	135	0.619	(0.399, 0.960)	0.0359*	excludes 2 early drug deaths both dosed
03/01/2021	135	0.629	(0.406, 0.973)	0.0413	extra event based on funeral record*
03/01/2021	137	0.644	(0.416, 0.995)	0.0518	includes 2 early drug deaths both dosed
03/01/2021	137	0.653	(0.423, 1.008)	0.0591	extra event based on funeral record
03/01/2021+	137	0.704	(0.460, 1.077)	0.1109	include death records after 03/01/21

*Applicant Excludes 2 early drug deaths both randomized and dosed
+ includes 4 drug and 1 placebo death dates after 03/01/2021
Source: Statistical Reviewer Analysis

The snapshot of extra death event based on the funeral record is shown in Table 36.

Table 36 A Snapshot of the Survival Sweep Dataset showing a Questionable Event

	STUDYID	DOMAIN	USUBJID	SSSEQ	SSTESTCD	SSTEST	SSORRES	SSSTRESC	VISITNUM	EPOCH	SSDTC	SSDY
170	AMX3500OLE	SS	AMX3500 (b) (6)	1	SURVSTAT	Survival Status	Alive as of (b) (6)	ALIVE			(b) (6)	717
171	AMX3500OLE	SS	AMX3500	2	SURVSTAT	Survival Status	Alive as of	ALIVE				811
172	AMX3500OLE	SS	AMX3500	3	SURVSTAT	Survival Status	Subject is deceased. Record of funeral for (b) (6) but no record of death found	ALIVE				988

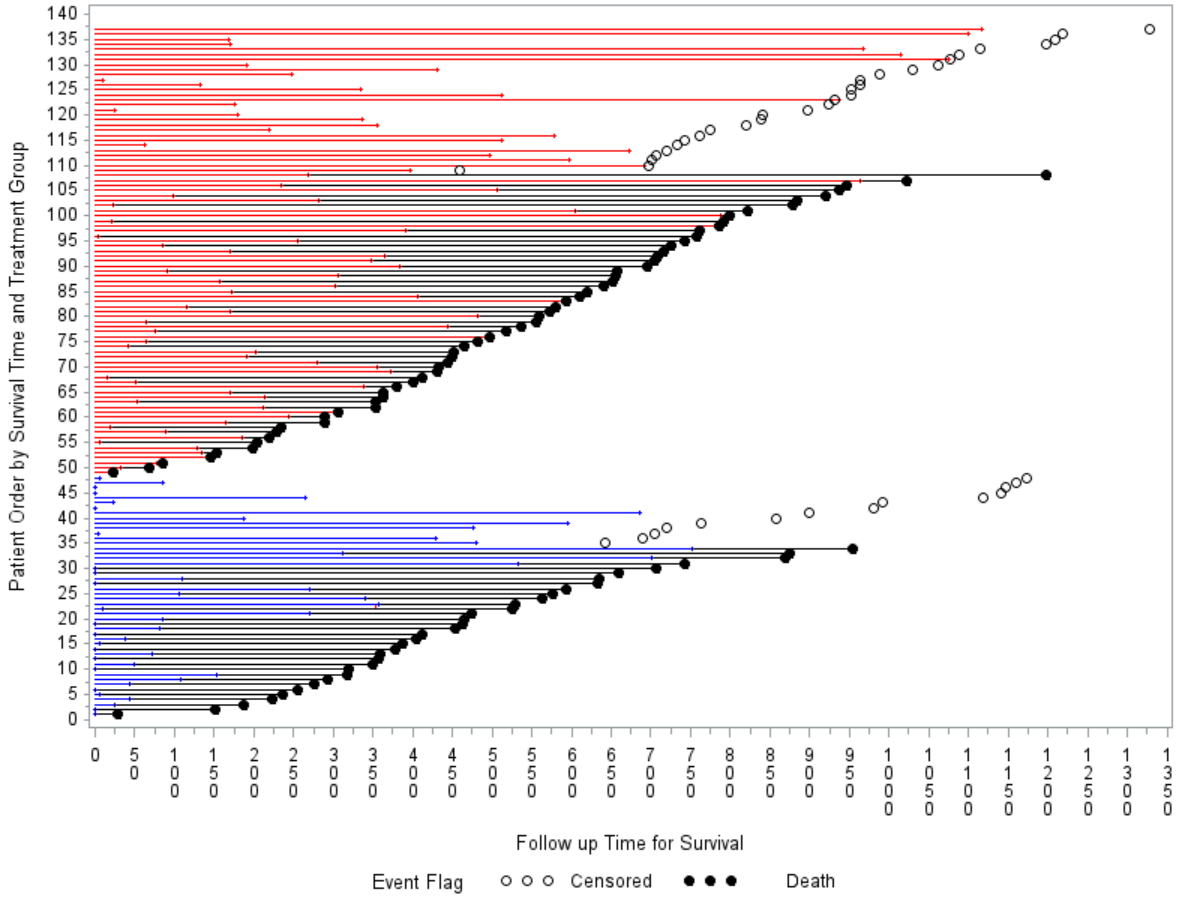
Counting **additional deaths after March 1, 2021,** the likelihood ratio test using the covariates in the supplementary SAP for survival (time to death in the ITT population) increases **p-value to 0.1109**, hazard ratio 0.70 (0.46, 1.08) as shown above in Table 34.

Corresponding to the March 1, 2021, cutoff for the ITT Cox proportional hazards model survival analysis of Time to Death, there is an estimated 152-day difference in median survival between the groups.

Figure 14 shows the exposure to study drug as well as survival follow-up time by patient through the OLE, with the patients ordered by treatment and survival time. The red lines indicate the time to treatment discontinuation for drug, and the blue lines indicate the time to treatment discontinuation for placebo/delayed start AMX. The black lines indicate the amount of time off treatment until death. Filled black circles indicate deaths and empty circles indicate

follow-up for survivors/censored patients. The first 168 days would be on placebo for the delayed group, i.e., during the double-blind period. One can see that both groups have patients with significant time off drug.

Figure 14 OLE Survival and Exposure Time by Treatment and Event Status (ITT)

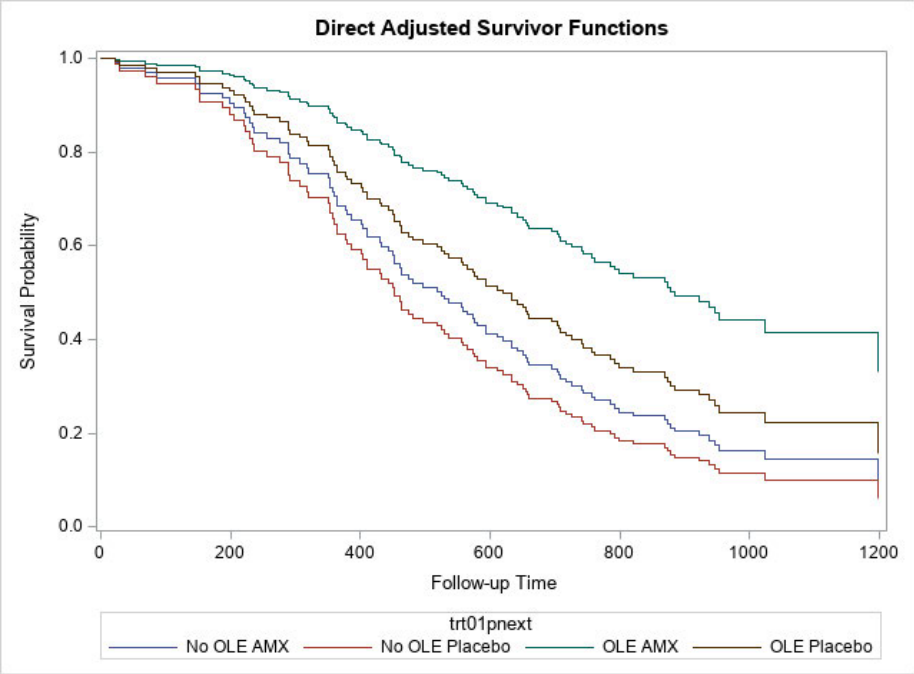


Source: Statistical Reviewer Analysis

The high proportion of subjects not entering the open label extension complicates the validity of the assumptions and interpretability of the OLE survival analysis. For example, the hazard is higher for those who did not enter the OLE (Figure 15), but the model does not account for this and this is a non-random subset, so it is not clear how to address this problem in a way that avoids possible bias (hazard ratio of no OLE over OLE is estimated as 2.304, 95% C.I. [1.513, 0.510]). There was no new randomization for the OLE. An effect of treatment on survival is also unclear for those who did not enter the OLE, the estimated hazard ratio in this non-random

subset is 0.7779 ,95% C.I. [0.3810,1.5886]. Figure 15 shows the exploratory estimated survival curves by both extension participation and treatment group.

Figure 15 Association of OLE Participation with Overall Survival (ITT)



Source: Statistical Reviewer Analysis

If we look at the hazard ratio over subsequent quarters of the randomized population defined by enrollment date, we see that the hazard ratio was much lower in the 2nd quarter of patient enrollment and highest in the last quarter (Table 37). Ideally, the hazard ratio should be relatively consistent across these time periods, but since it is much smaller in period 2, that could be related to an outlier.

Table 37 Relationship of Enrollment Quartile and Hazard Ratio

Enrollment Quartile	Placebo Deaths/N	Drug Deaths/N	Hazard Ratio	HR 95% CI
1	7/12	17/23	0.657	0.231 - 1.866
2	10/12	15/23	0.343	0.133 - 0.880
3	10/12	16/23	0.665	0.264 - 1.674
4	7/12	12/20	0.751	0.272 - 2.073

Source: Statistical Reviewer Analysis

* a placebo death at day 29 AMX3500-(b) (6) treated for 27 days according to EX dataset and with survival just 7 days longer than AMX3500 death excluded from mITT analysis due to lack of post-baseline ALSFRS-R assessment. According to compliance data (b) (6) consumed 21 of 98 doses dispensed

An analysis excluding the first 27 patients to assess sensitivity to the randomization implementation error, gave an estimated hazard ratio of 0.65 with a 95% confidence interval of (0.40, 1.05). The corresponding likelihood ratio test p-value is 0.0817.

Overall exposure across the OLE did not differ greatly by treatment group (Table 38).

Table 38 Exposure to Study Medication in the OLE (not counting DB)

Duration in Weeks	PA N (%)	AA N (%)
0-3	5 (14.7)	2 (3.6)
3-12	8 (23.5)	8 (14.3)
12-18	5 (14.7)	3 (5.4)
18-21	0 (0)	4 (7.1)
21-24	1 (2.9)	2 (3.6)
24-27	1 (2.9)	8 (14.3)
27-33	0 (0)	5 (8.9)
33-48	4 (11.8)	7 (12.5)
48-72	5 (14.7)	7 (12.5)
72-96	2 (5.9)	3 (5.4)
>96	3 (8.8)	7 (12.5)

Source: Statistic Reviewer Analysis

Death Equivalents

The number of death equivalents including PAV and tracheostomy are shown in Table 39. Given the low numbers of PAV and Tracheostomy, KM analyses were not performed.

Table 39 Death Equivalents (PAV and Tracheostomy)

	PA (N=34)	AA (N=56)
	n (%)	n (%)
Permanent Assisted Ventilation	2 (6%)	8 (14.3%)
Tracheostomy	1 (3%)	2 (3.6%)

Source: Clinical Reviewer

Statical Reviewers Comment:
Given the high proportions not participating in the open label extension it seems questionable whether the death equivalent analysis is reliable because death equivalent events may not have been captured in those subjects not participating in the OLE or who had discontinued from the open label extension.

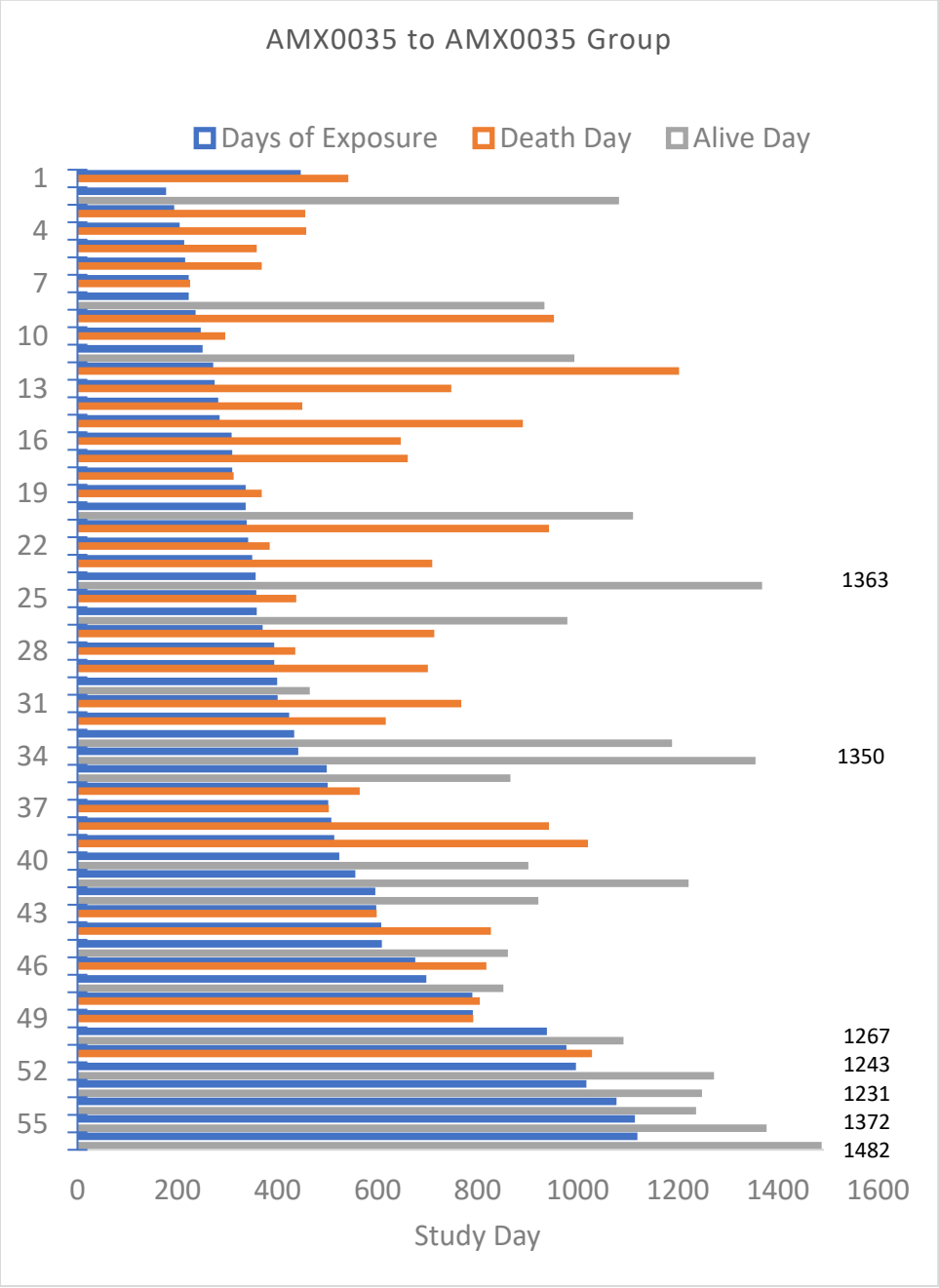
Clinical Reviewer's Comments:
The survival analyses were based on the vital sweep data obtained from all 137 subjects even if they discontinued the study. The following Figure 17 gives a visual appreciation of the dead/alive data in relation to the duration of treatment with AMX0035. These analyses are not based on statistical comparison but are visual representation of raw data. The blue bars represent the number of days of exposure to treatment in the study (Study Day plotted on the X-axis). The orange bars represent the death day of the subject whereas, the grey bars represent the day until which the subject was reported live based data cut-off date of March 1,2021, for the Applicant reported survival analyses.

Figure 16 (A, B, C) have been plotted for the following groups:

A. AMX0035 to AMX0035 group (All subjects that were on AMX0035 in the controlled study and remained on AMX0035 in the OLE Phase)
B. Placebo to AMX0035 group (All subjects that were on Placebo in the controlled study and transitioned to AMX0035 in the OLE Phase
C. All subjects that did not enter OLE phase, whether on AMX0035 or Placebo in the controlled phase

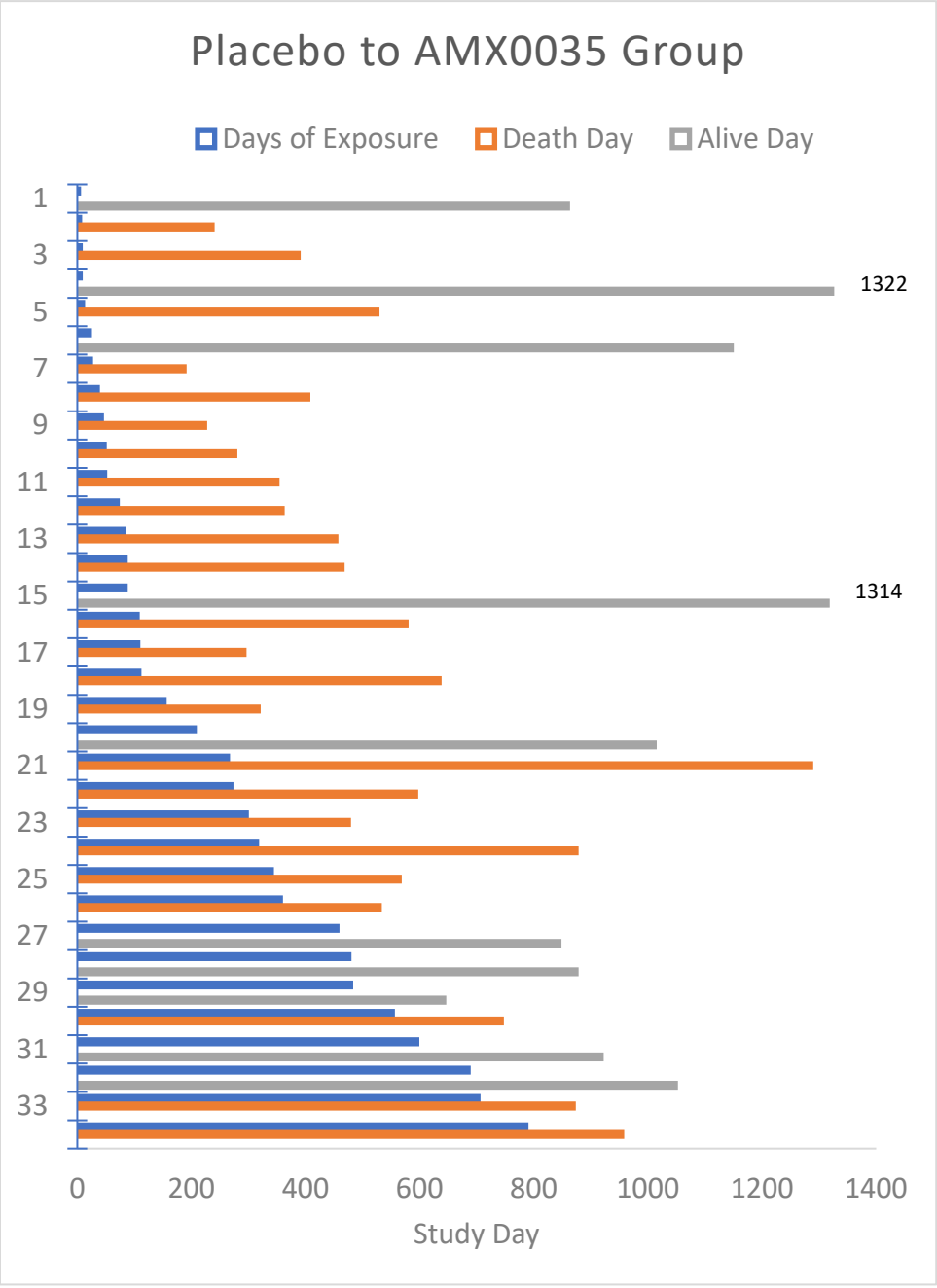
Figure 16 Relationship Between Days of Exposure and Dead/Alive Status

