Combined FDA and Applicant Briefing Document

NDA# 216660
Drug Name: AMX0035/ sodium phenylbutyrate (PB) and taurursodiol (TURSO)
Applicant: Amylyx Pharmaceuticals, Inc.

Peripheral and Central Nervous System Drugs Advisory Committee (PCNS) Meeting
March 30, 2022
Division of Neurology 1/Office of Neuroscience
Center for Drug Evaluation and Research

DISCLAIMER STATEMENT

The attached package contains background information prepared by the Applicant and the Food and Drug Administration (FDA) for the panel members of the Advisory Committee. The background package often contains assessments and/or conclusions and recommendations written by individual FDA reviewers. Such conclusions and recommendations do not necessarily represent the final position of the individual reviewers, nor do they necessarily represent the final position of the Review Division or Office. We have brought the drug AMX3005 to this Advisory Committee in order to gain the Committee's insights and opinions, and the background package may not include all issues relevant to the final regulatory recommendation and instead is intended to focus on issues identified by the Agency for discussion by the Advisory Committee. The FDA will not issue a final determination on the issues at hand until input from the Advisory Committee process has been considered and all reviews have been finalized. The final determination may be affected by issues not discussed at the Advisory Committee meeting.

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List of Abbreviations

Abbreviation	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
BMI	Body mass index
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 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 INTRODUCTION

This briefing document presents results from the AMX0035 amyotrophic lateral sclerosis (ALS) development program, Study AMX3500 (CENTAUR), along with discussion and analyses of the findings from the study. In general, this document includes the Applicant's position followed by the FDA's position, to reduce redundancy and improve readability.

1.1 Applicant Proposed Indication

Proposed indication: AMX0035 is indicated for the treatment of amyotrophic lateral sclerosis (ALS).

1.2 Purpose of the Meeting

The FDA's Position:

The purpose of this meeting is to discuss whether the data submitted by the Applicant is adequate to establish the effectiveness for AMX0035 in the treatment of ALS.

ALS is a rapidly progressive and fatal neurodegenerative disease that primarily affects motor neurons in the cerebral motor cortex, brainstem, and spinal cord, leading to loss of voluntary movement and the development of difficulty in swallowing, speaking, and breathing, ultimately leading to death. ALS patients can present with weakness and muscle atrophy in different areas of the body, with about 75 percent of patients first experiencing weakness in their limbs, and about 25 percent of patients presenting with difficulty swallowing and/or speaking (bulbar-onset ALS). Respiratory muscles are also affected, leading to respiratory failure and death of most patients within 3 to 5 years from the onset of symptoms. Approximately 10 percent of ALS patients survive for 10 or more years. Shorter survival may be associated with older age at onset, bulbar-onset, and faster rate of respiratory dysfunction. ALS is a heterogeneous disease, but all forms of the disease share the defining features of degeneration of both upper and lower motor neurons. ALS is also considered a multisystem neurodegenerative disorder that can include cognitive and behavioral changes in addition to muscle weakness.

The incidence of ALS is 2 per 100,000 per year, with approximately 6,000 new cases per year in the U.S. The estimated prevalence in the U.S. is 5 per 100,000 population, with approximately 16,000 cases. ALS most frequently affects people between 40 and 70 years of age (median age 55). Most cases of ALS are sporadic with no known cause or inheritance pattern. Five to ten percent of ALS cases are familial and are associated with approximately 50 different identified genes. Familial ALS generally has a 10-year earlier onset than sporadic ALS.

There is no cure for ALS. Available treatments are few and are intended to relieve symptoms, such as cramps and spasticity, and improve quality of life. There are two FDA-approved therapies for ALS: riluzole, which was shown to prolong survival by about 3 months and extend the time before ventilatory support is needed; and edaravone, which demonstrated a 33% smaller functional decline over 24 weeks of treatment, compared to placebo, in patients who were within 2 years of ALS diagnosis. Although these therapies provide some benefit, there is a continued need for new treatments for patients living with ALS.

To approve a drug, substantial evidence of effectiveness must be provided by the Applicant. Although two adequate and well-controlled clinical investigations are the typical standard for generating substantial evidence of effectiveness in many disease settings, there are scenarios in which a single, large, multicenter trial can be used to establish effectiveness. As described in the FDA draft guidance on "Demonstrating Substantial Evidence of Effectiveness for Human Drug and Biological Products", reliance on a single large

multicenter trial to establish effectiveness should generally be limited to situations in which the trial has demonstrated a clinically meaningful and statistically very persuasive effect on mortality, severe or irreversible morbidity, or prevention of a disease with potentially serious outcome, and confirmation of the result in a second trial would be impracticable or unethical.

Under certain circumstances, FDA can also conclude that one adequate and well-controlled clinical investigation plus confirmatory evidence is sufficient to establish effectiveness. The aforementioned FDA guidance provides a discussion of this approach, stating:

> "FDA will consider a number of factors when determining whether reliance on a single adequate and well-controlled clinical investigation plus confirmatory evidence is appropriate. 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 statutory standards for effectiveness apply to drugs developed for ALS, just as the standards apply for all other drug development. However, FDA has also long stressed the appropriateness of exercising regulatory flexibility in applying the statutory standards to drugs for serious disease with unmet medical needs, while preserving appropriate assurance of safety and effectiveness. (21 CFR 312.80 subpart E, Drugs Intended to Treat Life-Threatening and Severely Debilitating Illnesses).

In 2019, FDA published a Guidance for Industry "Amyotrophic Lateral Sclerosis – Developing Drugs for Treatment", which noted that survival time in ALS varies greatly, and that functional endpoints can be confounded by loss of data because of patient deaths. To address this, FDA recommends sponsors use an analysis method that combines survival and function in a single overall measure, such as the joint rank test.