(A) AMX0035 to AMX0035 Group

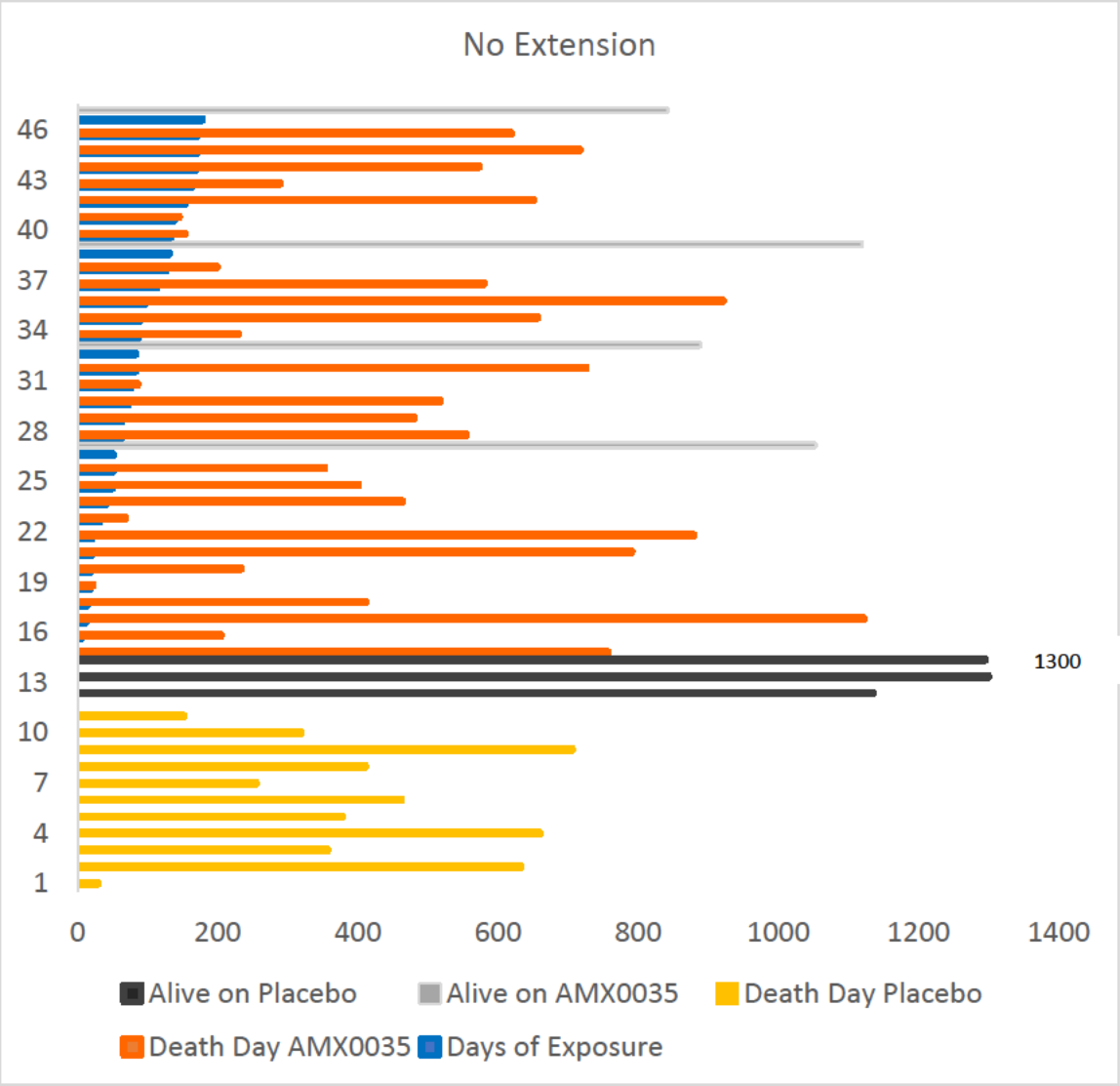


Source Clinical Reviewer Analyses

(B) Placebo to AMX0035 Group



(C) No extension group

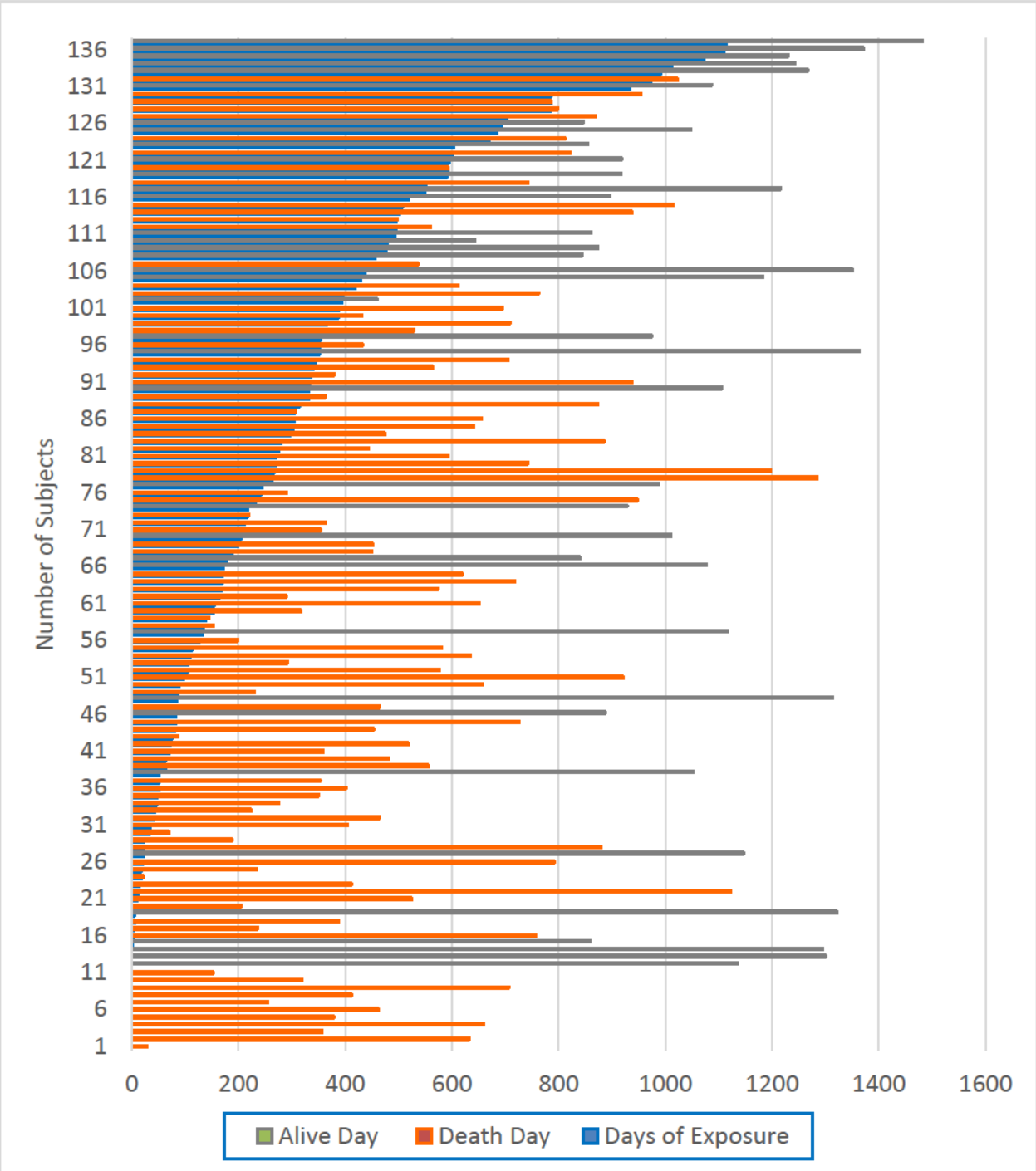


Note: The Dark and Light orange bars represent death day on AMX0035 and Placebo respectively and Black and Grey represents subjects alive on AMX0035 and Placebo respectively (see Legend)

These figures show that subjects survived to similar number of days irrespective of duration on AMX0035. There were subjects who lived for 1300 days on placebo without enrolling in the OLE, and there were subjects that were on AMX0035 >1000 days that lived for 1231-1267 days. This supports that the ALS disease trajectories are very heterogenous, therefore survival outcomes are challenging to interpret in small sample sizes. In addition, the clinical care of patients that discontinued from the study is not known since vital sweeps were taken long after

the subjects discontinued from the study. An overall graph including all subjects is shown in Figure 17.

Figure 17 Relationship Between Exposure and Survival Status of Subjects



It can also be seen that the two subject with similar maximum exposure have lived for 1372 and 1482 days showing a difference of 110 days (3.6 months). In conclusion, the survival benefit from this study is challenging to interpret as all subjects have received drug for varied durations and clinical care post discontinuations are not known. Therefore, it is unclear how much of the survival benefit is by chance alone or disease heterogeneity, rather than effect of the drug based on this small study sample size.

Clinical Reviewer's Comment:

There were 3 subjects in the Exposure dataset that had missing end of treatment date. This reviewer tried to verify the end of treatment date in the Drug Accountability dataset, however, the end of treatment date provided in the Survival Dataset could not be verified. Two of these subjects were amongst the top 4 that lived the longest on AMX0035 (Death Day 1363 and 1350). No CFR was provided for these subjects. An information request was sent to the Applicant. The Applicant indicated that these subjects were lost to follow-up. The end of treatment date was imputed based on that the lost to follow-up date. Therefore, the exposure duration in these cases may not be accurate.

Overall disease progression parameters (death, feeding tube, hospitalization, PAV and tracheostomy) based on treatment assignment in the controlled phase is shown in Table 40. The robustness of these data are unclear as there is no information regarding the clinical care that patients may or may not have received after discontinuation from the study, including the possibility of tracheostomy, additional hospitalizations, and/or other experimental treatments received.

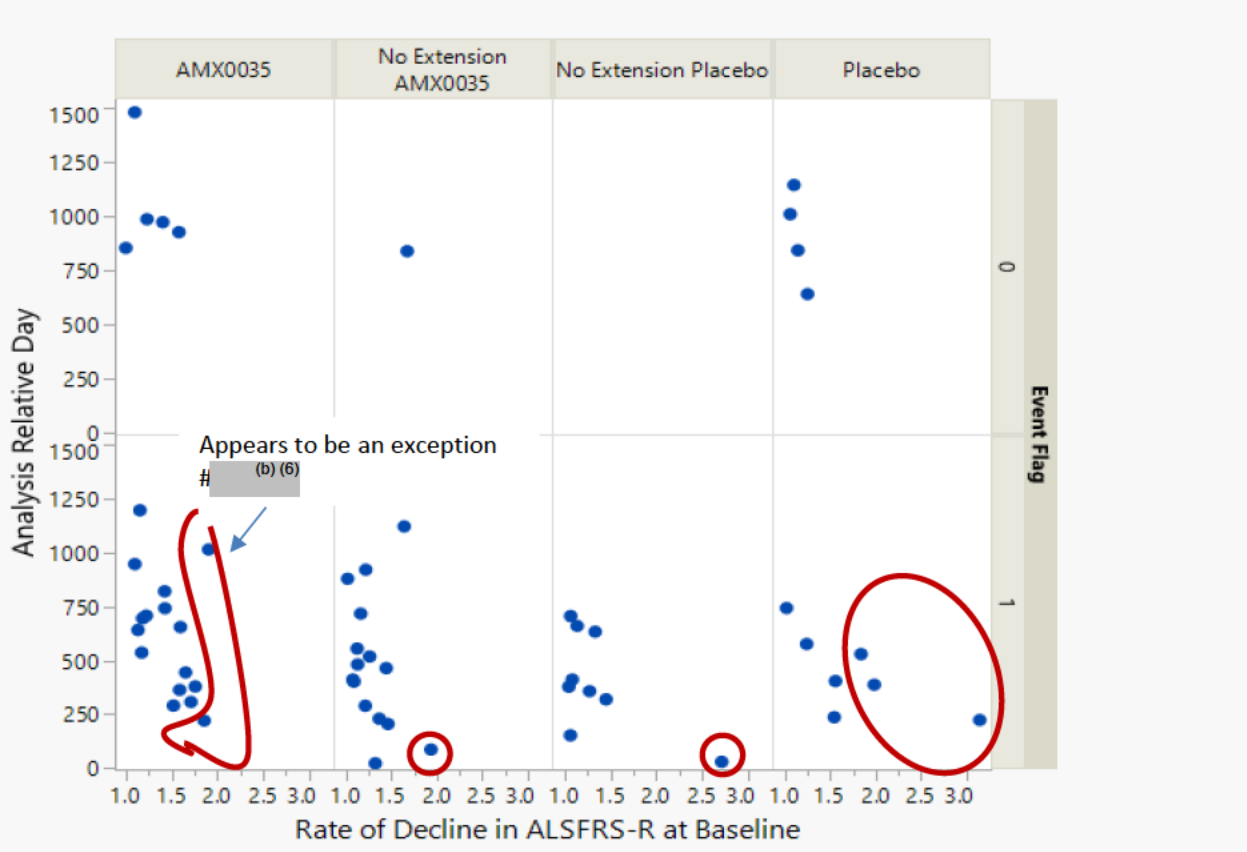
Table 40 Parameters of Disease Progression

Parameter	AMX0035 (N=56)	No Extension AMX0035 (N=33)	No Extension Placebo (N=14)	Placebo (N=34)
Death	35 (63%)	29 (88%)	11 (79%)	24 (71%)
Feeding Tube	26 (46%)	5 (15%)	3 (21%)	17 (50%)
Hospitalized	34 (61%)	6 (18%)	4 (28%)	22 (65%)
Permanent Assisted Ventilation	8 (14%)	0	1 (7%)	2 (6%)
Tracheostomy	2 (3.5%)	0	1 (7%)	1 (3%)

Source: Clinical Reviewer Analysis

I also explored if rate of decline in ALSFRS-R or the ALSFRS baseline score could predict prognosis in the subject, or if there were any imbalances or outliers between the groups (Figure 18). The exploratory analysis shows that a rate of decline >1.8 or 2 was predictive of earlier death regardless of treatment arm (based on the initial randomization in Study AMX0035). Subject (b) (6) *went on permanent assisted ventilation on Day 496, rate of decline in ALSFRS-R of 1.9, ALSFRS score of 18, on drug for 500 days but died on Day 953. This subject was censored in the survival analysis as the death occurred after the March 2021 cutoff date. No CRFs for this site have been provided.*

Figure 18 Higher Rate of Decline had Poor Prognosis



Source: Clinical Reviewer Analyses
Note: Rate of decline in ALSFRS-R <1 not shown in the figure
Event 0 indicates alive, and Event 1 indicates death
AMX0035 indicates AA group and placebo indicates PA group.

Analysis of Subgroups (Dr. Tristan Massie)

Only 7 (5%) of those randomized were non-White, 68% were Male, and 77% were < 65 years old. The power for detecting interactions (differential treatment effects by subgroups under the

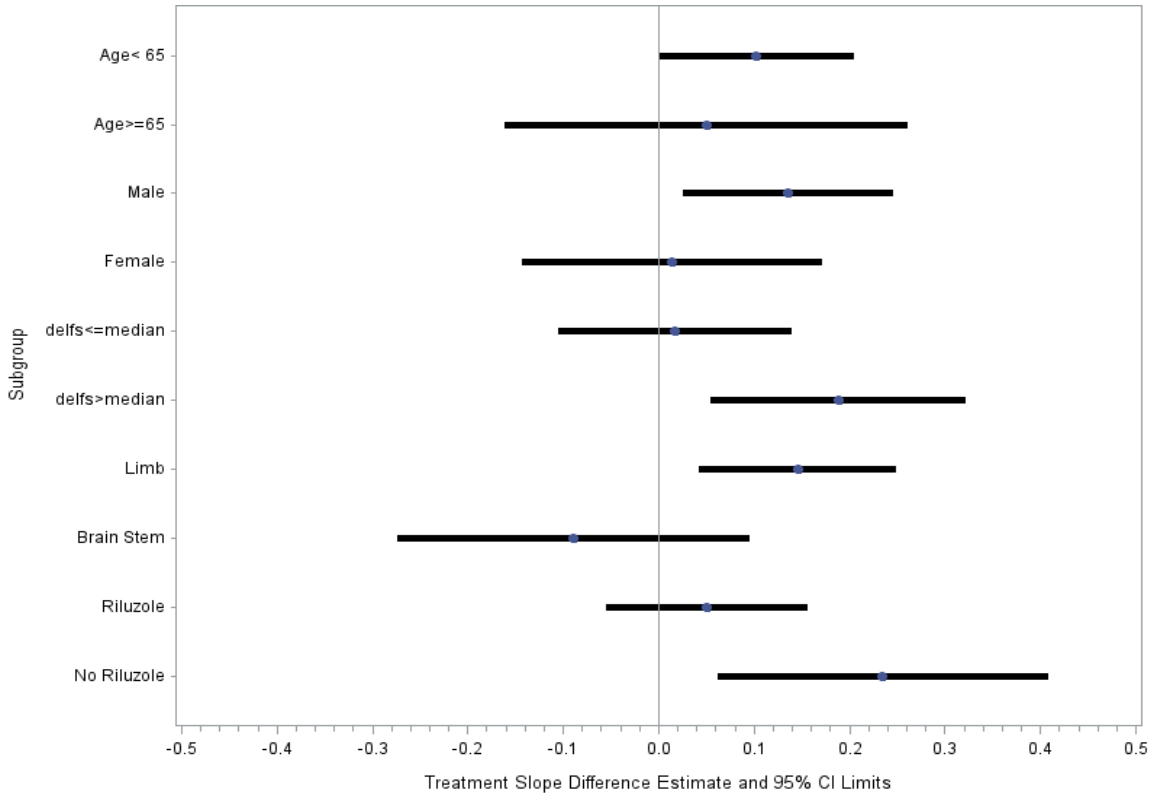
assumption that there is a treatment effect overall) may be limited since there were only 44 randomized Females.

For the analysis of slope, the estimated difference in ALSFRS-R at Week 24 was -0.296 for Females and -3.252 for Males. The test for a differential slope by gender was not nominally significant, p= 0.2105.

Based on the slope model the week 24 in ALSFRS-R difference was estimated as -2.46 for Age <65 and -1.19 for Age>65. The test for a differential treatment difference in slope by Age < 65 vs. Age > 65 was not nominally significant, p=0.6568.

Figure 19 shows estimated slope differences between Placebo and AMX0035 within various subgroups, based on the Applicant 's primary mixed effects slope model (mITT population) of ALSFRS-R.

Figure 19 Estimated Slope Differences in ALSFRS-R within Subgroups using Applicant 's Primary Slope Model (mITT population).



Subgroup analyses for covariate adjusted survival analysis of time to death using the March 1, 20201, cutoff in the ITT population are as follows. The Hazard ratio is closer to 1.0 for Age < 65 but not significantly different from Age > 65 subgroup. (Beta= -0.39 and -0.53 respectively ratios: 0.68 [0.42,1.1] and 0.59 [0.23, 1.50]). These are not significantly different though, as an interaction test for a difference between subgroups has p=0.7858.

The estimated Hazard ratio was numerically smaller for Males 0.52 (0.33, 0.96) vs. 0.82 (0.39, 1.75) for Females but these are also not significantly different (test for difference between subgroups in treatment effects: p=0.4112).

Assessment of the Impact of Individual Sites on the Overall Results (Dr. Tristan Massie)

Both the Applicant's primary ALSFRS-R slope analysis (or joint rank analysis) in the double-blind period as well as the survival analysis for time to death through the open label extension are sensitive to exclusion of some individual sites, i.e., there are some impactful sites. These are only done as an exploratory analysis to be considered when planning site inspections; unless an issue is found in the inspection there is no justification for their exclusion. Furthermore, the effect on the estimated treatment difference is of more interest than the effect on the p-value.

Sites 701 (n=13) and 713 (n=5) have the biggest impact on the Applicant's primary slope analysis (p>0**.**10 when either site is excluded). Slope difference estimates in case of these individual site exclusions are as follows (slope difference -0.097, corresponding Week 24 estimated difference of -2.32 with both sites included):
Without Site 701: slope difference -0.079 SE=0.049 p= 0.1027 (corresponding Week 24 difference of -1.90);
Without Site 713: slope difference -0.076 SE=0.046 p= 0.1008 (corresponding Week 24 difference of -1.82).

The joint rank p-values in the mITT population are also >0.05 with either of these exploratory site exclusions (with LOCF for missing data in survivors):
Without Site 701: p=0.057 and
Without Site 713: p=0.077.

Sites 701 (n=13) and 745 (n=10) have the biggest impact on survival (time to all-cause mortality in the ITT population with March 1, 2021 cutoff date) through the OLE (hazard ratio = 0.644, p = 0.0518 with both sites included):
Without Site 701: HR=0.684 p=0.118,
Without Site 745: HR= 0.682 p=0.106.

7 Integrated Review of Effectiveness

7.2 Assessment of Efficacy Across Trials

There is only one study and its extension (CENTAUR), therefore assessment of effectiveness across trials is not performed.

7.3 Additional Efficacy Considerations

A Phase 3 randomized, double-blind controlled study is ongoing in 600 ALS patients with read-out of results in late 2023 or early 2024.

AMX0035 is a fixed-dose combination of two drugs and must meet the requirements under 21 CFR 300.50. This regulation states that two or more drugs may be combined when each component makes a contribution to the claimed effects of the product. The Applicant has a submitted a conceptual basis for the combination of sodium phenylbutyrate and taurursodiol based on the role of phenylbutyrate as a pan-histone deacetylase (HDAC) inhibitor that ameliorates endoplasmic reticulum stress through upregulation of chaperone proteins, and the potential for taurursodiol to ameliorate mitochondrial stress by reducing mitochondrial permeability and increasing the apoptotic threshold of the cell. Although the Applicant has not performed additional clinical studies to assess these claims, applying a high degree of regulatory flexibility in the setting of the severity and unmet need in ALS, and given the functional benefits observed in the AMX3500 and suggestion of a survival benefit in long-term follow-up, this mechanistic argument suffices to address the requirement.

7.4 Integrated Assessment of Effectiveness

The Applicant is seeking approval of AMX0035 for the treatment of ALS based on a single study and with confirmatory evidence.

Under section 505(d) of the Federal Food, Drug, and Cosmetic Act (FD&C Act), a drug's effectiveness must be established by "substantial evidence." In section 115(a) of the Food and Drug Administration Modernization Act of 1997 (FDAMA), Congress amended section 505(d) of the FD&C Act to make it clear that the Agency may consider "data from one adequate and well controlled clinical investigation and confirmatory evidence" to constitute substantial evidence if FDA determines that such data and evidence are sufficient to establish effectiveness.

The Applicant submitted the 24 Week CENTAUR Study as one adequate, and well-controlled study. For confirmatory evidence, the sponsor submitted analyses from the OLE, and a survival analysis based on vital status on all patients enrolled in CENTAUR collected following the completion of the 132-week open label extension.

As described in the FDA draft guidance on "Demonstrating Substantial Evidence of Effectiveness for Human Drug and Biological Products", reliance on only a single study will generally be limited to situations in which a trial has demonstrated a clinically meaningful effect on mortality, irreversible morbidity, or prevention of a disease with potentially serious outcome and confirmation of the result in a second trial would be practically or ethically impossible". The guidance further suggests factors when a single study plus confirmatory evidence will be adequate to support an application. "These factors may include the persuasiveness of the single trial; the robustness of the confirmatory evidence; the seriousness of the disease, particularly where there is an unmet medical need; the size of the patient population; and whether it is ethical and practicable to conduct more than one adequate and well-controlled clinical investigation". The guidance further suggests that "the strength of the single trial will affect the extent of confirmatory evidence required."

Considering these regulatory standards for approval, the following assessment can be made on how the data included in the Application meets the requirement for approval based on a single study plus confirmatory evidence or single study alone.

ALS is a serious disease and there is an unmet medical need for new treatments. Given the serious and life-threatening nature of ALS and the substantial unmet need, it is appropriate to exercise regulatory flexibility in considering the data and information submitted to the application, including the evidence intended to establish the effectiveness of the product. FDA's regulations allow for FDA to exercise regulatory flexibility in applying the statutory standards for establishing the safety and effectiveness of new therapies intended to treat persons with life-threatening and severely debilitating illnesses, especially where no satisfactory alternative therapy exists. This approach is consistent with that outlined in the ALS: Developing Drugs for Treatment Guidance for Industry (September 2019), which states: "The statutory standards for effectiveness apply to drugs for ALS just as the standards apply for all other drugs. However, FDA has long stressed the appropriateness of exercising regulatory flexibility in applying the statutory standards to drugs for serious diseases with unmet medical needs, while preserving appropriate assurance of safety and effectiveness."

I also note that although the CENTAUR study was conducted in only 137 ALS patients, a much larger Phase 3 study in 600 ALS is ongoing at the time of the submission of this application with results available in late 2023 or early 2024. Therefore, although there is an unmet need in this population, another study is neither unethical nor impractical.

The review of all data in its entirely leads to the conclusion that the statutory standards of effectiveness based on the strength of the evidence from CENTAUR as a single study alone appear to be unmet. Therefore, additional confirmatory evidence would be required.

There are limitations and weaknesses to the efficacy from the controlled study CENTAUR study and the proposed confirmatory evidence that are summarized below.

CENTAUR as the single adequate well controlled study:

Primary endpoint, Rate of decline in ALSFRS-R at Week 24:

The **Applicant reports a statistically significant mean treatment difference of 2.32 points with a modest p-value of 0.034** in favor of AMX0035 on the primary endpoint, ALSFRS-R rate of decline, between the treatment arm and placebo in the mITT population. The slope analysis assumes linearity of ALSFRS-R over time. The Applicant assessed for quadratic effects to determine whether a linear or quadratic model was more appropriate, and there were no significant quadratic effects for the prespecified quadratic model although this quadratic term is very close to the p=0.10 cutoff which would have required its use with a p=0.1016. In addition, the slope analysis does not account for death in the study. The Applicant's slope analysis also excludes 2 patients that were treated with AMX0035 and died before ALSFRS-R measurement which can introduce bias in comparisons of treatment arms.

Dr. Massie has performed a number of sensitivity analyses to explore how alternate analytical methods could impact the results from this study.

The Applicant had proposed a backup analysis plan in the SAP for a model with a quadratic term, had the linearity assumption not been met. A sensitivity **analysis performed by Dr. Massie based on this analysis plan, the Week 24 treatment difference is estimated as 1.68 points with a p-value of 0.1134.**

In addition**, in a traditional Mean-By Visit MMRM analysis of change from baseline ALSFRS-R at Week 24 performed by Dr. Massie, the mean treatment difference of 1.86 points with a p-value of 0.0749 was observed in the mITT population. Note that both this analysis and the Applicant's primary slope analysis may be optimistic due to their ignoring deaths.**

FDA guidance for Industry on ALS drug development recommends the use of joint rank analysis method for ALSFRS-R change from baseline analysis if deaths are observed in the study. There were 7 deaths in the study, 5 on AMX0035 and 2 on placebo. The joint rank analysis combines survival and function in the analysis which is more appropriate if

deaths are observed in the study. The **Applicant's post-hoc joint rank analysis reports a p-value of 0.037, that used an inappropriate missing data handling method of Last Observation Carried Forward (LOCF).** This is especially problematic in a degenerative disease such as ALS because ALSFRS-R scores tend to worsen over time in ALS whereas LOCF imputes no change from the last observed time to the final time. **Dr. Massie's joint rank analysis with a more appropriate method of handling missing data (multiple imputation based on a missing-at-random assumption) but the latter method to a lesser degree still involving untestable assumptions (thus, may still be optimistic but provides a more reasonable starting point in lieu of assuming a specific missing not at random missing data mechanism) of ALSFRS-R and death has p-value of 0.063 for the mITT population and a p-value of 0.079 for the ITT population** that includes the 2 deaths in the treatment arm who were dosed but had no post-baseline ALSFRS-R assessments.

Additionally, in the Applicant's analyses on individual domains of ALSFRS-R, only Fine Motor Domain had a nominally positive p-value lacking consistent support from all domains of ALSFRS-R.

Therefore, the evidence of effectiveness based on the primary endpoint ALSFRS-R at Week 24 is modest. Assessed by any statistical method the p-value is not highly persuasive.

Secondary endpoints:

All secondary endpoints were also slope analyses, assume linearity in change, and do not include death in the analysis; therefore, all the secondary analyses have similar concerns as the primary endpoint.

- On the pre-specified slope analysis of rate of change of ATLIS, the **Applicant reports a non-significant difference of 2.8 points in Total ATLIS scores.** Upon exploratory analyses of the ATLIS, the Applicant reports a nominally significant treatment difference of 4.3 points in favor of AMX0035 with a p-value of 0.0420 in the Upper ATLIS, and a non-significant difference of 2.1 in Lower ATLIS scores. The Statistical Analysis Plan did not pre-specify which ATLIS component (Total, Upper or Lower ATLIS) would be analyzed first, but it appears the Total ATLIS was intended as the secondary endpoint. Only the Upper ATLIS score was nominally positive using the Applicant's prespecified analysis method. **Dr. Massie conducted a sensitivity analysis using a traditional MMRM analysis which showed a treatment difference of 3.09 points in favor of AMX0035 with a p-value of p=0.1483 for Upper ATLIS. This finding suggests that the finding of nominal significance is not robust, as it is no longer significant using a different statistical approach.**

- There was a statistically non-significant treatment difference of 32.7 pg/mL in favor of placebo for the rate of decline in pNF-H with a p-value of 0.260.

- There was a statistically non-significant treatment difference of 5% in favor of AMX0035 for rate of decline in SVC with a p-value of 0.076, which is not felt to be consistent with a clinically meaningful change in SVC.

- There were no statistical differences observed in the first 24 weeks of the study between treatment groups in percentage of events for the survival outcomes including: Death events only, Death or Death Equivalents that include tracheostomy and permanent assisted ventilation, and Hospitalizations.

Overall, there is weak support from other secondary endpoints.

There were also study conduct concerns including randomization implementation error where first 18 subjects were randomized to AMX0035 and subsequent 9 to placebo. There is also concern of potential for functional unblinding due to bitter taste and adverse effects (i.e., GI symptoms occurring in higher proportion of AMX0035 patients in the first 3 weeks of the study) of the drug, and post-baseline initiation of ALS medications that were higher in the AMX0035 arm. In the double-blind period 63% of the subjects on placebo guessed accurately that they were on placebo. There were 14% missing data on guesses which add to the uncertainty on how many may have guessed accurately. These observations suggest a potential for unblinding.

The impact of these study conduct issues on the study results are difficult to assess; however, taken together, these review issues raise concerns in a small study such as CENTAUR with an endpoint that can have subjectivity in assessments.

The statutory requirement for the evidence of effectiveness from a single pivotal study would generally require statistically compelling and clinically relevant results with robust findings across centers/countries, positive findings on independent secondary endpoints, and no indications of bias within the study. In summary, although CENTAUR is an adequate and well controlled study the strength of the evidence of effectiveness from the primary and secondary endpoints and the assessments of bias within the CENTAUR study do not provide statistically persuasive evidence of effectiveness but are suggestive of potential treatment benefit in a single study.

However, given the unmet need in ALS, one is willing to seek support from confirmatory evidence to strengthen the weakness of a single trial that is not persuasive on its own. The Applicant has provided various analyses of the open label extension phase and

follow-up vital status as confirmatory evidence to support the application, which are discussed below.

Confirmatory evidence:

- CENTAUR Open-label extension as confirmatory evidence of effectiveness:

The primary endpoint for the OLE phase was safety. Several analyses were conducted with the CENTAUR Open-label extension data as supportive evidence that are discussed below:

i. Extended slope-analysis for ALSFRS-R at Week 48:
The first efficacy endpoint was the ALSFRS-R Extended slope analysis at week 48. The Applicant reports a statistically significant extended slope analysis in favor of those randomized to AMX0035 group with a p-value of 0.0239. However, open-label efficacy results on a functional endpoint are difficult to interpret. Enrollment in the OLE was optional. Only 66% of the subjects enrolled in OLE – that included 56 AMX0035-treated subjects and 34 placebo subjects. There was higher non-participation in the OLE in AMX0035 group. There were additional discontinuations during the open-label phase with only 40% patients that remained in the study at Week 48. Additionally, there were 23 deaths by Week 48, which are ignored in the slope analysis at Week 48.

Therefore, the extended slope analyses of ALSFRS-R are not interpretable to establish supportive evidence of effectiveness.

ii. Extended Slope Analyses of ATLIS and SVC at Week 48:
The extended slope analyses for ATLIS and SVC were 3rd and 4th in the hierarchy of secondary endpoints for the OLE phase. The Applicant reports a statistically significant extended slope analysis in favor of those randomized to AMX0035 group for upper ATLIS and SVC with a p=0.029 and 0.0372, respectively. There are similar concerns regarding the interpretation of extended slope analyses of ATLIS and SVC as that for ALSFRS-R.

iii. Survival Analyses:

Overall survival analyses were performed from the initial randomized phase comparing patients randomized to AMX0035 to patients randomized to placebo in a vital status sweep that was conducted multiple times during and after the controlled phase up to the 132-week open label extension phase. The Applicant's pre-specified survival analysis included a composite time to survival event analysis including death, tracheostomy, permanent-assisted ventilation (PAV), hospitalization. This survival analysis was 2nd in the hierarchy of endpoints in the open-label phase.

Survival analyses were done after multiple data cutoff dates including: September 25, 2019, February 29, 2020, July 20, 2020 and March 1, 2021. A professional firm, Omnitrace, was contracted to conduct a search (vital status sweep) based on subject's family, clinic notes, CDC national death index, social security index. The vital sweeps were completed after most subjects discontinued from the study, which reduces the rigor of any survival analyses performed on the extension study, the limitations of which are explained in the subsequent paragraphs.

The **Applicant reports a statistically significant increase in the composite time to survival events** (including death, tracheostomy, permanent-assisted ventilation, and hospitalization) in patients initially randomized to AMX0035 compared to those initially randomized to placebo in the mITT population with **a difference of 4.8 months, hazard ratio of 0.62 and p value of 0.0196.**

This analysis was performed after a few survival analyses had been performed that reported p-values of 0.0621 (with prespecified covariates of age and del-fs) and 0.0380 (with additional covariate of baseline ALSFRS-R).

In addition, the Applicant's composite survival analysis is difficult to interpret as there were a large number of dropouts during OLE, in addition to 34% non-participation in OLE. There are limitations of including tracheostomy and hospitalization data in a composite survival endpoint due to subjectivity involved in timing of tracheostomy placement and hospitalization due to differences in standard of care. These were also not systematically collected in the OLE. Therefore, there may be missing data on tracheostomy and hospitalizations after subjects terminated from the study, which were not captured in vital status sweeps. A rigorously defined outcome of permanent-assisted ventilation would have also been acceptable to be included in the definition of survival because there is less subjectivity regarding this definition. However, this data was not available for many patients in the vital status check.

There was also no information on clinical care of patients after study discontinuation, including the possibility that some patients may have received other investigational treatments which could have influenced survival.

Additional **post hoc survival analysis** including **time to death alone** was also performed after CENTAUR study was unblinded with July 20, 2020 and March 1, 2021, cut-off dates. The Applicant reports a statistically significant survival benefit on this supplemental time to death only analysis showing a median difference **of 4.8 months, HR=0.644, p=0.0475 in the ITT population, and a median difference of 4.8**

months, HR=0.62, p=0.0324 in the mITT population for the March 1, 2021, cut-off date.

Survival analysis with death alone was not included in the initial Statistical Analysis Plan as a key endpoint, but in a supplemental SAP for survival analysis of time to death alone. This analysis was performed after survival analyses from the controlled phase and multiple survival analyses from the OLE phase were performed, and the additional covariate of baseline ALSFRS-R was added. In this analysis, the Applicant reports the p-value from a Cox proportional hazard model and not the pre-specified likelihood ratio test in the supplemental SAP. **With the supplemental SAP specified likelihood ratio test, the p-value is 0.0518, with a corresponding hazard ratio of 0.64**.

There were 5 additional deaths captured after the March 1, 2021, cut-off. **Dr. Massie's exploratory survival analyses in the ITT population, including these deaths results in a hazard ratio of 0.70 and a p- value of 0.1109.**

An additional concern with the results of these survival analyses is that many patients discontinued participation in either the controlled phase or during the OLE phase; therefore, many subjects did not receive AMX0035 for long durations but have contributed to the overall survival analyses results. The March 1, 2021, cut-off date based on vital status sweep occurred long after most patients had discontinued from the study. These survival analyses are difficult to interpret as all subjects receive AMX0035 for varied durations. It was observed that some placebo patients that never received AMX0035 survived longer than those that received AMX0035 for any duration. This raises concerns whether the observed survival benefit is by chance alone or due to underlying disease heterogeneity. It is known that the time from symptom onset to death ranges from 20 to 48 months and 10–20% of ALS patients have a survival longer than 10 years (Chio 2009[10]), indicating heterogeneity in overall ALS survival.

Therefore, there is lack of statistical persuasiveness of the survival benefit.

iv. Additional survival analyses submitted on June 17, 2022 post late-cycle meeting

The Applicant reported an additional post hoc survival analysis based on Rank Preserving Structural Failure Time Model (RPSFTM) with a survival benefit of 9.7

[10] Chio A, Logroscino G, Hardiman O, et al. Prognostic factors in ALS: A critical review (nih.gov) Amyotroph Lateral Scler. 2009 Oct-Dec; 10(5-6): 310–323.

months in favor of AMX0035 (p=0.045). A methodological reference article cited by the Applicant[11] indicates that the reported new post-hoc rank preserving structural failure time survival analysis may be biased in favor of drug and increasingly so, as the proportion of placebo group switching to drug increases. The article states, "We found that analyses which re-censored usually produced negative bias (i.e., underestimating control group restricted mean survival and overestimating the treatment effect)"[1]. Further it states that "The increased switching proportion had an important impact, leading to increased bias, with the relative effect on the different adjustment methods dependent on the size of treatment effect."[1]. This switching proportion is quite high in this trial at 71% due to placebo completers switching to AMX by design of the OLE. The reference paper only studied lower switching proportions. The reference also recommended a complementary analysis in order to assess the bias. However, according to the Applicant's reference article on CENTAUR analysis, "AF [acceleration factor] could not be estimated in assessments of on-treatment RPSFTM without applying re-censoring". Thus, bias of the reported post-hoc RPSFTM analysis (and the magnitude of the bias) remains in question.

v. <u>Additional natural history analyses submitted on June 17, 2022 post late-cycle meeting:</u>

The Applicant conducted and reported additional post-hoc analyses after the March 30 advisory committee meeting. For the first of these analyses, the Applicant applied a survival prediction model developed by the European Network for the Cure of ALS (ENCALS) to the CENTAUR AMX0035 treatment group. This model was developed based on data from select European patients from 1992 to 2016 and reported a survival benefit of 9.9 months (p<0.0001). In the second analysis, the Applicant used a post hoc propensity score matching model to select a subgroup from the external Pooled Resource Open-Access ALS Clinical Trial Database (PRO-ACT) for a survival comparison to the CENTAUR AMX0045 group. This PRO-ACT database contains data from patients from ALS clinical trials from 1990 through 2010 and reported a survival benefit of 11 months in favor of AMX0035 (p=0.0002).

These are non-randomized comparisons to external data for which there was no common treatment protocol or prespecified analysis plan. Therefore, patients in CENTAUR may differ from those in ENCALS and PRO-ACT cohorts. In particular, they may differ in measured prognostic factors e.g., stage or severity of disease.

[11] Latimer et. al. 2019; Causal inference for long-term survival in randomised trials with treatment switching: Should re-censoring be applied when estimating counterfactual survival times? Statistical Methods in Medical Research 2019, Vol. 28(8) 2475–2493. https://pubmed.ncbi.nlm.nih.gov/29940824/

Furthermore, they may also differ in unmeasured prognostic factors. Additionally, Patients in the external control or population for model development may have received different supportive care and/or available therapies, yet another possible confounder of these post-hoc non-randomized analyses.
Both ENCALS and PRO-ACT analyses were post hoc analyses only planned and conducted after having knowledge of unblinded CENTAUR trial data. Ideally, for these to be reliable the analysis plans would have been in place before the conduct of the CENTAUR trial. Note also that the analysis plans and datasets for these analyses were not submitted to the Application.

vi. Biomarker data submitted on June 17, 2022 post late-cycle meeting as additional confirmatory evidence of effectiveness:

In addition, the Applicant submitted mechanistic evidence for impact on neurodegeneration and neuroinflammation in CSF of Alzheimer's disease (AD) patients. The Applicant indicates that the improvement in select CSF biomarkers supports the mechanistic activity of AMX0035 in the central nervous system (CNS). It is unclear if these findings, even if they were demonstrated to be indicative of benefit in AD, would be generalizable to ALS as the underlying pathophysiology of AD and ALS are different. Moreover, neurofilament light chain (nFL), a biomarker of neuronal degeneration that is usually evaluated in ALS studies, did not show a nominally significant change during the 24-week study in both AD and ALS patient populations. Therefore overall, the use of biomarker data as confirmatory evidence in unclear.

Therefore, the various components of the confirmatory evidence provided by the Applicant are also suggestive of a potential treatment benefit with AMX0035, but are not persuasive. The data provide only limited support for the evidence observed on the primary endpoint in the CENTAUR study.

Conclusion

As noted above, there are notable limitations and weaknesses to the efficacy data from the controlled CENTAUR study and its open label extension submitted by the Applicant. However, FDA's regulations allow for FDA to exercise regulatory flexibility in applying the statutory standards for establishing the safety and effectiveness of new therapies intended to treat persons with life-threatening and severely debilitating illnesses, especially where no satisfactory alternative therapy exists. For example, FDA's regulation at 21 CFR 312.80 notes, "while the statutory standards of safety and effectiveness apply to all drugs, the many kinds of drugs that are subject to them, and the wide range of uses for those drugs, demand flexibility in applying the standards. The Food and Drug Administration (FDA) has determined that it is

appropriate to exercise the broadest flexibility in applying the statutory standards, while preserving appropriate guarantees for safety and effectiveness." This approach is consistent with that outlined in the ALS: Developing Drugs for Treatment Guidance for Industry (September 2019), which states: "The statutory standards for effectiveness apply to drugs for ALS just as the standards apply for all other drugs. However, FDA has long stressed the appropriateness of exercising regulatory flexibility in applying the statutory standards to drugs for serious diseases with unmet medical needs, while preserving appropriate assurance of safety and effectiveness."

In addition, FDA's draft Guidance for Industry: Demonstrating Substantial Evidence for Effectiveness for Drugs and Biological Products, December 2019, cites examples of clinical circumstances where additional flexibility may be warranted and exercised. The guidance states that: "In all cases, FDA must reach the conclusion that there is substantial evidence of effectiveness to approve a drug; however, the degree of certainty supporting such a conclusion may differ, depending on clinical circumstances (e.g., severity and rarity of the disease and unmet medical need)" reflecting that "a somewhat greater risk….. of false positive conclusions may be acceptable when balanced against risk of rejecting or delaying the marketing" of a potentially effective therapy in the situation of a medical unmet need for a severe life-threatening disease. This guidance further cites the aforementioned FDA regulations and states that "Subpart E regulations promulgated in 1988 call for FDA to exercise its broad scientific judgment in applying the evidentiary approval standards to drugs for life-threatening and severely debilitating diseases, especially where there is no satisfactory alternative therapy."

There are a number of limitations to the statistical analyses in this submission. Although the prespecified statistical analyses on the primary endpoint rate of reduction in the ALSFRS-R total scores from baseline to Week 24 gave a p-values of 0.034, there were concerns with the linearity assumption and the analysis ignored deaths. The statistical reviewer conducted a number of analyses to address these limitations, and an analysis that addressed the linearity assumption showed a p-value of 0.1134 and a joint rank analysis with multiple imputation accounting for death showed a p-value of 0.079, demonstrating the prespecified analysis was not robust to different assumptions. As noted above, the various components of the confirmatory evidence provided by the Applicant are also suggestive of a potential treatment benefit with AMX0035, but are not persuasive.

The previously referenced 2019 Effectiveness Guidance also states that, "The data supporting effectiveness could, despite the greater risk of error, support a conclusion that there is substantial evidence of effectiveness." These guidance statements acknowledging the potential for some uncertainty even when FDA has concluded that a drug provides substantial evidence of effectiveness are consistent with the FDA regulations cited above, which make clear that it is appropriate to exercise regulatory flexibility in applying our statutory standards in the context

of a serious and life-threatening disease with significant unmet medical need, such as the current case.

ALS is a severely debilitating disease with unmet need, and the exercise of regulatory flexibility in applying our statutory standards is appropriate. It is also notable that at the second AC meeting, the majority of committee members (7 vs. 2) supported approval of AMX0035 in ALS. Although the data from the CENTAUR study and survival analyses are not statistically persuasive, given the seriousness of ALS, the unmet need, and the generally acceptable safety profile; I (Clinical Reviewer) find the data may be considered adequate to support the approval of AMX0035.

8 Review of Safety

8.3 Safety Review Approach

The safety population consisted of all randomized patients who received at least 1 dose of AMX0035.
The evidence of safety of AMX0035 is based on data in adults with ALS from a Phase 2 study:

- Controlled Study AMX3500
- Open-label extension AMX3500OLE

Overall safety will be assessed in the following groups:

1. Analysis of controlled safety database (Study AMX3500): duration of 24-weeks
2. Long-term safety: Pooled analysis of controlled and open-label data (Study AMX3500+AMX3500OLE): duration up to 132 weeks
3. Long-term Safety: Open-label data only (Study AMX3500OLE)

The following terminology is used in this review to describe the treatment groups in the open label extension AMX3500OLE where all subjects received active treatment:

- **"Group PA"** refers to the group of subjects randomized to placebo in the controlled or main phase and who switched to AMX0035 upon enrollment in the OLE (i.e., Placebo to Active)

- **"Group AA"** refers to the group of subjects randomized to AMX0035 in the main phase and who stayed on AMX0035 upon enrollment in the OLE (i.e., Active to Active)

8.4 Review of the Safety Database

8.4.1 Overall Exposure

Exposure in Controlled Phase and Open-label Extension Phase

In the controlled phase, 89 subjects received AMX0035, and 48 subjects received placebo. The mean (SD) duration of exposure in the 24-week controlled study was slightly lower in the AMX0035 group (19.7 [7.89] weeks) compared with the placebo group (21.5 [5.82] weeks). The median duration of treatment was 23.9 weeks in both treatment groups.

In the AMX0035 group, 79 of 89 (88.8%) subjects were able to increase the dose from 1 to 2 sachets after 3 weeks of treatment.

A total of 90 subjects transitioned from the controlled phase to the open-label extension phase. In the open-label extension phase, 56 subjects transitioned from AMX0035 to AMX3500 group (referred to as AA, i.e., those subjects who received AMX0035 in the main 24-week randomized study and received AMX0035 in the OLE) and 34 subjects transitioned from placebo to AMX3500 group (referred as PA, i.e., those subjects who received placebo in the main 24-week randomized study and received AMX0035 in the OLE). The mean (SD) duration of exposure in the safety population was higher in the AA group; (44 weeks) than the PA (33 weeks) (Table 41).

Table 41 Exposure in the controlled and open-label phase

Parameter	Controlled Phase (N=137)		Subjects who entered Open-Label Phase (N=90)		
	Placebo (N=48)	AMX0035 (N=89)	PA (N=34)	AA (N=56)	All Subjects in OLE (N=90)
Overall Duration of Exposure (weeks)					
Mean (SD)	21.5 (5.8)	19.7 (7.9)	32.9 (33.5)	43.7 (36.7)	39.6 (35.7)
Median	23.9	23.9	15.4	31.4	27.7
Min, Max	1.0, 25.9	0.6, 31.6	0.4, 112.4	0.6, 134.6	0.4, 134.6
Duration of exposure (by exposure category) (n [%])					
0 to ≤3weeks	1 (2.1%)	7 (7.9%)	5 (14.7%)	2 (3.6%)	7 (7.8%)
>3 to ≤12 weeks	4 (8.3%)	12 (13.5)	8 (23.5%)	8 (14.3%)	16 (17.8%)
12 to ≤18 weeks	3 (6.2%)	4 (4.5%)	5 (14.7)	3 (5.3)	8 (8.8%)
>18 to ≤21 weeks	2 (4.2%)	5 (5.6%)	0	4 (7.1)	4 (4.4%)
>21 to ≤ 24 weeks	17 (35.4%)	22 (24.7)	1 (2.9)	2 (3.6)	3 (3.3%)
>24 to ≤27 weeks	21 (43.8%)	36 (40.4)	1 (2.9%)	8 (14.3)	9 (10%)
>27 to ≤33 weeks	0	3 (3.4)	0	5 (8.9)	5 (5.5%)
>33 to ≤48 weeks			4 (11.8)	7 (12.5%)	11 (12.2%)
>48 to ≤72 weeks			5 (14.7%)	7 (12.5%)	12 (13.3%)
>72 to ≤ 96 weeks			2 (5.8%)	3 (5.3%)	5 (5.5%)

> 96 weeks			3 (8.8%)	7 (12.5%)	10 (11.11%)
Number of Subjects (n [%])					
Increased Dose to 2 Sachets	45 (93.8)	79 (88.8)			
Did Not Increase Dose to 2	3 (6.3)	10 (11.2)			

Source: Clinical Reviewer Analysis

Table 40 shows that 27 (30%) of subjects that entered the OLE stayed in this phase for >48 weeks, and 15 (17%) stayed in the OLE >72 weeks.

120-Day Safety Update:
Safety data from 3 ongoing studies were included in the 120-day safety update: AMX800 in Alzheimer's disease (N=41 with average exposure of 21 weeks), A35-003 in ALS (compassionate use, N=4 for <3 months, N=17 for 18-<48 months), Study A35-005 in ALS (PK-PD study, N=14 for 28 days). No new safety signals were observed.

8.4.2 Relevant characteristics of the safety population

The demographic and baseline characteristics of the safety population is summarized in Table 42. Overall, 68% of the ALS patients were males and 32% females. Majority of the subjects were White (95%) and Not Hispanic (93%), and the mean age of the subjects was 58 years.

Table 42 Demographic and baseline characteristics (Safety Population)

Demographic or Baseline Characteristic	Main Phase			OLE Phase	
	Placebo (N=48)	AMX0035 (N=89)	Overall (N=137)	PA (N=34)	AA (N=56)
Gender (n [%])					
Male	32 (66.7)	61 (68.5)	93 (67.9)	24 (70.6)	43 (76.8)
Female	16 (33.3)	28 (31.5)	44 (32.1)	10 (29.4)	13 (23.2)
Age at Enrollment					
n	48	89	137	34	56
Mean (SD)	57.3 (7.56)	57.9 (10.57)	57.7 (9.60)	58.5 (7.41)	57.8 (10.22)
Median	57.5	60.0	59.0	59.0	60.0
Race (n [%])					
White	46 (95.8)	84 (94.4)	130 (94.9)	33 (97.1)	52 (92.9)
Asian	1 (2.1)	2 (2.2)	3 (2.2)	1 (2.9)	2 (3.6)
Black or African American	1 (2.1)	2 (2.2)	3 (2.2)	0	1 (1.8)
Unknown	0	1 (1.1)	1 (0.7)	0	1 (1.8)
Ethnicity (n [%])					
Hispanic or Latino	1 (2.1)	6 (6.7)	7 (5.1)	1 (2.9)	4 (7.1)
Not Hispanic or Latino	47 (97.9)	83 (93.3)	130 (94.9)	33 (97.1)	52 (92.9)

Source: Clinical Reviewer Analysis

8.4.3 Adequacy of the safety database

Given the orphan nature of the disease, the patient exposure appears adequate and generalizable to the US population.

8.5 Adequacy of Applicant's Clinical Safety Assessments

8.5.1 Issues Regarding Data Integrity and Submission Quality

Overall, the safety database was adequate in format and quality for review.

8.5.2 Categorization of Adverse Events

For Study AMX3500, an Adverse Event (AE) was defined as any untoward medical occurrence in a subject who received investigational study medication (i.e., AMX0035 or placebo) that did not necessarily have a causal relationship with study medication and that occur after informed consent was signed and up to 28 days (+5 days) after the study medication discontinuation. Symptoms of progression/worsening of ALS, including 'normal' progression, were to be recorded as AEs. Investigational sites were instructed to not capture medication bad taste as an AE, but instead to capture emergent AEs relating to challenges of the oral delivery of study medication if they had a clinically untoward effect (e.g., burning, vomiting, anxiety).

AEs were coded into system organ classes and preferred terms using the MedDRA v23.1. A treatment-emergent adverse event (TEAE) was defined as an AE with an onset date on or after the start of study medication dosing. Adverse event summaries generated included only TEAEs. If a preferred term or system organ class was reported more than once for a subject, each subject was only counted once in the incidence count for each category. If a subject had the same AE on multiple occasions, the highest severity (severe > moderate > mild) recorded for the event was presented.

The verbatim terms were manually reviewed for accuracy of coding. The applicant's coding resulted in appropriate translation of verbatim terms to preferred terms. However, TEAEs were often coded to multiple different equivalent Preferred Terms. The grouping of closely related terms or pooling of preferred terms (AEDECOD) was not accurately conducted by the applicant for a few preferred terms. These were re-coded by the reviewer as shown in Table 43. The recoded dataset was used in the AE analyses summarized in the review. Data Analyst Rui Li assisted in generating AE tables for this review.

Table 43 Grouping of Closely Related Preferred Terms to Avoid Splitting

Groupings for AEDECOD	AEDECOD re-coded AS

Abdominal discomfort Abdominal pain Abdominal pain upper Abdominal distension	Abdominal pain
Blood urine Blood urine present	Blood urine
Cough Productive cough	Cough
Depression Adjustment disorder with depressed mood Depressed mood	Depression
Frequent bowel movements Diarrhea Diarrhea hemorrhagic Gastrointestinal hypermotility	Diarrhea
Diaphragmatic disorder Diaphragmatic spasm	Diaphragmatic disorder
Amyotrophic lateral sclerosis Disease progression Muscular weakness	Disease progression
Diverticular perforation Diverticulitis	Diverticulitis
Dyspnea Dyspnea exertional	Dyspnea
Dysarthria Speech disorder	Dysarthia
Peripheral swelling Edema peripheral Swelling Edema	Edema
Aspartate aminotransferase increased Alanine aminotransferase increased Transaminase increase Transaminase increased	Elevated liver enzymes
Asthenia Fatigue Malaise	Fatigue
Increased upper airway secretion Increased viscosity of upper respiratory secretion	Increased upper airway secretion
Muscle twitching Muscle contractions involuntary	Muscle contractions involuntary

Muscle spasms Muscle Spasticity Muscle strain	Muscle spasms
Musculoskeletal chest pain Musculoskeletal discomfort Musculoskeletal pain Myalgia	Musculoskeletal pain
Neutrophil count increased White blood cell disorder Neutrophilia	Neutrophil count increased
Oral discomfort Oropharyngeal pain	Oral discomfort
Pneumonia Pneumonia aspiration Pneumonia respiratory syncytial viral	Pneumonia
Pain Pain in extremity	Pain
Viral infection Viral upper respiratory tract infection Upper respiratory tract infection Upper-airway cough syndrome Acute sinusitis	Respiratory tract infection
Respiratory failure Acute respiratory failure	Respiratory failure
Restless sleep Sleep disorder Poor quality sleep Insomnia	Sleep disorder
Urinary incontinence Automatic bladder	Urinary incontinence
Vision blurred Visual impairment	Visual impairment
Vertigo Vertigo positional	Vertigo
White blood cells urine White blood cells urine positive	White blood cells urine

8.5.3 Routine Clinical Tests

The routine clinical tests included anthropometrics, vital signs, hematology, chemistry, urinalysis, ECG, physical and neurological exam, and Columbia Suicide Severity Rating Scale (C-SSRS):

The following vital signs were collected: systolic and diastolic blood pressure, pulse rate (radial artery) (per minute), respiratory rate (per minute), temperature, and weight. Height was also collected at the Screening Visit only. Vital signs collection occurred after the subject had been in a seated position for several minutes.

The following laboratory tests were performed by the site laboratories as part of routine safety monitoring:

- Hematology with differential panel: complete blood count with differential (hematocrit, hemoglobin, platelet count, red blood cell [RBC] indices, total RBC count, total white blood cell [WBC] count, and WBC with differential)
- Blood chemistry panel/liver function tests (LFTs): alanine aminotransferase (ALT) aspartate aminotransferase (AST), 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

The physical examination included an assessment of general appearance and a review of the following systems: neurological; musculoskeletal; respiratory; cardiovascular; gastrointestinal; genitourinary; head, ears, eyes, nose, and throat; and skin, hair, and nails.

The neurological examination included an assessment of mental status, cranial nerves, motor and sensory function, reflexes, coordination, and stance/gait. As part of the baseline neurological examination, the patient was evaluated for a definite diagnosis of ALS as defined by the World Federation of Neurology revised El Escorial criteria.

The reference range of some laboratory tests differed amongst laboratory tests. These were taken into consideration when evaluating outliers.

8.6 Safety Results

The overall summary of fatal and serious TEAEs (SAEs) in both the controlled and open-label phases of the study is tabulated in Table 44 and will be elaborated in subsequent sections of this review.

Table 44 Overall Summary of Treatment-Emergent Adverse Events and Serious Adverse Events in AMX3500 and AMX350OLE – Safety Population

Controlled Phase AMX3500

Preferred Terms	Placebo (N = 48)	AMX0035 (N = 89)
Subjects with Fatal TEAE	2 (4.2%)	5 (5.6%)
Subjects with Serious TEAE (SAE)	8 (16.7%)	11 (12.4%)
Subjects who had drug withdrawn due to a SAE	4 (8.3%)	2 (2.2%)
Subjects who had drug withdrawn due to a TEAE	5 (10.4%)	18 (20.2%)

Open-label Study AMX3500OLE

Preferred Terms	PA (N = 34)	AA (N = 56)	Combined (N = 90)
Subjects with Fatal TEAE	9 (26.5%)	6 (10.7%)	15 (16.7%)
Subjects with Serious TEAE (SAE)	13 (8.2%)	18 (32.1%)	31 (34.4%)
Subjects who had drug withdrawn due to a SAE	7 (20.6%)	7 (12.5%)	14 (15.6%)
Subjects who had drug withdrawn due to a TEAE	15 (44.1%)	11 (19.6%)	26 (28.9%)

Safety population and TRTEMFL = Y
* By Former Randomized Treatment (PA= placebo to AMX3400; AA=AMX3500 to AMX3500

8.6.1 Deaths

All deaths appeared to be related to disease progression, as summarized below for both the controlled and open-label phases.

Controlled Study AMX3500

Seven of the 137 subjects enrolled in Study AMX3500 died during the study (5 [5.6%] in the AMX0035 group, and 2 [4.2%] in the placebo group) during the 24-week study period (See Table 44). In majority of the subjects, the cause of death was consistent with manifestations or complications of ALS. The reasons for death included respiratory failure (n=4), respiratory arrest (n=1), subdural hematoma (n=1), perforated diverticulum (n=1). The subdural hematoma was related to a fall with head strike.

Narrative for Patient (b) (6) (perforated diverticulum): Subject was on AMX0035. Subject started dosing on (b) (6) and stopped on (b) (6). The subject had diarrhea starting Day 2 that was resolved on (b) (6) (Day 11) which was considered mild in intensity. The subject was also having abdominal discomfort which was eventually found to be diverticulitis (Day 4). The drug was withdrawn. The narrative mentions that the subject only took 1-3 days course of study drug after baseline visit and stopped it due to the taste. The subject was hospitalized and had surgery for a perforated diverticulum on (b) (6) (Day 60). She was still hospitalized and in ICU as of (b) (6) and died on (b) (6) (Day 69). The subject was septic with diverticulitis.

Reviewer's Comment: *The Applicant assigns this event as not drug related; however, the event could be drug related. The subject's actual exposure was unclear from the narrative and the dataset. The narrative indicates that the subject took the drug from 1-3 days, but the ADSURV.xpt, dataset indicates that the subject has been on AMX3500 for 4.5 weeks, as do other datasets (EX.xpt and DS.xpt). The dataset indicates that the subject's date of first study treatment was (b) (6) and last treatment was (b) (6), however it appears that the drug was withdrawn after the event of diarrhea and diverticulitis on Day 2-4. Subsequently abdominal discomfort continued which was eventually fatal. The subject was reported to have discontinued from the study on day 33. It was clarified from the Applicant that the subjects only took 5 sachets of the drug, and the remaining sachets were returned unopened due to bad taste of the drug product. Therefore, the Applicant notes that the subject received treatment for only 3 days.*

Open label Study AMX3500OLE

Fifteen of the 90 subjects (16.7%) died during the study (9 [26.5%] subjects in the PA group and 6 [10.7%] subjects in the AA group). The reasons for death included respiratory failure (n=10), aspiration pneumonia (n=1), disease progression (n=3), cardiac arrest (n=1), which were all consistent with the complications of ALS.

8.6.2 Serious Adverse Events

A serious AE (SAE) was defined as resulting in death, was life-threatening, required hospitalization, resulted in significant disability, resulted in birth defect, necessitated medical or surgical intervention.

Controlled Study AMX3500

SAEs occurred more frequently in the placebo group (8 [16.7%]) compared with the AMX0035 group (11 [12.4%]) as summarized in Table 45. I reviewed the narratives of all SAEs. All SAEs,

unless fatal, resolved without sequelae. Most SAEs were related to ALS, treatment procedure (e.g., port-a-cath, g-tube placement), or secondary to falls due to disease progression.

Table 45 Serious Adverse Events in AMX3500

Preferred Terms	Placebo (N = 48)	AMX0035 (N = 89)
Total Subjects with any Adverse Events	8 (16.7%)	11 (12.4%)
Respiratory failure	3 (6.3%)	2 (2.2%)
Bacteremia	1 (2.1%)	1 (1.1%)
Nephrolithiasis	1 (2.1%)	1 (1.1%)
Pneumonia	0 (0.0%)	2 (2.2%)
Catheter site infection	1 (2.1%)	0 (0.0%)
Device dislocation	1 (2.1%)	0 (0.0%)
Diverticulitis	0 (0.0%)	1 (1.1%)
Implant site cellulitis	0 (0.0%)	1 (1.1%)
Pelvic fracture	1 (2.1%)	0 (0.0%)
Pneumoperitoneum	0 (0.0%)	1 (1.1%)
Pulmonary embolism	1 (2.1%)	0 (0.0%)
Respiratory arrest	0 (0.0%)	1 (1.1%)
Skull fracture	0 (0.0%)	1 (1.1%)
Stoma site hemorrhage	0 (0.0%)	1 (1.1%)
Subdural hematoma	0 (0.0%)	1 (1.1%)
Visual impairment	0 (0.0%)	1 (1.1%)

Safety population and TRTEMFL = Y

Only 2 SAEs of nephrolithiasis (occurring in 1 subject each in the AMX0035 and placebo group) were assessed as possibly related to study medication by the investigator. The narratives and discussion of these patients are summarized below. In addition, the narrative for the subject with diverticulitis has been described under section 8.5.1 (deaths), as it subsequently became perforated diverticulitis that was fatal.

Narrative for Patient (b) (6) **(nephrolithiasis):** This subject was on placebo. Subject presented to the local emergency department after experiencing abdominal pain and hematuria. A foley was placed and bladder irrigation performed with only clots coming out. A CT scan of urinary bladder showed that he still had urine retention. He was admitted to the hospital after a CT scan of his abdomen showed a right mid ureteric stone measuring 0.3 cm. The patient was

discharged after 2 weeks, and the kidney stone passed 5 days later. There was no action taken with respect to study medication and the subject recovered from the event while on study medication.

Reviewer's comment: *This is not a drug related event as patient was receiving placebo.*

Narrative for Patient (b) (6) **(nephrolithiasis):** Patient was on AMX3500 for 1 week and had a previous history of nephrolithiasis, was diagnosed with bilateral kidney stones. An abdominal/pelvic CT with IV contrast showed bilateral non-obstructive nephrolithiasis, largest 10 mm in diameter on the left side and bilateral renal cysts. She had no urinary retention (fluid input +2L since admission), and her creatinine was within normal limits.

Reviewer's comment: *Patient was on AMX3500; however, she had a history of nephrolithiasis. Since the subject was on AMX3500 for only 1 week, it is unclear if stones could develop and increase in size so rapidly. Attribution of the event to AMX3500 is unclear; however, I noticed that "crystal urine present" was a TEAE reported in 4 patients in the AMX3500 group only. Occurrence of crystals in the urine could therefore potentially be drug related effect.*

Open label Study AMX3500OLE

A total of 40 SAEs were reported in 31 (34.4%) subjects overall. SAEs occurred at similar rates in the PA group (13 [38.2%] subjects) and in the AA group (18 [32.1%] subjects). Overall, there was 20 (22%) in the 'Respiratory, thoracic and mediastinal' SOC primarily including respiratory failure, aspiration pneumonia/pneumonia, dyspnea, hypoxia, pneumothorax, pleural effusion, pulmonary embolism. Other included diverticulitis (n=1) that occurred in the PA group. Others included ALS complications, including dysphagia, device site infections, fractures, cardiac arrest and disease progression.

8.6.3 Dropouts and/or Discontinuations Due to Adverse Effects

Controlled Study AMX3500

Overall, 23 of the 137 enrolled subjects prematurely discontinued study medication due to TEAE(s), with a higher percentage discontinuing in the AMX0035 group (18 [20.2%]) compared with the placebo group (5 [10.4%]) as shown in Table 46.

Table 46 Dropout Summary for Study AMX3500

Preferred Terms	Placebo (N = 48)	AMX0035 (N = 89)
Subjects Who Had Drug Withdrawn Due to a SAE	4 (8.3%)	2 (2.2%)

Preferred Terms	Placebo (N = 48)	AMX0035 (N = 89)
Subjects Who Had Drug Withdrawn Due to a TEAE	5 (10.4%)	18 (20.2%)

Safety population and TRTEMFL = Y

TEAEs that led to withdrawal are summarized in Table 47. Abdominal pain, diarrhea and nausea were the main TEAE that led to discontinuation, in addition to TEAEs that appeared to be related to complications of ALS.

Table 47 TEAEs that Led to Dropouts in Study AMX3500

TEAE	Placebo (n)	AMX3500 (n)	Severity	Outcome
Respiratory failure	3		severe (3)	Fatal
Respiratory arrest		1	severe	Fatal
Subdural hematoma		1	severe	Fatal
pelvic fracture secondary to fall	1		severe	Recovered
Peripheral edema, joint swelling, arthralgia		1	mild	Recovered
Abdominal pain		3	mild (1), moderate (2)	Not recovered
Diarrhea		5	mild (1), moderate (3) and severe (1)	Recovered
Diverticulitis		1	severe	Not recovered
Nausea		1	severe	Recovered
Nausea	1		mild	Recovered
Fatigue		2	mild (1), severe (1)	Recovered
Fatigue		1	mild	Recovered
Atrial fibrillation		1	moderate	Recovered
Depressed mood		1	severe	Not recovered
Neck and Ear Rash		1	moderate	Recovered
Dysgeusia		2	mild/moderate	Recovered
Weight and appetite decreased		1	mild	Recovered
Sinus infection		1	moderate	Recovered

Open label Study AMX3500OLE

Overall, 29% of subjects discontinued the study due to a TEAE and 16% discontinued due to a SAE (Table 48).

Table 48 Dropout Summary for Study AMX3500OLE

Preferred Terms	PA (N = 34)	AA (N = 56)	Combined (N = 90)
Subjects who had drug withdrawn due to a SAE	7 (20.6%)	7 (12.5%)	14 (15.6%)
Subjects who had drug withdrawn due to a TEAE	15 (44.1%)	11 (19.6%)	26 (28.9%)

Safety population and TRTEMFL = Y
* By Former Randomized Treatment (PA= placebo to AMX3400; AA=AMX3500 to AMX3500

In addition to the TEAEs that led to withdrawal in the controlled phase, TEAEs that led to withdrawal in the open-label extension include vomiting, dizziness, pneumonia, dysphonia, migraine, laryngospasm, pulmonary embolism, sinus tachycardia, atelectasis, disease progression, bronchial secretion retention that occurred in 1 patient each. TEAEs that led to withdrawal that occurred in ≥ 2subjects include respiratory failure, nausea, vomiting and diarrhea. Most of these SAEs leading to withdrawal are likely due to complications of ALS as well.

8.6.4 Significant Adverse Events

Other than gastrointestinal TEAEs, most other AEs appeared to be manifestations or complication of ALS, including respiratory failure, falls, fractures secondary to falls, fatigue, muscular weakness, and dysarthria.

8.6.5 Treatment Emergent Adverse Events and Adverse Reactions

Controlled-Study AMX3500

Treatment emergent AEs that occurred in more than ≥2 % of AMX3500 treated subjects and a difference of >1% from placebo is shown in Table 49 . The highest percentage of common TEAEs occurring in >5% of AMX3500 treated patients and >1% difference from placebo belonged to the gastrointestinal SOC (including diarrhea, abdominal pain, nausea, dyspnea, salivary hypersecretion). Others included respiratory tract infection and fatigue, dizziness, dyspnea, dysarthria, decreased appetite/weight)

Table 49 TEAEs in ≥2% of AMX3500 Treated Subjects and >1% Difference Compared to Placebo in Controlled Phase (Safety Population)

Preferred Terms	Placebo (N = 48)	AMX0035 (N = 89)
Total Subjects with any Adverse Events	46 (95.8%)	86 (96.6%)
Diarrhea	9 (18.8%)	22 (24.7%)
Abdominal pain	6 (12.5%)	19 (21.3%)
Nausea	6 (12.5%)	16 (18.0%)
Respiratory tract infection	5 (10.4%)	16 (18.0%)
Fatigue	3 (6.3%)	11 (12.4%)
Dyspnea	4 (8.3%)	10 (11.2%)
Salivary hypersecretion	1 (2.1%)	10 (11.2%)
Dizziness	2 (4.2%)	9 (10.1%)
Decreased appetite	2 (4.2%)	7 (7.9%)
Dysarthria	2 (4.2%)	7 (7.9%)
Proteinuria	2 (4.2%)	6 (6.7%)
Arthralgia	2 (4.2%)	5 (5.6%)
Weight decreased	1 (2.1%)	5 (5.6%)
Ketonuria	1 (2.1%)	4 (4.5%)
Pyrexia	1 (2.1%)	4 (4.5%)
Vomiting	1 (2.1%)	4 (4.5%)
Crystal urine present	0 (0.0%)	4 (4.5%)
Muscle contractions involuntary	1 (2.1%)	4 (4.5%)
Pain	0 (0.0%)	4 (4.5%)
Decubitus ulcer	1 (2.1%)	3 (3.4%)
Dysgeusia	1 (2.1%)	3 (3.4%)
Flatulence	1 (2.1%)	3 (3.4%)
Pneumonia	1 (2.1%)	3 (3.4%)
Tremor	1 (2.1%)	3 (3.4%)
Dyspepsia	0 (0.0%)	3 (3.4%)
Hot flush	0 (0.0%)	3 (3.4%)
Rib fracture	0 (0.0%)	3 (3.4%)
Skin odor abnormal	0 (0.0%)	3 (3.4%)
Somnolence	0 (0.0%)	3 (3.4%)
Atrial fibrillation	0 (0.0%)	2 (2.2%)
Balance disorder	0 (0.0%)	2 (2.2%)
Blood creatinine increased	0 (0.0%)	2 (2.2%)
Blood urine	0 (0.0%)	2 (2.2%)
Chest pain	0 (0.0%)	2 (2.2%)

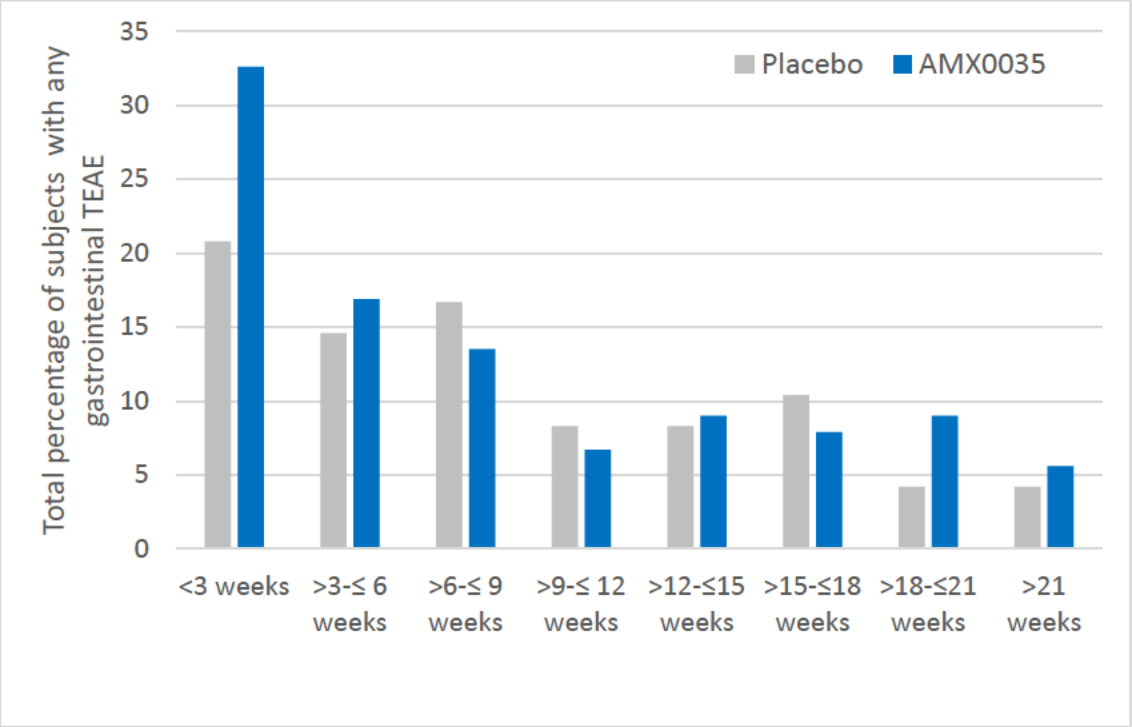
Preferred Terms	Placebo (N = 48)	AMX0035 (N = 89)
Humerus fracture	0 (0.0%)	2 (2.2%)
Hyperhidrosis	0 (0.0%)	2 (2.2%)
Hypertension	0 (0.0%)	2 (2.2%)
Hypoesthesia	0 (0.0%)	2 (2.2%)
Migraine	0 (0.0%)	2 (2.2%)
Muscle contractions involuntary	0 (0.0%)	2 (2.2%)
Musculoskeletal stiffness	0 (0.0%)	2 (2.2%)
Oral discomfort	0 (0.0%)	2 (2.2%)
Palpitations	0 (0.0%)	2 (2.2%)
Postoperative wound infection	0 (0.0%)	2 (2.2%)
Retching	0 (0.0%)	2 (2.2%)
Sputum increased	0 (0.0%)	2 (2.2%)
Throat irritation	0 (0.0%)	2 (2.2%)
Visual impairment	0 (0.0%)	2 (2.2%)

Safety population and TRTEMFL = Y
>2% of AMX0035-Treated Patients and >1% Difference vs. Placebo

Reviewer's Comment:
This table differs from that proposed in the label because the Applicant has proposed to include only drug related AEs in the proposed label. Increased salivary hypersecretion in ALS patients could be related to higher percentage of bulbar-onset ALS patients in the AMX0035 group (30%) compared to the placebo group (21%).

Incidence of gastrointestinal TEAEs were significantly more in the AMX3500 group compared to the placebo group. This is part of the known safety profile of TUDCA. Any gastrointestinal TEAE occurred in >5% of the patients throughout the study in any group; however, the GI TEAEs were the greatest in the AMX0035 treated patients in the first 3 weeks of treatment as shown in Figure 20 The major gastrointestinal adverse events included diarrhea, abdominal pain, and nausea.

Figure 20 Total percentage of patients with any gastrointestinal TEAE by study duration



Long-term Safety (cumulative from controlled phase to open-label phase Studies AMX3500+AMX3500OLE)

Treatment emergent AEs that occurred in more than ≥5 % of AMX3500 treated subjects in the pooled controlled + open-label phase is summarized in Table 50. Note that the placebo arm presented in this Table is not placebo only, as all subjects received AMX0035 in the OLE phase. However, the groups are assigned based on original assignment in the controlled phase. In addition, not all subjects transitioned to OLE. Therefore, this Table is not very quantitatively interpretable. However, it does shows that the major TEAEs remained the same in the two groups.

Table 50 TEAEs in ≥5 % of AMX3500 Treated Subjects in the Combined Controlled and Open Label Phase

Preferred Terms	Placebo (N = 48)	AMX0035 (N = 89)	Combined (N = 137)
Total Subjects with any Adverse Events	46 (95.8%)	89 (100.0%)	135 (98.5%)
Fall	19 (39.6%)	33 (37.1%)	52 (38.0%)

Preferred Terms	Placebo (N = 48)	AMX0035 (N = 89)	Combined (N = 137)
Diarrhea	15 (31.3%)	28 (31.5%)	43 (31.4%)
Disease progression	16 (33.3%)	23 (25.8%)	39 (28.5%)
Nausea	12 (25.0%)	20 (22.5%)	32 (23.4%)
Abdominal pain	9 (18.8%)	22 (24.7%)	31 (22.6%)
Constipation	15 (31.3%)	16 (18.0%)	31 (22.6%)
Headache	11 (22.9%)	15 (16.9%)	26 (19.0%)
Respiratory tract infection	5 (10.4%)	18 (20.2%)	23 (16.8%)
Respiratory failure	10 (20.8%)	10 (11.2%)	20 (14.6%)
Dyspnea	4 (8.3%)	15 (16.9%)	19 (13.9%)
Dizziness	4 (8.3%)	13 (14.6%)	17 (12.4%)
Musculoskeletal pain	5 (10.4%)	12 (13.5%)	17 (12.4%)
Fatigue	3 (6.3%)	13 (14.6%)	16 (11.7%)
Cough	6 (12.5%)	8 (9.0%)	14 (10.2%)
Elevated liver enzymes	5 (10.4%)	8 (9.0%)	13 (9.5%)
Salivary hypersecretion	2 (4.2%)	11 (12.4%)	13 (9.5%)
Decreased appetite	3 (6.3%)	9 (10.1%)	12 (8.8%)
Dysarthria	3 (6.3%)	9 (10.1%)	12 (8.8%)
Skin laceration	4 (8.3%)	8 (9.0%)	12 (8.8%)
Urinary tract infection	4 (8.3%)	8 (9.0%)	12 (8.8%)
Anxiety	3 (6.3%)	8 (9.0%)	11 (8.0%)
Rash	4 (8.3%)	7 (7.9%)	11 (8.0%)
Weight decreased	3 (6.3%)	8 (9.0%)	11 (8.0%)
Back pain	4 (8.3%)	6 (6.7%)	10 (7.3%)
Contusion	4 (8.3%)	6 (6.7%)	10 (7.3%)
Dysphagia	5 (10.4%)	5 (5.6%)	10 (7.3%)
Vomiting	3 (6.3%)	7 (7.9%)	10 (7.3%)
Arthralgia	2 (4.2%)	7 (7.9%)	9 (6.6%)
Depression	2 (4.2%)	7 (7.9%)	9 (6.6%)
Muscle spasms	3 (6.3%)	6 (6.7%)	9 (6.6%)
Neck pain	5 (10.4%)	4 (4.5%)	9 (6.6%)
Proteinuria	2 (4.2%)	7 (7.9%)	9 (6.6%)
Sleep disorder	3 (6.3%)	6 (6.7%)	9 (6.6%)
Dry mouth	5 (10.4%)	3 (3.4%)	8 (5.8%)
Pneumonia	3 (6.3%)	5 (5.6%)	8 (5.8%)
Skin abrasion	3 (6.3%)	5 (5.6%)	8 (5.8%)
Pyrexia	2 (4.2%)	5 (5.6%)	7 (5.1%)
Stoma site pain	2 (4.2%)	5 (5.6%)	7 (5.1%)
Edema peripheral	3 (6.3%)	5 (5.6%)	8 (5.8%)

Open-label Extension Study AMX3500OLE

Treatment emergent AEs that occurred in greater than or equal to 5 % of subjects in the OLE phase alone is summarized in Table 51. This table shows that the gastrointestinal adverse events were the major TEAEs, with diarrhea and nausea occurring in 18% of the subjects, and abdominal pain and constipation in 10-11% of the subjects. This was followed by dizziness and headache in 8% of the subjects. This table also shows that percentage of patients experiencing diarrhea were more in the PA group compared to the AA group, as these subjects started AMX0035 in the OLE phase, confirming that incidences of diarrhea occur more frequently on initiation of treatment with AMX0035. Overall, more than 30% of subjects in the PA group reported the first occurrence of Gastrointestinal disorder TEAEs within 3 weeks after starting treatment.

Table 51 TEAEs in ≥5 % of AMX3500 Treated Subjects in the Open Label Phase

Preferred Terms	Placebo/AMX0035 (N = 34)	AMX0035/AMX0035 (N = 56)	Combined (N = 90)
Total Subjects with any Adverse Events	32 (94.1%)	49 (87.5%)	81 (90.0%)
Fall	6 (17.6%)	14 (25.0%)	20 (22.2%)
Diarrhea	10 (29.4%)	6 (10.7%)	16 (17.8%)
Nausea	7 (20.6%)	9 (16.1%)	16 (17.8%)
Disease progression	6 (17.6%)	7 (12.5%)	13 (14.4%)
Respiratory failure	6 (17.6%)	6 (10.7%)	12 (13.3%)
Constipation	4 (11.8%)	6 (10.7%)	10 (11.1%)
Abdominal pain	4 (11.8%)	5 (8.9%)	9 (10.0%)
Dyspnea	0 (0.0%)	8 (14.3%)	8 (8.9%)
Anxiety	1 (2.9%)	6 (10.7%)	7 (7.8%)
Dizziness	2 (5.9%)	5 (8.9%)	7 (7.8%)
Headache	1 (2.9%)	5 (8.9%)	6 (6.7%)
Urinary tract infection	2 (5.9%)	4 (7.1%)	6 (6.7%)
Weight decreased	2 (5.9%)	4 (7.1%)	6 (6.7%)
Cough	3 (8.8%)	2 (3.6%)	5 (5.6%)
Elevated liver enzymes	1 (2.9%)	4 (7.1%)	5 (5.6%)
Pneumonia	3 (8.8%)	2 (3.6%)	5 (5.6%)
Pyrexia	1 (2.9%)	4 (7.1%)	5 (5.6%)
Vomiting	2 (5.9%)	3 (5.4%)	5 (5.6%)

8.6.6 Laboratory Findings

There were no clinically meaningful changes from baseline in any hematology, chemistry, or urinalysis result between treatment groups or any clinically relevant trends for change over time in either treatment group.

Changes, if they occurred, were transient, occurred at one or more time points during the study, and mostly occurred in subjects that had abnormal values at baseline/screening. There were no significant differences between treatment arms.

8.6.7 Vital Signs

No statistically significant differences between treatment groups were observed for most vital sign parameters, except systolic blood pressure where change from baseline was -6.7 mmHg (p=0.029) only at week 24 (which arm was lower – treatment or placebo?). Blood pressure increase was recorded as a TEAE in 1 subject in the placebo group. Fluctuations if observed were transient in nature and occurred at one or more time points.

8.6.8 Electrocardiograms (ECGs)

Standard 12-lead electrocardiograms (ECGs) were performed that included: global PR, global QRS, global QT, global QT corrected for heart rate using Bazett's formula (QTcB), global QT corrected for heart rate using Fridericia's formula (QTcF), and global respiration rate (RR) in Clinical Pharmacology Food Effect Study A35-002 and the CENTAUR Study).

Majority of the subjects had normal ECGs. Shifts from baseline to Week 12 or 24 of the CENTAUR study were infrequent and there was no difference between treatment groups in percent of subjects interpreted as abnormal ECG in CENTAUR Study.
Two subjects in placebo group (4.2%) and 8 subjects in the treatment group (9.0%) had treatment emergent ECG abnormalities (TEAE). The most common ones were flat T waves (4 subjects), sinus tachycardia (3 subjects), left anterior hemiblock (3 subjects), and inverted T-wave (2 subjects).

In Study A35-002, none of the subjects experienced PR >220 msec with and without 25% increase over baseline in any of the treatment groups. None of the subjects experienced QRS >120 msec with and without 25% increase over baseline in any of the treatment groups.

Also refer to Interdisciplinary Review Team for Cardiac Studies (IRT) and section 8.7.3 for discussion on cardiac events.

8.6.9 QT

None of the subjects experienced QTcF >450 msec with or without a change from baseline >60 msec and none of the subjects experienced ΔQTcF >60 msec in any of the treatment groups. Overall, no large mean increases (i.e., >20 msec) in the QTcF interval was detected in this QT assessment of AMX-3500 at the therapeutic dose. However, the Interdisciplinary Review Team for Cardiac Studies was reluctant to draw conclusions of lack of an effect in an absence of a positive control, large exposure margin, or an integrated nonclinical safety assessment conduct according to best practices.
Study # A35-002 was acceptable to exclude large mean increases (>20 msec) in the QTc interval at the therapeutic dose level. Because the high clinical exposure scenario has not been identified, potential effects of parent drugs and metabolites on QTc at higher exposure are unknown.

During the randomized controlled period and open-label extension (OLE) period of AMX3500, there was 1 case of electrocardiogram QT prolonged, 5 cases of syncope (5 subjects), 1 case of seizure, and 1 case of cardiac arrest. There were no cases of ventricular arrhythmias, ventricular tachycardia, ventricular fibrillation, or Torsades de Pointes. One seizure suffered by a 57-year-old female occurred during the respiratory failure was due in part to ALS progression and in part to pneumonia and it was considered not related to the study drug (refer to IRT Review). The IRT Reviewer concludes that none of the events identified to be of clinical importance per the ICH E14 guidelines (i.e., significant ventricular arrhythmias) occurred in this study. The one seizure and one cardiac arrest were considered unlikely related to the study drug.

Also refer to Interdisciplinary Review Team for Cardiac Studies (IRT) and section 8.7.3 for discussion on cardiac events.

8.6.10 Columbia Suicide Severity Rating Scale (C-SSRS)

Patients with ALS are known to be at higher risk for depression; therefore, C-SSRS questionnaires were administered during the double-blind period of study. The C-SSRS involves a series of probing questions to inquire about possible suicidal thinking and behavior.

At Baseline, ≥10% of subjects in both treatment groups (15 [16.9%] in the AMX0035 group and 5 [10.4%] in the placebo group) reported experiencing suicidal ideation. The percent of subjects reporting suicidal ideation did not increase after initiation of treatment with AMX3500 as shown in Table 52. There was no active suicidal ideation reported in any group during the study.

At baseline, one subject in each treatment arm reported suicidal behavior. After Week 6, no subject reported suicidal behavior (not shown in the Table).

Table 52 Suicide Ideation (% of subjects)

Time point	Placebo (N at visit)	Placebo (%)	AMX3500 (N at visit)	AMX3500
Baseline	48	10.4%	89	16.9%
Week 3	47	4.2%	82	11.2%
Week 6	44	4.2%	80	11.2%
Week 12	44	6.3%	73	12.4%
Week 18	39	6.3%	68	9%
Week 24	38	6.3%	67	9%

The TEAEs showed that 4.2% subjects in the placebo group and 3.4% subjects in the AMX3500 group reported depression as an TEAE. One subject (Subject (b) (6)) in the AMX0035 group experienced depression beginning Day 2 that was assessed as severe in intensity and the study drug was withdrawn permanently.

These data do not suggest the AMX3500 contributes to worsening of depression.

8.7 Analysis of Submission-Specific Safety Issues

8.7.1 Sodium Retention with Edema

Since AMX0035 contains (b) (4) mg of sodium, the Applicant proposes to include "(b) (4) " under "Warnings and Precautions" section of the product label consistent with the BUPHENYL label. The proposed label recommends caution in use in patients with congestive heart failure, severe renal insufficiency, or other conditions associated with sodium retention with edema. I looked for preferred terms related to edema in the ADAE.xpt dataset. There was no difference in the occurrence of edema between treatment groups in ALS patients. The preferred term "peripheral edema" occurred 3 (6.3%) in the placebo group and 5 (5.6%) in the AMX0035 group indicating no increase in edema associated with treatment. However, it could be a concern if compounded with other conditions associated with sodium retention. This caution should be included in the "Warning and Precautions" section of the label.

8.7.2 Enterohepatic Circulation, Pancreatic and Intestinal Disorders

The Applicant has proposed to include "Enterohepatic circulation, pancreatic and intestinal disorders in the "Warnings and Precautions" section of the label that recommends caution in use in patients with enterohepatic circulation disorders (e.g., frequent biliary colic, biliary infection), severe pancreatic disorders or intestinal diseases which may alter the concentration of bile acids (e.g., ileal resection and stoma, regional ileitis), and affect ursodoxicoltaurine

levels. Pancreatic insufficiency or intestinal malabsorption may also reduce phenylbutyrate absorption. This is also included as a Warning and Precaution in the RAVICTI (glycerol phenylbutyrate) Label. RAVICTI is a prodrug for phenylbutyrate containing 3 molecules of phenylbutyrate. Inclusion of this warning in the RELYVRIO label is acceptable.

8.7.3 Cardiac events

The potential cardiovascular risk associated with use of phenylbutyrate can be related to the sodium load and fluid retention in patients with heart failure. Elevated serum bile acids could also reduce heart rate and contractility, with recent findings indicating that high bile acid levels are linked to arrhythmias and cardiac dysfunction in cirrhosis cardiomyopathy and in obstetric cholestasis (Hanafi 2018; Desai 2017)[12].

The review of the cardiac disorder SOC indicated that cardiac TEAEs were reported in only subjects who were receiving AMX0035, including cardiac arrest (SAE, fatal, 1 subject), tachycardia (5 subjects), atrial fibrillation (2 subjects), palpitation (2 subjects), tachycardia (1 subject), first degree AV block (1 subject), left bundle branch block (1 subject) and sinus tachycardia (1 subject). Three cardiac events were assessed as possibly related to AMX0035 (first degree AV block, bundle branch block left, and one atrial fibrillation). They are outlined below in further detail.

Subject (b) (6) **(Cardiac arrest):** A 74-year-old patient had a cardiac arrest at home 19 days after starting treatment. She died 3 days later in the hospital. The patient was found to have aspiration pneumonia that led to the pulseless electrical activity (PEA). There were no known cardiac risk factors except for hypertension. The patient also developed paroxysmal atrial fibrillation (AF) while in the study, though it precise timing with regards to the cardiac arrest is not known. The
PI considered the cardiac arrest unlikely related to AMX0035 and the AF possibly related.

Subject (b) (6) **(Left bundle branch block):** Left bundle branch block was noted as a new ECG abnormality at week 24. The baseline QRS width was normal and increased to 110 msec on week 24 (diagnosed as an intraventricular conduction delay). There were no medical diagnoses or concomitant drugs that could have been responsible for this change.

Subject (b) (6) **(First degree AV block):** At week 24 of the OLE this 55-year-old patient developed first degree AV block. Examination of the recordings reveals only a slight change from baseline, probably a result of higher vagal tone (HR 77 vs 85 bpm). He had been on a stable dose of metoprolol for years before this event.

[12] Hanafi 2018; Biomolecules 8(4):159
Desai 2017, Hepatology, 65(1):189-201

During the randomized, controlled period and open label extension (OLE) period of AMX3500, there was 1 case of electrocardiogram QT prolonged, 5 cases of syncope (5 subjects), 1 case of seizure, and 1 case of cardiac arrest. There were no cases of ventricular arrhythmias, ventricular tachycardia, ventricular fibrillation or Torsades de Pointes. One seizure suffered by a 57-year-old female occurred during the respiratory failure was due in part to ALS progression and in part to pneumonia and it was considered not related to the study drug.

Subject (b) (6) **(Cardiac arrest):** This 56-year-old patient had no treatment-emergent ECG abnormalities. The patient was originally assigned to placebo and received AMX0035 for 10 months in the OLE. She was hospitalized for pneumonia, and AMX035 was stopped 9 days prior to her death. PI considered death to be related to respiratory failure.

IRT Reviewer's Comment (refer to Independent IRT Review):
None of the events identified to be of clinical importance per the ICH E14 guidelines (i.e., significant ventricular arrhythmias) occurred in this study. The one seizure and one cardiac arrest were considered unlikely related to the study drug.

8.7.4 Neurotoxicity

Published studies (Gilbert 2001, Phuphanich 2005)[13] on high doses (>400 mg/kg) of sodium phenylbutyrate and phenylacetic acid (PAA) taken intravenously in clinical studies with advanced cancer has been associated with CNS effects such as memory loss, sedation, and confusion. Intravenous administration of PAA (major metabolite of phenylbutyrate) to cancer patients (125 - 150 mg/kg BID) included CNS effects such as somnolence, fatigue, light-headedness, headache, dysgeusia, hypoacusis, disorientation, impaired memory, and exacerbation of a pre-existing neuropathy at PAA levels ranging from 499 to 1285 µg/mL. Oral administration of sodium phenylbutyrate was also associated with fatigue, slurred speech, decreased concentration and confusion reported following oral administration at doses of 9 to 45 g/kg/day in 3 divided doses.

In healthy subjects, after administration of RAVICTI (glycerol phenylbutyrate) at doses 13.2 g/day and 19.8 g/day for 3 days, a dose-dependent increase in non-serious nervous system adverse reactions were observed.

Although all of the total daily doses mentioned in the above examples are higher than the amount of sodium phenylbutyrate in AMX0035 (3g BID, or 6g a day), the Applicant includes a discussion of the potential neurotoxicity with AMX0035 and asserts that although a positive

[13] Gilbert 2001, Clinical Cancer Research, 7, 2292-2300
Phuphanich 2005, Neuro Oncology, 7, 177-182

relationship between plasma peak PAA level and the incidence of nervous system AEs was previously observed, the Cmax in ALS patients treated with AMX0035 under the current BID regimen was lower (40 µg/mL, Study A35-002) compared to the concentrations at which a significant increase in nervous system AEs were observed in healthy volunteers (>80 µg/mL). The incidence of nervous system adverse event was elevated when the PAA Cmax exceeded 80 µg/mL (90%) compared to when PAA levels were lower than 80 µg/mL (32%) (source: RAVICTI FDA Medical Review). Applicant does not propose including 'Neurotoxicity" under "Warnings and Precautions" of the proposed label.

Reviewer's Comment:
The RAVICTI label includes a statement that subjects who had non-serious nervous system adverse reactions, plasma concentrations of PAA ranged from 8 to 56 µg/mL, which were not always measured at symptom onset. The plasma concentration range is similar to that observed with AMX0035. Discussed further with the Clinical Pharmacology Reviewer, Dr. Xiaohan Cai to explore the relationship between nervous system AEs and PAA concentrations observed during the FDA RAVICTI review. A similar relationship between PAA(?) concentration and Nervous system AEs as seen in healthy volunteers was not found in Urea Cycle Disorder patients for whom RAVICTI is indicated. This discrepancy in exposure-response relationship between healthy volunteers and Urea Cycle Disorder patients was thought to be either due to Urea Cycle Disorder patients being more tolerant to nervous systems side effects, or that the patients were on stable doses of BUPHENYL prior to receiving RAVICTI. Nevertheless, in healthy volunteers the incidences of Nervous System Disorder AEs were <40% at PAA concentrations <50 µg/mL.

Given these observations regarding phenylbutyrate moiety and PAA, I reviewed the Nervous System Disorder SOC for comparisons of TEAES between treatment arms. The TEAEs in this SOC that occurred with 1% difference greater than placebo is summarized in Table 53. Other than dizziness, somnolence, and migraine, all TEAEs that were greater in the AMX0035 group appear to be ALS related, hence it is difficult to ascertain if treatment with AMX0035 is worsening these symptoms in ALS patients. Often ALS patients have ALS plus other neurological condition such as parkinsonism or neuropathy, which may contribute to some of the treatment emergent AEs observed. Among these, dizziness does appear to be more in the AMX0035 group.

Table 53 TEAEs in the Nervous System Disorder SOC in ≥2% of Subjects and >1% Difference from Placebo

Preferred Terms	Placebo (N = 48)	AMX0035 (N = 89)
Dizziness	2 (4.2%)	9 (10.1%)
Dysarthria	2 (4.2%)	7 (7.9%)

Preferred Terms	Placebo (N = 48)	AMX0035 (N = 89)
Muscle contractions involuntary	1 (2.1%)	4 (4.5%)
Tremor	1 (2.1%)	3 (3.4%)
Somnolence	0 (0.0%)	3 (3.4%)
Dysgeusia	1 (2.1%)	3 (3.4%)
Balance disorder	0 (0.0%)	2 (2.2%)
Migraine	0 (0.0%)	2 (2.2%)

I also noted that females have higher concentrations of PAA compared to males, therefore explored if nervous system adverse events were higher in the females compared to males treated with AMX0035 for key events as shown in Table 54. The Table does show that dizziness appears to occur more in females (25%) compared to males (3%). However, these are small numbers based on number of patients, hence the reliability of these differences may not be certain.

Table 54 Nervous System TEAEs in Females Compared to Males

Preferred Terms	Males (N = 61)	Females (N = 28)
Dizziness	2 (3.3%)	7 (25%)
Dysarthria	3 (4.9%)	4 (14.3%)
Muscle contractions involuntary	2 (3.3%)	2 (7.1%)
Tremor	2 (3.3%)	1 (3.6%)
Somnolence	2 (3.3%)	1 (3.6%)
Dysgeusia	2 (3.3%)	1 (3.6%)
Balance disorder	1 (1.6%)	1 (3.6%)
Migraine	1 (1.6%)	1 (3.6%)

8.8 Safety Analyses by Demographic Subgroups

Due to the small sample sizes of the subgroups and the relatively low occurrences of many of the TEAEs within the subgroups, firm conclusions regarding any differences in occurrences of TEAES in any of the subgroups cannot be reliably made.

Age: There were no age-related differences in TEAEs in subgroups of patients less than or equal to 59 and greater than 59 years of age.

Sex: The TEAEs occurring in more than 1 subject and occurring in higher percentage in AMX0035 treated subjects compared to placebo that showed differences between males and females are summarized in Table 55. The table suggests dizziness, dysarthria, abdominal pain, nausea and vomiting occurred more in females than males. However, these conclusions are drawn on a small sample size.

Table 55 TEAE Comparisons in Males and Females for AEs Occurring in Higher Percentage of AMX0035 Treated Patients Compared to Placebo

Preferred Terms	Males (N = 61)	Females (N = 28)
Dizziness	2 (3.3%)	7 (25%)
Dysarthria	3 (4.9%)	4 (14.3%)
Abdominal Pain	12 (19.7%)	7 (25%)
Diarrhea	17 (27.9%)	5 (17.9%)
Nausea	2 (3.3%)	8 (28.6%)
Vomiting	1 (1.6%)	3 (10.7%)

8.9 Specific Safety Studies/Clinical Trials

None

8.10 Additional Safety Explorations

8.10.1 Human Carcinogenicity or Tumor Development

No neoplasms were reported in the Application.

8.10.2 Human Reproduction and Pregnancy

There are no human data on the use of AMX0035 in pregnancy or mild production.

8.10.3 Pediatrics and Assessment of Effects on Growth

Not applicable.

8.10.4 Overdose, Drug Abuse Potential, Withdrawal, and Rebound

No case of overdose was observed. The potential for drug abuse has not been studied. No studies examining withdrawal or rebound were conducted.

8.10.5 TEAEs suggesting disease progression

From the reported TEAEs, I looked for preferred terms that would indicate ALS progression and included preferred terms of:"disease progression, fall, respiratory failure, salivary hypersecretion, dyspnea, dysphagia, and dysarthria" to assess if prolonged treatment with AMX0035 has fewer reporting of terms that would suggest disease progression in a subject.

Table 56 shows the percentage of subjects that have key TEAEs that would suggest differences in disease progression in the two groups: Placebo transitioned to AMX0035 (PA) and AMX0035 transitioned to AMX0035 (AA) in the open-label extension. This analysis does not unequivocally suggest that longer treatment with AMX0035 reduces the disease related events. Although respiratory failure appears to be significantly more in the PA group (17.6%) compared to the AA group (10.7%), this includes one subjects in the PA group with highest rate of decline of 3.8 that received treatment for 6 weeks and died. There were no subjects with this high rate of decline in the AA group. Without this subject the percentage of subjects with respiratory failure in the PA group is 14.7% compared to 10.7% in the AA group. The percentage of subjects with disease progression was also greater in the PA group (17.6%) compared to the AA group (12.5%). However, three of the subjects (2 in the PA and 1 in the AA group) had a reported preferred term of disease progression, but the events were fatal and should not also be reported as disease progression. Removing these fatal TEAEs, the percentage of patients with preferred term of disease progression were similar (11.7 % in the PA groups and 10.7% in the AA group). In addition, TEAEs of "Fall, dyspnea, dysphagia, and dysarthria were greater in the AA group.

Several patients discontinued the study due to disease progression or other reasons which are not accounted for in the analysis in Table 55. In addition, these events were reported at various times during the study. Study day from double-blind baseline that the event occurred ranged from 193-307 days in the AA group and between 174-954 days in the PA group (event start day for 2 subjects in the AA group and 2 subjects in the PA group were missing), suggesting some subjects in the PA group may have been treated with AMX0035 for similar or longer than the subjects in the AA Group. Therefore, clear conclusion regarding overall disease progression differences between patients that were on AMX0035 longer and those that were on transitioned to AMX0035 can be made based on TEAE terms suggesting differences in disease progression.

Table 56 TEAEs that Occurred in the Open Label Phase in the Two Groups: PA and AA

Preferred Terms	Placebo/AMX0035 (N = 34)	AMX0035/AMX0035 (N = 56)	Combined (N = 90)
Fall	6 (17.6%)	14 (25.0%)	20 (22.2%)
Respiratory failure	6 (17.6%)	6 (10.7%)	12 (13.3%)
Disease progression*	6 (17.6%)	7 (12.5%)	13 (14.4%)
Salivary hypersecretion	2 (5.9%)	2 (3.6%)	4 (4.4%)
Dyspnea	0 (0.0%)	8 (14.3%)	8 (8.9%)
Dysphagia	1 (2.9%)	2 (3.6%)	3 (3.3%)
Dysarthria	0 (0.0%)	2 (3.6%)	2 (2.2%)

*removing subjects that had preferred term disease progression, but the events were fatal, show 4 (11.7%) in the PA group and 6 (10.7%) in the AA group.

8.11 Safety in the Postmarket Setting

8.11.1 Safety Concerns Identified Through Postmarket Experience

AMX0035 is not marketed in any country.

8.11.2 Expectations on Safety in the Postmarket Setting

It is difficult to predict the safety profile in the postmarket setting given the complications and manifestations of ALS.

8.11.3 Additional Safety Issues From Other Disciplines

None.

8.12 Integrated Assessment of Safety

An integrated assessment of safety was not performed given the small sample size of the safety database.

In summary, AMX0035 generally appears safe. There were no major differences in fatal and serious adverse events between AMX0035 and placebo. Most of these adverse events were secondary to manifestations and complications of underlying ALS.

Common TEAEs belonged to the gastrointestinal SOC (including diarrhea, abdominal pain, nausea, salivary hypersecretion). Other common TEAEs included dizziness, respiratory tract

infection, fatigue, and dyspnea.

There were no differences in laboratory abnormalities or vital signs between AMX0035 and placebo-treated participants

9 Advisory Committee Meeting and Other External Consultations

The Advisory Committee Meeting was held on March 30, 2022. The committee's advice was sought on the following question.

VOTE: Do the data from the single randomized, controlled trial and the open-label extension study establish a conclusion that sodium phenylbutyrate/taurursodiol is effective in the treatment of patients with amyotrophic lateral sclerosis (ALS)?

a. If you voted "no", please discuss what additional information you would consider necessary to establish a conclusion that sodium phenylbutyrate/taurursodiol is effective in the treatment of patients with ALS

Vote Result: Yes: 4 No: 6 Abstain: 0

All members who voted expressed similar sentiments that the decision was difficult. Those who voted "Yes" admitted that it was a difficult decision and could have decided either way. Amongst the 4 members that voted "yes", one was a consumer representative who wanted the consumers voice to be heard and the other a patient representative who believed that the Agency should exercise regulatory flexibility given the lack of material harm with AMX0035 but looked forward to more stronger data from the ongoing study. One member thought to establish a conclusion on effectiveness (as stated in the voting question) was not quite the same bar as substantial evidence of effectiveness of the drug. This member agreed with the Applicant's statistical analyses using a shared baseline linear random effects model and though there were not many deaths in the study.

Those who voted "No" concluded that the data from the CENTAUR study did not meet the statutory and regulatory threshold for substantial evidence and persuasiveness. Some key considerations on voting "No" included: lack of persuasive evidence required for approval based on a single study based on concerns on trial conduct, sample size, treatment of missing data, modest effect on primary endpoint with no support on secondary endpoints.

There were concerns on the exploratory nature of the open-label study to provide support as

confirmatory evidence with serious limitations such as high rate of non-participation and dropouts, treatment of tracheostomy or hospitalization as death equivalents as composite endpoint, post-hoc analyses of death alone and overall interpretability of the results.

Importantly, all those who voted "No" acknowledged that the ongoing larger PHOENIX trial would resolve the uncertainties on effectiveness of AMX0035.

A second Advisory Committee Meeting was held on September 7, 2022. The committee's advice was sought on the following question.

1. DISCUSSION: Discuss the strength of the currently available data regarding the effectiveness of sodium phenylbutyrate/taurursodiol (AMX0035), to include the new information submitted and the information presented at the March 30, 2022, PCNS meeting. The discussion may include considerations regarding the unmet need in amyotrophic lateral sclerosis (ALS), the status of the ongoing Phase 3 trial, and the seriousness of ALS.
2. VOTE: Considering the new information submitted and the information presented at the March 30, 2022, PCNS meeting, is the available evidence of effectiveness sufficient to support approval of sodium phenylbutyrate/taurursodiol (AMX0035) for the treatment of patients with ALS? In addition to the prior and new evidence presented, you may take into account in your vote the unmet need in ALS, the status of the ongoing Phase 3 trial, and the seriousness of ALS.

Vote Result: Yes: 7 No: 2 Abstain: 0

The Committee members expressed a range of viewpoints when discussing the strength of the currently available data (including the new information submitted and the information presented at the March 30, 2022, PCNS meeting) regarding the effectiveness of sodium phenylbutyrate/taurursodiol (AMX0035). Some members were in agreement that the overall evidence presented from both meetings was mild to moderately persuasive of the effectiveness of AMX0035, noting that while the data presented has its limitations and challenges, the endpoints trend in the same direction and may support the finding of prolonged survival with the product. Other members expressed being reassured by the absence of a safety signal, suggesting that AMX0035 is not likely to harm patients even if the Phase 3 PHOENIX trial fails to demonstrate a benefit.

Other members found the overall evidence less compelling. Several members were in agreement that the biomarker analysis did not add much to support evidence of effectiveness, with members pointing to shortcomings such as measurements being taken from one time point, and the unclear relevance of biomarker data derived from patients with Alzheimer's Disease to ALS. The committee members were divided when discussing the strength of the new

sensitivity analyses using external natural history data presented by the Applicant. Some members were less compelled, pointing to the analyses being conducted post-hoc and not pre-specified, and questioning the source and population base used. Other members noted the analyses were supportive of a real-world difference in patients treated with AMX0035 and the observed survival benefit seemed to make sense, but acknowledged the limitations.

During the Committee's discussions, several members recognized the unmet medical need for treatment options for a rare and life-threatening condition such as ALS, with one member pointing to the importance of listening to the patient community and highlighting FDA's ability to exercise regulatory flexibility in this context. A few members discussed the financial aspect of market approval for AMX0035, and FDA clarified that the drug cost would not be a relevant consideration in the FDA's scientific deliberations and assessment of the scientific evidence.

10 Labeling Recommendations

10.3 Prescription Drug Labeling

Labeling is reviewed separately.

11 Risk Evaluation and Mitigation Strategies (REMS)

Not Applicable.

12 Postmarketing Requirements and Commitments

There are no clinical recommendations for PMRs or PMCs..

13 Appendices

14.1 References

See footnotes throughout document.

14.2 Financial Disclosure

Covered Clinical Study (Name and/or Number): CENTAUR AND CENTAUR-OLE

Was a list of clinical investigators provided:	Yes ☒	No ☐ (Request list from Applicant)
Total number of investigators identified: 130		
Number of investigators who are Applicant employees (including both full-time and part-time employees): 0		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): 0		
If there are investigators with disclosable financial interests/arrangements, identify the number of investigators with interests/arrangements in each category (as defined in 21 CFR 54.2(a), (b), (c) and (f)): Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: _____ Significant payments of other sorts: _____ Proprietary interest in the product tested held by investigator: _____ Significant equity interest held by investigator in S Applicant of covered study: _____		
Is an attachment provided with details of the disclosable financial interests/arrangements:	Yes ☐	No ☐ (Request details from Applicant)
Is a description of the steps taken to minimize potential bias provided:	Yes ☐	No ☐ (Request information from Applicant)
Number of investigators with certification of due diligence (Form FDA 3454, box 3) _____		
Is an attachment provided with the reason:	Yes ☐	No ☐ (Request explanation from Applicant)

14.3 Endpoint Details

Coefficients and Intercepts for ATLIS Standardization*

Sex	Maneuver	Age (years) Coefficient	Weight (lb) 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

Coefficients and Intercepts for ATLIS Standardization*

Sex	Maneuver	Age (years) Coefficient	Weight (lb) Coefficient	Height (in) Coefficient	Intercept
	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

* Coefficients and intercepts were modified from the originally published values, as necessary, based on use of ATLIS Version 2.
Data from Andres 2013.

For example, the predicted value for the left grip maneuver for a 41-year-old woman who is 62 inches tall and weighs 126 pounds would be calculated as follows:

$$Predicted = -28.91 - 0.15*Age + 0.16*Weigh\,t + 1.18*Heigh\,t$$
$$Predicted = -28.91 - 0.15*41 + 0.16*126 + 1.18*62$$
$$Predicted = 58.26$$

(Andres, P. et al. Developing normalized strength scores for neuromuscular research. Muscle and Nerve. 2013.)

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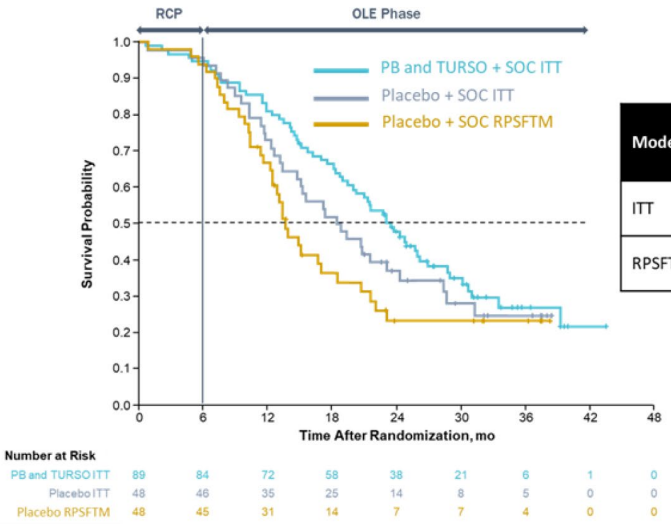
14.4 Applicant's New post-hoc Survival Analysis

The new AMX survival analysis[14] is post-hoc, counterfactual, heavily dependent on untestable assumptions, known to be biased and not accompanied by the sensitivity analysis to gauge the bias that was recommended[15] due to the sensitivity analysis failing to have a solution for this data. The OLE design requires this analysis to rely on counterfactuals for 71% of the placebo arm, increasing the reliance on the untestable assumptions. The following best practices for such analysis identified by a coauthor of 14 in an earlier paper[16]were not implemented by this applicant.

- Carefully consider whether to include switch in trial design; it is preferable not to include this from a data interpretation perspective.
- Include the planned method to handle switching up front in protocol and analysis plan.
- Consider contemporaneous collection of data on treatment without switch outside the trial, in a comparable setting, to provide external validation.
- Consider sensitivity analyses using other methods.

Figure 21 Applicant's post-hoc Rank Preserving Structural Failure Time Model to account for Placebo switching treatments



Model	Median Survival Duration Difference, mo	HR (95% CI)	*P* Value
ITT	4.8	0.64 (0.42–1.00)	.048
RPSFTM	9.7	0.42 (0.18–0.99)	.048

Note: Figure above was copied from the sponsor submission "survival analyes as confirmatory evidence"

[14]Paganoni 2022 Muscle and Nerve article doi: 10.1002/mus.27569
[15] NR Latimer,1 IR White,2 KR Abrams3 and U Siebert4,5,6 Causal inference for long-term survival in randomised trials with treatment switching: Should re-censoring be applied when estimating counterfactual survival times? Statistical Methods in Medical Research 2019, Vol. 28(8) 2475–2493
[16] C Watkins, X Huang, N Latimer,Y Tang, and E Wright, Adjusting overall survival for treatment switches: commonly used methods and practical application, Pharmaceutical

The study design does not support and is at odds with the intention of the new analysis to compare unswitched placebo to AMX through the open label extension because placebo double blind period completers switched by design. The new analysis comparison is counterfactual/hypothetical and not for just a small proportion but for a very high proportion (this is similar to an ALSFRS-R analysis with 75% missing data). The basis for estimating survival for counterfactual placebo, i.e., what would have happened if they hadn't switched, is weak because most eligible placebo switched (by design) and, furthermore, the ineligible placebo group, those who didn't complete the double blind (DB) period, is not a random subset. In particular, double blind period dropouts actually have a worse baseline average than eligible patients for switching (for placebo the mean baseline ALSFRS-R is 3.7 points higher for completers [37.5, n=37] than dropouts [33.8, n=11]). Thus, the analysis is biased as indicated in the 2019 Latimer reference article [15] and reliant on hypothetical imputation for a very high proportion of placebo subjects.

The SAP also mentioned several comparisons of interest, in addition to PA vs. AA, including placebo only to pooled AMX/AMX and placebo/AMX, within group comparisons between main phase and extension phase. Therefore, there are multiplicity issues and comparing PP vs. AA was not a priority as evidenced by the design. There were also no prespecified analyses to adjust the placebo arm for switching to AMX in the placebo vs. AMX comparison as has been done in this post-hoc analysis. Note again that switching was mandated for continuing completers by the study design. It is also not clear that the Applicant's new analysis accounted for the fact that placebo dropouts had no chance of receiving AMX in the extension due to not completing the double-blind phase and completing being a requirement for switching and the analysis seems to assume exchangeability of placebo dropouts and completers despite double blind period dropouts having a lower average baseline ALSFRS-R score.

The Applicant's new survival analysis relies on strong untestable assumptions. It uses re-censoring which one of the cited references states leads to an optimistically biased treatment effect and the sensitivity analysis without re-censoring, recommended by the reference to accompany the re-censoring analysis as a check on the bias of the re-censoring analysis, failed to converge to a solution for this data (which may suggest no treatment benefit). The paper also suggested based on a simulation study that the bias of the re-censoring analysis increases with the proportion switching, which is very high for the placebo arm.

In particular, one of the applicant's reference papers[15] states that "We found that analyses which re-censored usually produced negative bias (i.e. underestimating control group restricted mean survival and overestimating the treatment effect), whereas analyses that did not re-censor consistently produced positive bias which was often smaller in magnitude than the bias associated with re-censored analyses, particularly when the treatment effect was high and the switching proportion was low." Furthermore, the Applicant journal article reporting the results of the new survival analysis [14] states that "in the RPSFTM analysis, it was not possible to estimate the AF for the RPSFTM without re-censoring using the on-treatment duration of effect assumption." Thus, the presented result is known to be biased in favor of treatment and the reference's recommendation that analyses without re-censoring should be also presented and

factored in, to account for this bias was not done because the latter model did not converge to a solution. It seems that this lack of convergence could be consistent with an acceleration factor of 0, i.e., no treatment benefit, and/or the analysis' untestable assumptions, such as the same proportional treatment effect applying regardless of treatment start time.
By design of the OLE, placebo non-completers had no opportunity to switch to AMX because they were not eligible for the OLE and if the treatment is effective for survival, they would likely be the most disadvantaged subgroup for survival. Overall, DB period completers have a better double-blind baseline ALSFRS-R score than non-completers (36.5 vs. 34.4, p=0.059). The latter were not allowed to continue into the OLE. Thus, opportunity for longer treatment and placebo switching is tied to completion of the DB period. Those who were not switched are not a random subset.
Median weeks on treatment is 12 for placebo switchers and 9 for AMX DB dropouts (70% of placebo switchers have <=39 weeks of treatment). While these aren't random subsets the placebo switchers appear to have significantly better survival than DB AMX dropouts. This may violate the structural model assumption that there is the same extension of survival time proportional to time on AMX regardless of when it is started, which seems like a highly questionable assumption to begin with, e.g., the drug patient dosed for 3 days would've gotten some proportional survival benefit under this assumption (but actually died at day 22).
The Applicant states "probability of censoring weighting and two-stage models are often used in oncology to adjust overall survival for switching and were considered here; however, these methods are not suitable when most participants switch, as was the case in the CENTAUR trial, in which only three of the placebo-randomized participants who were eligible to enroll in the OLE phase did not do so." This argument against the Oncology models seems misleading (i.e., the claim that most placebo switched), because the placebo non-completers of the DB accounting for 17% of placebo, were by design not eligible to switch. These oncology methods are also exploratory but might shed a different light on the data. However, they were dismissed based on what the statistical reviewer believes is a design flaw, not allowing placebo DB dropouts to switch or continue into the OLE (same for AMX0035 dropouts), and so these methods were not presented. This design element may compromise the ability to make unbiased inference about survival. Also, it seems questionable whether the recensoring method is applicable when the switching proportion is very high, greater than .70 in this case.

Excerpt from the Applicant article on lack of convergence of the analysis without recensoring:
"No factor could be selected that balanced the counterfactual survival (ie, the model-estimated survival time in the absence of PB and TURSO) without recensoring between the treatment arms. It may be due to a prognostic imbalance at baseline between randomized arms, though this seems unlikely as baseline characteristics were generally well balanced and the AF could be estimated from other models. Alternatively, it may be that the on-treatment duration of effect assumption is not suitable for this study, but this was judged to be a more plausible assumption than the treatment group approach. Similar results were seen for the analyses using both duration of effect assumptions, suggesting that varying this assumption does not have a large

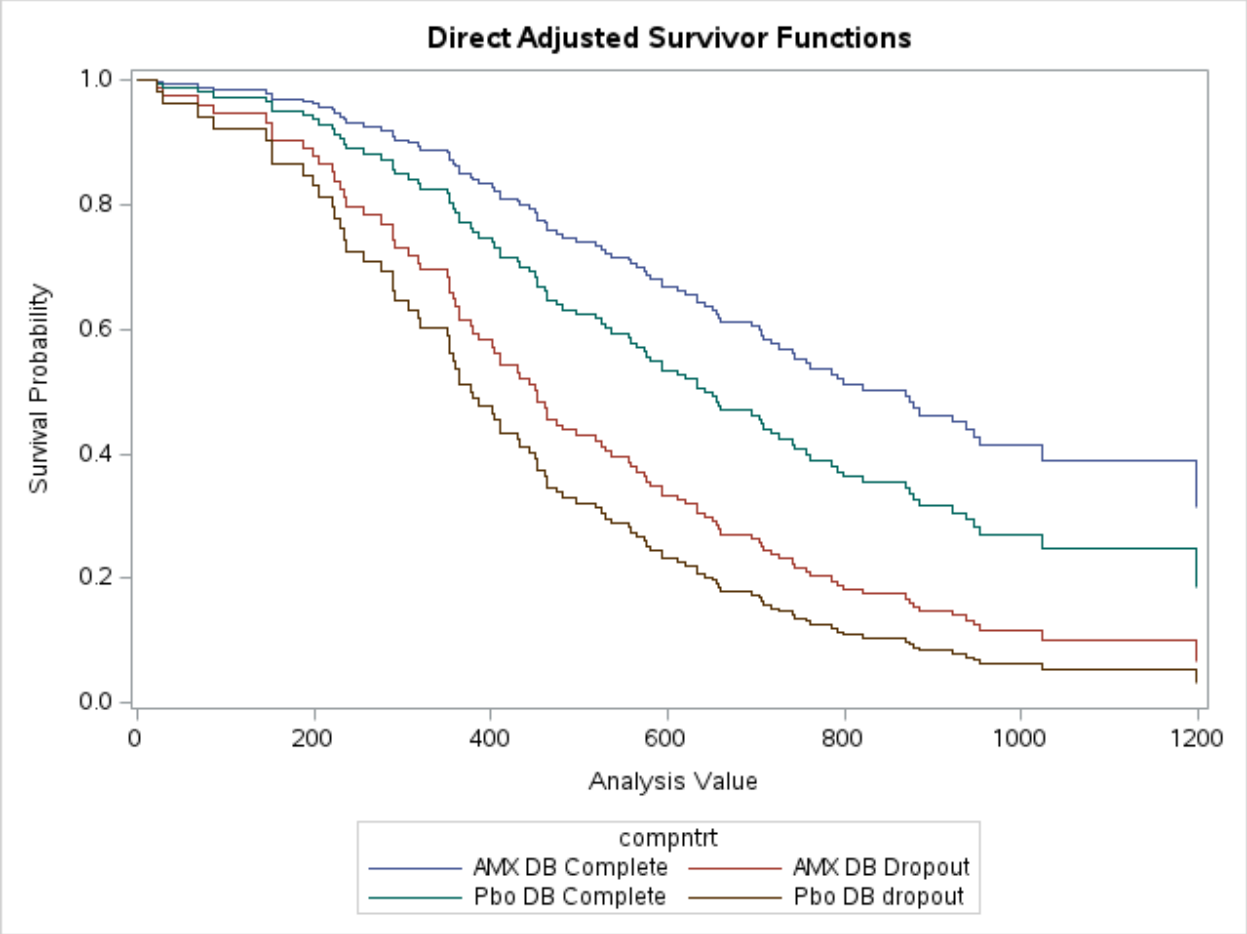
impact. Another important point about the RPSFTM method is that it assumes a common treatment effect (ie, exposure-response is the same, no matter when the treatment is received).[17]This assumption may be unreliable in degenerative conditions such as ALS, though the time between randomization and crossover in CENTAUR was relatively short (only 6 months)".

The Applicant acknowledges some limitations of the new survival analysis: "It is important to note that the post hoc subgroup analysis applied herein has limitations as the subgroups are small, and potential confounding differences among the groups were not controlled for, although covariates of age at randomization, pre-baseline ALSFRS-R slope, and baseline ALSFRS-R total score attempted to control for some of this bias."

Figure 22 shows that based on a reviewer exploratory analysis, placebo switchers (green, N=37) appear to have better survival than AMX arm double blind dropouts (red, N=21), but note that standard errors and number at risk are not shown.

[17]Latimer NR, Abrams KR. NICE DSU Technical Support Document 16: Adjusting Survival Time Estimates in the Presence of Treatment Switching. Published July 2014.

Figure 22 Reviewer Exploratory Time to Death Alone Survival Time Comparison between AMX and Placebo subgrouped by completion of the double-blind period.



Another applicant publication[18] is focused on the time to composite survival endpoint analyses which are problematic due to significant loss to follow-up on tracheostomy and hospitalization events (35% did not participate in the open label extension).
A survival as confirmatory evidence document submitted to the NDA covers these analyses as well as a summary of a reportedly positive comparison of the CENTAUR AMX arm to natural history (section IV, page 5-6). However, the document provides only very limited details on the specifics of this natural history comparison.

Assumptions and Details of Rank Preserving Structural Failure Time method (RPSFTM)

Assumptions:

[18]Paganoni 2022a Paganoni S, Hendrix S, Dickson SP, et al. J Neurol Neurosurg Psychiatry Epub ahead of print: [please include Day Month Year]. doi:10.1136/jnnp-2022- 329024

- Experimental treatment effect is same regardless of when it is given (at randomization or at switch)
- Counterfactual survival time is balanced between treatment groups due to randomization

Limitations:

- Assumption of constant treatment effect may not be realistic in many diseases.
- Simulations show this can then lead to large biases.
- Methods and results can be difficult for non-experts to understand.
- Unstable if amount of treatment is similar in both arms.

Applicant's article supporting model documentation:

Supplementary Methods. Rank-Preserving Structural Failure Time Model

The rank-preserving structural failure time model (RPSFTM) was used to estimate, for each trial participant, the counterfactual survival time in the absence of active treatment (sodium phenylbutyrate and taurursodiol), and an active treatment effect that extended the survival time while the participant was on treatment. The model is structured as follows:

Patient i

Observed time on active treatment $T_{i,on}$ (= 0 for participants originally randomized to placebo who did not switch)

Observed time off active treatment $T_{i,off}$

Observed survival time $T_{i,obs} = T_{i,on} + T_{i,off}$

Counterfactual survival time in the absence of active treatment $T_i(0)$

Counterfactual and observed survival times are related through a treatment effect ψ as follows:

$$T_i(0) = T_{i,off} + e^{\psi} T_{i,on}$$

(1)

e^{ψ} is sometimes referred to as an acceleration factor (AF), as it speeds up remaining life.

The RPSFTM makes a strong untestable assumption: that the active treatment effect ψ is the same whenever the treatment is given, whether given at randomization in the active arm or upon entry into the OLE phase in the control arm.

The AF was estimated via G-estimation using a grid search with a step size of 0.01, with the same test statistic as the intent-to-treat (ITT) analysis (Cox test statistic with covariates for age at randomization, pre-baseline Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised [ALSFRS-R] slope, and baseline ALSFRS-R total score).

Censoring flags are generally passed through from the observed time scale to the counterfactual time scale. However, censoring indicators that are noninformative on the observed time scale may be informative on the counterfactual time scale.[15,16] Recensoring can be applied to guard against informative censoring in the counterfactual dataset. However,

recensoring also causes a loss of longer-term survival information.[18] Analyses are often conducted both with and without recensoring.
Recensoring was applied where relevant to reduce bias, in the following way:
C_i was set to be the potential censoring time for participant i . This is usually the time from study entry to administrative data cutoff. For the small number of participants who were censored in the observed data for reasons other than reaching the administrative data cutoff, we set C_i to be the observed date of censoring.[14]
For a given value of ψ, a participant's counterfactual survival time is $T_i(0, \psi)$, and we re-censor this at the earliest possible censoring time on the counterfactual time scale across possible treatment profiles for that arm, which is

$$D_i(\psi) = \min(C_i, e^{\psi} C_i) \quad .$$

Recensoring can be applied in the estimation of the AF only or in both the estimation of the AF and the comparison of adjusted survival times. The first approach has less loss of long-term survival information.
Analyses were conducted both without and with recensoring.
Using the CI and P value directly from a Cox analysis based on the adjusted survival data is not correct, as this approach does not reflect the uncertainty in the ψ parameter. Therefore, symmetric CIs were constructed for the log hazard ratio using the ITT P value.[15]

Excerpts from Latimer et al (2019) [15] and Watkins et al., Pharmaceutical Statistics (2013) 12, 348-357[16]:

The simple one-parameter version of the RPSFTM splits the observed event time, T_i, for each patient into time spent on the control treatment, T_{Ai} , and time spent on the intervention treatment, T_{Bi} . And

$$T_i = T_{Ai} + T_{Bi} \quad .$$

(eq1)
For patients who are randomized to the intervention treatment, and who do not switch onto the control treatment, T_{Ai} is equal to zero. For patients randomized to the control group who do not switch onto the intervention, T_{Bi} is equal to zero. However, for patients who switch treatments, both T_{Ai} and T_{Bi} will be greater than zero. The RPSFTM method relates T_i to the counterfactual survival time (U_i) with the following causal model Rank-preserving structural failure time method.

$$U_i = T_{Ai} + e^{\Psi} T_{Bi}$$

(eq2)
Here $e^{-\Psi}$ represents the acceleration factor (AF) associated with the intervention – the factor by which treatment increases an individual's expected survival time. The RPSFTM assumes

- there is a common treatment effect associated with the experimental treatment (i.e., that the treatment effect, e^{Ψ}, is the same no matter when the treatment is received) and

- if no patients received the experimental treatment average survival times in the randomized groups would be equal.

Given these assumptions, g-estimation is used to estimate ψ, with the true value being that for which counterfactual survival times (U_i) are independent of randomized group This is done by computing U_i for a range of values of ψ and each time testing whether the U_i are independent of randomized group.
The RPSFTM was initially developed by Robins and Tsiatis to adjust patient survival time for patients switching from a randomized treatment allocation to another study treatment. A patient who switches treatment has, in theory, an unknown counterfactual event time: the time-to-event if no experimental treatment were received. The RPSFTM is a semiparametric approach that estimates the counterfactual event time of patients.
Calculating the counterfactual event time. A patient's observed individual time-to-event duration (T_i) can be split into the duration of time a patient spends on (T_{Bi}) and off (T_{Ai}) the experimental study treatment (eq1). Estimating the acceleration factor. The acceleration factor from the counterfactual event time equation (2) is estimated using G-estimation[19]. This is an iterative process of searching a grid of possible ψ values and the corresponding test statistic for the null hypothesis: U_i is independent of randomized treatment. The test statistic could be taken from any standard survival analysis model, for example, log rank, Wilcoxon, and Cox, with or without covariates. It may be preferable to use the same model as the ITT analysis. The value of ψ that satisfies the null hypothesis (test statistic = 0) is selected. Care should be taken to ensure this is a unique solution. If no unique solution can be found, the plausibility of the different values should be considered. An unweighted test statistic such as log rank can result in uncertainty in estimating ψ.
The estimated acceleration factor is then applied in (4) to estimate counterfactual survival times of switched control arm patients. Re-censoring may be needed (see the supporting information for details). These are then combined with the observed survival times from experimental and non-switched control arm patients and analyzed using standard survival analysis techniques. However, the uncertainty in the estimate of the acceleration factor should be carried through to the estimation of the standard error (and hence the p-value and the confidence interval [CI]) of the RPSFTM hazard ratio (HR). An improvement in the RPSFTM HR compared with the ITT HR will be countered by a widening of the CI. As proposed by White[20], the RPSFTM p-value can be set as the ITT p-value and the confidence limits calculated accordingly, because the ITT p-value is preserved by this method as long as the same test is

[19]Robins JM, Blevins D, Ritter G, Wulfsohn M. G-estimation of the effect of prophylaxis therapy for Pneumocystis carinii pneumonia on the survival of AIDS patients. Epidemiology 1992; 3:319–336.

[20] White IR, Babiker AG, Walker S, Darbyshire JH. Randomization-based methods for correcting for treatment changes: examples from the Concorde trial. Stat Med. 1999;18(19):2617-2634

used for both. Alternatively, the confidence limits and p-value can be estimated using bootstrap techniques.

Censoring is problematic for the RPSFTM and two-stage method due to an association between treatment received, counterfactual censoring time, and prognosis. For ease of exposition, we assume the experimental treatment is beneficial, though similar arguments apply if it is harmful. The counterfactual survival model then involves shrinking survival times for all patients who receive the experimental treatment. For some patients, the event time (usually death) may not be observed – instead it is censored. For these patients, the RPSFTM and two-stage methods involve shrunken censoring times. The amount by which survival or censoring times are shrunk depends upon the size of the treatment effect and the duration for which the experimental treatment is received. Counterfactual censoring times will be prone to informative censoring bias if either/both of the two following criteria are met:

. If treatment switching decisions are related to prognostic factors;

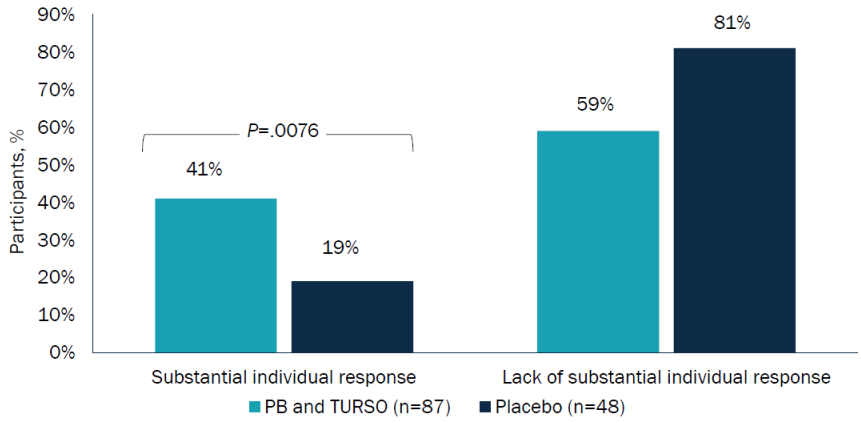
. If the duration of treatment is related to prognostic factors.

Whilst both the RPSFTM and two-stage estimation seek to account for prognostic differences between switchers and non-switchers in their estimation of ψ, the potential for informative censoring in the counterfactual dataset remains because censoring times will be related to switching times, which may be related to prognostic factors. It has been suggested that possible bias associated with informative censoring can be avoided by breaking the dependence between the counterfactual censoring time and treatment received by re-censoring the counterfactual survival time associated with a given value of ψ (that is, $U_i(\psi)$) for all patients at the minimum of the administrative censoring time C_i and $C_i \exp(\psi)$, representing the earliest possible censoring time over all possible treatment trajectories, $D_i^*(\psi)$.

$U_i(\psi)$ is then replaced by $D_i^*(\psi)$ if $D_i^*(\psi) < U_i(\psi)$. In the context considered in this paper, where switching is from the control group onto the experimental treatment, survival or censoring times in the control group are re-censored at $D_i^*(\psi)$ (i.e. $C_i \exp(\psi)$, when the treatment prolongs survival) if this is less than the observed survival or censoring time for non-switchers (since, for patients who did not switch, $U_i(\psi) = T_i$), or less than counterfactual survival or censoring times $U_i(\psi)$ for switchers. It is straightforward to appreciate that the greater the treatment effect ψ, the greater the impact of re-censoring, and the more control group events will be lost as the survival data are artificially censored at a time-point earlier than the follow-up times observed in the trial. A treatment effect calculated by comparing counterfactual control group survival times and observed experimental group survival times is therefore based upon shorter-term data for the control group. If the treatment effect is not constant over time, using the recensored survival data would result in bias if the objective is to estimate the overall longer-term treatment effect.

Additional Post-hoc analysis of ALSFRS-R slope

Comparing pre-randomization retrospective estimated slope (ΔFS) to slope from baseline to Week 18 the outcomes can be subgrouped into 1) Participants whose actual rate of change in the ALSFRS-R at week 18 was greater than or equal to their own prebaseline progression rate (ΔFS) (Fig 2) and 2) Participants who died before Week 18 or withdrew before Week 12 were included in this group. Participants whose actual rate of change in the ALSFRS-R at week 18 was less than their own prebaseline progression rate (ΔFS).
The applicant claims that substantial individual response was observed in a greater proportion of participants receiving PB and TURSO (41%; 95% CI, 31%–52%) versus placebo (19%; 95% CI, 8%–30%); odds ratio, 3.06; 95% CI, 1.32–7.09; P=0.00768 (see Figure below).



Source: ENCALS 2022 poster

This analysis is post-hoc and correlated with the primary analysis, not independent data, because change from baseline slope and prerandomization slope were both used in the primary analysis.
The applicant claims it uses participants as their own control and thus may provide independent evidence of effectiveness. However, the comparison is still between the treatment groups rather than within groups and the design did not include the treatment sequence (drug then placebo) needed for a proper crossover design and within patient analysis."
For a crossover analysis you need to have the sequence drug/placebo which the applicant does not have for the data analyzed here. In fact, the pre-randomization period outcome is retrospective, this outcome does not come from a well-controlled design. There could be a period or carryover effect but the drug/placebo sequence is missing from this design. They compared to week 18, rather than week 24 without any justification, and effect size on the primary endpoint was bigger at week 18 than week 24. The delfs or pre-randomization slope is retrospectively collected. The variability of the change in slope may not be constant because delfs depends on time from symptom onset which varies across patients. Linearity seems questionable for pre-randomization slope because this slope is over a period of up to 608 days (median=418, 25th percentile=337, minimum=92), much longer than 24 weeks (for which the review of the primary analysis found may not be linear) and there is no way to check linearity,

the pre-randomization slope is based on a baseline measurement and a presumed score of 48, maximum normal ALSFRS-R score, at disease onset. Note that in a true crossover design the treatment in each period is typically the same duration, which is not the case here, the duration actually varies by patient because the data is retrospective to time of diagnosis. There is also post-baseline starting of ALS medications, slightly more in the drug arm, which could also confound this post hoc analysis. In summary, this post-hoc analysis does not consist of independent well controlled data, that could provide independent confirmatory evidence.

14.5 Details of Statistical Reviewer's Exploratory Analyses

As described in the AC briefing document and presentation, our questions about the sufficiency of the evidence of effectiveness are based on a variety of factors, including the presence of only a single study, the lack of highly persuasive primary and secondary analysis results in that single study, and the uncertainty about the robustness of those results. The questions about robustness are based on several different assumptions and limitations of the data and do not hinge on any specific sensitivity analysis. Nevertheless, because you have raised questions about the details of a few of the sensitivity analyses conducted and presented by FDA, we have described the chosen models below. Also note that this document is not intended to be a comprehensive response to all of the concerns you have raised in recent submissions and that were discussed at the recent late-cycle meeting. Rather, this is specifically focused on addressing any remaining uncertainties about the details of statistical models that were fit by FDA and the corresponding results of those analyses.

Mean-by-visit MMRM

Because of questions about the linearity assumption, the reviewer conducted a traditional mean-per-visit MMRM analysis that does not rely on an assumption of linearity in ALSFRS-R over time. There were no prespecified model details in the SAP for a traditional mean-per-visit MMRM model. The reviewer only explored a traditional MMRM as a sensitivity analysis to explore robustness to the primary slope model linearity assumption while recognizing that neither of these models handles deaths appropriately, so should not be primary. The reviewer did not exclude any records after Day 182 for comparability with the slope model since these were not excluded from the slope analysis (the SAP mentioned doing this for windows but the primary analysis did not use windows). The reviewer's model included age and pre-randomization slope (DELFS) as covariates and interactions between pre-randomization slope and Visit (categorical), and age and Visit, as well as effects for treatment, Visit, and the treatment by Visit interaction. An unstructured covariance matrix was used for the variance covariance of repeated measures within subjects. The model was analyzed in the mITT population and did not include the baseline assessment of ALSFRS-R as the first measure of the dependent variable (since the outcome was change from baseline, unlike the primary analysis). The estimated difference (95% CI; p-value) from this analysis was 1.86 (-0.19, 3.91; p=0.0749).

Quadratic

Because of questions about the linearity assumption and because a quadratic analysis was prespecified as a backup analysis in the SAP, the reviewer conducted quadratic analyses that allow more flexibility in the nature of the ALSFRS-R change over time. The pre-specified backup quadratic model included a random effect for the quadratic weeks term but did not include a treatment by quadratic weeks interaction. The estimated difference at Week 24 (95% CI; p-value) from this analysis was 1.68 (-0.40,3.75; p=0.1134). The reviewer also conducted an additional quadratic analysis that was the same as the model prespecified in the note on the quadratic model except it added one more fixed effect term, weeks^2*treatment, to the model. More specifically, the reviewer's additional quadratic model included fixed effects for Weeks, Age*Weeks, DELFS*Weeks, Treat*Weeks, Weeks^2, Age*Weeks^2, DELFS*Weeks^2, Treat*Weeks^2, and random effects for Intercept, Weeks, and Weeks^2, with an unstructured covariance among random effects within subject. Although this analysis may imply different curvature between groups, the basis for assuming equal curvature is also not clear and could cause bias if the true model is quadratic. In fact, for the given data, the weeks^2*treatment term p-value is well below the level of nominal significance (p=0.0060) which raises questions about linearity and equal curvature. The estimated difference at Week 24 (95% CI; p-value) from this analysis was 1.97 (-0.12, 4.06; p=0.0644). Note also that the mean-per-visit MMRM analysis does not enforce equal curvature between groups.

Joint rank (with multiple imputation)

Because of concerns about the handling of deaths in the primary analysis, and about the handling of missing data in survivors via last observation carried forward (LOCF) in the applicant's joint rank analyses, the reviewer conducted an additional joint rank analysis with multiple imputation of missing data based on a missing-at-random assumption. The reviewer's joint rank analysis used 50 multiple imputations for missing Week 24 ALSFRS-R data in survivors (observed data for deaths were not excluded for the basis of multiple imputation of missing data, e.g., including the modeling of earlier non-monotone missing ALSFRS-R data). First, a monotone missing data pattern was achieved using PROC MCMC in SAS for the variables AGE, DELFS (pre-randomization slope), and ALSFRS-R at Weeks 3, 6, 9, 12, 15, 18, 21, and 24. Next, with a monotone missing pattern established, the missing Week 24 ALSFRS-R data were imputed once for each of the 50 monotone patterns through successive regression models of ALSFRS-R at each Visit Week with regressors of AGE, DELFS, and ALSFRS-R at all previous visits to the given week until any missing Week 24 ALSFRS-R data for survivors were imputed. The multiple imputation was performed separately for each treatment group. Next, the imputed datasets for separate treatment groups were combined and the joint ranks were obtained for each imputation as follows.
if patient i died and j did not, then i gets +1
if j died and i did not, then i gets -1

if both died and i had shorter survival, then i gets +1, if i had longer survival, then i gets -1, or if the pair is tied on survival time then i gets 0
if both survived, rank on ALSFRS-R change at Week 24 (more positive change is better), if i is better, i gets -1; if i is worse than j, then i gets +1; or if they are tied, then i gets 0 for this comparison.
For each i, sum these individual comparison scores/ranks over pairs with patient i.
Rank the joint rank and the covariates of age, and DELFS.
Perform an ANCOVA of the ranked joint rank with covariates of ranked age and ranked DELFS and treatment group as an explanatory variable.
Perform the ANCOVA for each of the 50 multiple imputations and then summarize the treatment estimate using Rubin's method as implemented in SAS in PROC MIANALYZE.

This analysis was conducted in both the mITT and ITT population. In the mITT population, the mean difference in ranks was 12.58, with a p-value=0.0626. In the ITT population, the mean difference in ranks was 12.00, with a p-value=0.0785.

14.6 Applicant's Natural History Analyses

A new post-hoc survival analysis based on a survival prediction algorithm created from natural history data:

According to the applicant another method to account for the issue of placebo participants crossing over to active treatment is to analyze survival versus predicted control data based on natural history data instead of comparing with the concurrent placebo group from the CENTAUR study.
ALS researchers at UMC Utrecht in the Netherlands have developed a model to predict survival of patients with ALS based on 16 different baseline characteristics. This model was published in Lancet Neurology and validated across multiple datasets (Westeneng 2018). The model uses over 15,000 patient records collected from across the European Union (EU) where survival according to the applicant has generally observed to be similar as compared to the United States. The researchers remained blinded to the CENTAUR treatment groups while producing results that included an individualized survival prediction for every patient in the CENTAUR study.

In this analysis, the ITT active group showed a 9.9 month benefit (23.5 compared to 13.6 months model predicted survival).

In a second post-hoc analysis presented in the September 7th, 2022 Advisory Committee briefing document a Cox proportional hazards survival analysis was used to compare mortality of the ITT AMX0035-treated participants from CENTAUR with a subset of the Pooled Resource Open-Access ALS Clinical Trial database (PRO-ACT) matched controls. The outcome of the analysis demonstrated an 11.0-month median survival benefit for participants randomized to AMX0035 as compared to the propensity score matched population in the PRO-ACT group (mOS 23.5 months in AMX0035 group vs mOS 12.5 months from PROACT population prediction: 11.0-month median survival benefit, HR = 0.48, p=0.00017).

Statistical Reviewer's Comments:
The external data and analysis plans for these post-hoc analyses were not submitted to the NDA.
The first analysis is a post-hoc comparison to predicted outcomes from a survival prediction model developed from a database without a common treatment protocol with CENTAUR. The prediction model was developed based on selected European patients from 1992 to 2016.
The applicant only reported the analysis results without any analysis details. It is not clear if this post-hoc analysis takes into account the uncertainty of the survival predictions involved in the comparison. More importantly, this was only planned and conducted after having unblinded CENTAUR survival data results. There was no prespecified analysis plan for this analysis. Therefore, it doesn't account for multiplicity, and it is essentially uninterpretable.
For example, patients in CENTAUR may differ from those in ENCALS and PRO-ACT cohort as follows.

- *Patients may differ in the measurement of prognostic factors (stage/severity of disease)*
- *Patients may differ in unmeasured prognostic factors*
- *Patients may have received different supportive care and available therapies*

Any such differences may confound the analysis or have introduced bias into the prediction model.
The applicant states that "Of note, there was no detected difference in the AMX0035 naïve group versus (i.e., the subjects in the placebo group who did not crossover to AMX0035 treatment in the OLE) versus predictions (p=0.4001), suggesting the validity of this model". This statement fails to recognize that the placebo group who did not crossover is not a representative subgroup for the whole placebo group. For example, placebo who did not cross over, the majority of whom did not complete the double-blind period, had a double-blind period ALSFRS-R average baseline score 3.7 points lower than placebo who did crossover, i.e., they were worse at baseline.

The second analysis compared the CENTAUR AMX0035 group to a selected subset of the PRO-ACT database, selected by means of a post-hoc propensity score matching model. Propensity score matched analysis involves numerous analysis choices and assumptions which were not prespecified. Note that the PRO-ACT database used for selecting matched controls contains patients from ALS clinical trials from 1990-2010. The applicant claims that a comparison of the baseline covariates used in the propensity score matching shows the groups to be generally well-balanced. However, the 1.3 month difference in time since onset (13.6 S.D.=3.8 for AMX0035 vs. 12.3 SD=3.4 for PRO-ACT matches) appears to be nominally significant if the reported standard deviations are correct (the corresponding standard error of the difference is 0.6). This suggests that there could be other differences in other prognostic factors measured or unmeasured as well. Furthermore, only 74 of 89 CENTAUR subjects randomized to AMX were matched which may create bias.

Both ENCALS and PRO-ACT analyses were post-hoc, unblinded analyses.
Ideally, analysis plans for these analyses would have been in place before the conduct of the CENTAUR trial. In the absence of prespecification there are multiplicity and interpretability issues with these analyses. In addition, all these analyses may be substantially biased for the reasons given above.

14.7 Applicant's New Biomarker Data

The Applicant also submitted new biomarker results from a recently completed Phase 2 study of AMX0035 in Alzheimer's disease (PEGASUS).
The PEGASUS study enrolled 95 patients, with 51 patients on AMX0035, and 44 patients on placebo. Overall, 80% of AMX0035 patients and 96% of placebo patients completed the study. Patients received AMX0035 or placebo twice daily for 24 weeks. The study assessed 18 CSF biomarkers that were felt to be core AD biomarkers or targets of the presumed mechanism of action of AMX0035. These biomarkers were prospectively specified as exploratory endpoints. Results of the change from baseline at 24 weeks in the selected CSF biomarkers are reported in Table 57.

Table 57 Change from Baseline in CSF Biomarkers after 24 weeks

Biomarker	AMX0035	Placebo	LSMEAN Difference (95% CI)	p-value
Neurodegeneration				
Total Tau (pg/mL)	-64.93	8.82	-73.74 (-106.84, -40.65)	<0.0001
Phosphorylated Tau (pg/mL)	-14.63	-0.27	-14.36 (-21.51, -7.21)	0.0002
FABP3 (pg/mL)	-344.62	102.90	-447.52 (-684.59, -210.45)	0.0004
NfL (pg/mL)	169.48	63.61	105.87 (-119.74, 331.47)	0.35
Synaptic Function				
Neurogranin (pg/mL)	-81.19	-8.34	-72.85 (-220.82, -34.89)	0.0003

Inflammation				
YKL-40 (pg/mL)	-14635.39	1507.88	-16143.27 (-26995.89, -5290.65)	0.004
IL-15 (pg/mL)	-0.02	0.25	-0.28 (-0.49, -0.06)	0.01
IL-6 (pg/mL)	644.38	565.93	78.45 (-1042.5, 1199.40)	0.89
IL-8 (pg/mL)	1.54	1.17	0.37 (-4.37, 5.11)	0.88
GFAP (pg/mL)	821.68	488.15	333.53 (-2080.17, 2747.22)	0.78
MCP-1 (pg/mL)	-1.97	-0.79	-1.18 (-21.15, 18.79)	0.91
Core AD Pathology				
AB_{42}/AB_{40} ratio	0.0039	-0.0051	0.0090 (0.0029, 0.0151)	0.005
AB_{42} (pg/mL)	-8.09	-41.46	33.37 (-38.37, 105.11)	0.36
AB_{40} (pg/mL)	-752.7	-754.81	2.11 (-1007.67, 1011.88)	1.0
Metabolism/Oxidative Stress				
8-OHdG (pg/mL)	0.31	-0.13	0.44 (0.13, 0.74)	0.006
24=OHC (pg/mL)	-0.20	-0.07	-0.13 (-0.67, 0.41)	0.63
Leptin (pg/mL)	0.45	4.53	-4.09 (-25.71, 17.54)	0.71
sIR (pg/mL)	-0.04	-0.19	0.15 (-0.25, 0.55)	0.47
Neurovascular				
MMP-10 (pg/mL)	-3.13	-0.92	-2.21 (-8.5, 4.1)	0.48

Source: Applicant Submission Table 3

The Applicant indicates that the improvement in select CSF biomarkers supports the mechanistic activity of AMX0035 in the central nervous system (CNS). AMX0035 lowered levels of CSF total tau, p-tau 181, neurogranin, and YKL-40, and raised the ratio of Aβ42/Aβ40, markers of neurodegeneration that are felt to be relevant to AD pathology.

Reviewer's Comments:
Neurofilament light chain (nFL), a frequently measured biomarker of neuronal degeneration in ALS, did not show a nominally significant change during the 24-week study in AD patients as well. It is unclear if the changes observed in some markers of inflammation and neurodegeneration in AD patients relate to ALS patients as the underlying pathophysiology of AD and ALS are different. These were exploratory markers in the PEGASUS study, so the nominally significant p-values are representative of the actual p-values had they been adjusted for multiplicity.

Additionally in a submission dated August 19, 2022, the Applicant provided additional biomarker data in ALS patients on Chitinase-3-like protein 1 (CHI3L1 or YKL-40) and Chitinase 1 (CHIT1) presented as markers of inflammation that are elevated in ALS and other neurodegenerative diseases. Applicant asserts a significant difference at week 24 in YKL-40, however there were baseline differences in treatment groups, therefore the differences observed at Week 24 are not interpretable.

Observed Value			Change from Baseline		
Analyte Visit Statistics	AMX0035	Placebo	AMX0035	Placebo	p-value [1]
YKL40/CHI3L1 (ng/mL)					
Baseline					
N	81	45			
Mean (SD)	43.89 (47.639)	36.64 (24.060)			
Median	29.46	28.22			
(Q1, Q3)	(20.53, 40.42)	(21.60, 41.87)			
Min, Max	10.0, 268.6	11.4, 126.1			
p-value for normality test [2]	<0.0001	<0.0001			
Week 12					
N	68	38	68	38	
Mean (SD)	34.75 (23.274)	41.28 (26.045)	-5.56 (25.743)	5.02 (15.383)	
Median	27.97	32.48	-1.27	4.22	0.0046
(Q1, Q3)	(20.93, 39.16)	(23.10, 50.98)	(-8.52, 3.55)	(-2.44, 14.80)	
Min, Max	10.7, 129.2	14.2, 111.5	-179.0, 30.6	-44.3, 42.3	
p-value for normality test [2]	<0.0001	<0.0001	<0.0001	0.1047	
Week 24					
N	69	38	69	37	
Mean (SD)	44.46 (56.573)	47.90 (38.491)	1.05 (42.317)	7.64 (27.949)	
Median	27.71	34.78	-0.98	4.83	0.0234
(Q1, Q3)	(21.79, 39.97)	(25.83, 59.89)	(-8.08, 5.20)	(-0.53, 12.28)	
Min, Max	13.2, 340.6	17.4, 231.1	-149.9, 207.8	-49.3, 129.8	
p-value for normality test [2]	<0.0001	<0.0001	<0.0001	<0.0001	

[1] Wilcoxon rank test is performed using non-parametric method.
[2] Shapiro-Wilk test is used for normality test.
Source: Applicant's report on Submission Dated 8/19/22

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/s/

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I concur with the statistical review.

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