The Applicant conducted a single double-blind, placebo-controlled Phase 2 Study (AMX3500, also titled CENTAUR) in 137 patients with ALS. The Applicant reported a positive result on a prespecified but non-preferred analysis of a functional endpoint in ALS, which did not appropriately account for deaths that occurred during the study. The prespecified statistical result was not exceptionally persuasive and there were analytical and interpretative issues associated with its consideration. The Division expressed concerns that the data may not be adequate to serve as a single study capable of providing substantial evidence of effectiveness and encouraged the Applicant to urgently begin work on a Phase 3 study to confirm the findings.

The Applicant continued to evaluate the data from the open-label extension study (AMX3500OLE) and subsequently reported and published findings of a survival benefit in patients who initially received AMX0035 compared to those patients who originally received placebo in the CENTAUR study. In discussions with the Applicant, the Division noted concern about the interpretability of the survival benefit given the large number of dropouts in the open-label extension period. The Applicant has recently initiated a Phase 3 study in 600 patients worldwide, which is currently enrolling patients and is expected to complete in 2024.

When making regulatory decisions about drugs intended to treat serious and life-threatening conditions, FDA recognizes the importance of considering patient tolerance for risk and the nature of the condition in the context of statutory requirements for safety and efficacy. Although FDA had lengthy and thoroughly discussed pre-submission reservations about the strength of the data provided by the sponsor's development program and strongly suggested the conduct of an additional Phase 3 study (again, which is now underway), given the reported identification of a possible longer-term survival benefit to accompany the earlier placebo-controlled results, we invited the Applicant to submit an NDA prior to completion of the ongoing Phase 3 study in order to provide a consideration of the data available to date in the context of an application review, including discussion with this committee.

The FDA is charged with determining whether the data from the CENTAUR study and the open-label extension study meet the statutory requirements for substantial evidence of effectiveness for AMX0035 in the treatment of ALS and seeks input from the committee on the strength of those data.

1.3 Draft Points for Consideration by the Advisory Committee

The FDA's Position:

Discuss whether the data from Study AMX3500 randomized controlled phase and open-label phase provide substantial evidence of effectiveness for AMX0035 in the treatment of ALS.

2 BACKGROUND

2.1 ALS

The Applicant's Position

ALS is a rapidly progressive paralytic neurodegenerative disease that affects nerve cells in the brain and spinal cord. While the median age of onset is 55, ALS affects a broad range of people, from those in their early 20s to those in their 80s. ALS is universally fatal with a median survival of ~2 years from diagnosis (Traxinger et al, 2013). Rapid progression of symptoms results from degeneration of motor neurons causing the loss of motor function resulting in loss of speech, fine motor skills, and mobility. Most people with ALS eventually need assistance with activities of daily living, with subsequent progression leading to respiratory compromise and eventually to respiratory failure, which is the leading cause of death in ALS (Brown and Al-Chalabi, 2017).

Although the precise etiology of ALS is unknown, the disease is characterized by widespread neuroinflammation and motor-neuron death.

The FDA's Position:

The Applicant's overview accurately describes the nature of ALS and the lack of clear understanding of the etiology of the disease.

FDA notes that there is often a delay in ALS diagnosis, and the median survival from time of diagnosis is approximately 2 years; however, time from symptom onset to death is more variable and may range from 20 to 48 months. FDA also notes that 10–20% of ALS patients have a survival longer than 10 years (Chio 2009), indicating heterogeneity in overall ALS survival.

2.2 Unmet Need

The Applicant's Position

Although there are currently two approved products for ALS in the US, riluzole (Rilutek™) and edaravone (Radicava™), the disease remains rapidly progressive and fatal. As such, there remains a high unmet medical need for new treatments for those with ALS.

The FDA's Position:

FDA agrees that there is a pressing unmet medical need for treatments for ALS.

2.3 Regulatory and Development History

The Applicant's Position

The IND was accepted in April 2017 and AMX0035 was granted Orphan Drug Designation in July 2017.

Amylyx submitted to FDA a New Drug Application (NDA) in October 2021 for approval to market of AMX0035.

Amylyx sought guidance from FDA during the development of AMX0035 as a treatment for ALS:

- 12 March 2020 (Type C meeting) – discussion of the functional and survival results from Study AMX3500 (CENTAUR)
- 04 February 2021 (Type C meeting) – continued discussion of functional and survival results from Study AMX3500 and proposed additional clinical study
- 15 July 2021 (Type B [pre-NDA] meeting) – format and content of NDA.

The FDA's Position:

FDA notes the following additional regulatory history:

- Initial pre-IND meeting on March 21, 2016, at which time the Division advised the Applicant to use a joint-rank analysis of survival and change from baseline in ALSFRS-R.
- Fast track designation was denied on April 20, 2018, and September 17, 2018, because the Phase 2 CENTAUR study was not adequately designed to show benefit over currently available therapies.
- The initial statistical analysis plan (SAP) for the double-blind period was reviewed on March 6, 2019, and the Agency commented that "if there are deaths, then the joint rank analysis of function and survival should be the primary analysis." The Agency also commented on the importance of backup/sensitivity analyses for assumptions about linearity and missing data. The Applicant provided responses to these comments on August 26, 2019, and a revised SAP on October 15, 2019. The Agency did not review the revised SAP prior to data unblinding in November 2019.
- The Applicant submitted a final version prior to any data unblinding of a separate SAP for the OLE on November 5, 2019. This SAP included an analysis of a composite survival outcome based on tracheostomy, hospitalization, and death (listed second after rate of progression of ALSFRS-R in the hierarchy of efficacy outcome measures). However, death alone was not listed in the hierarchy of endpoints. The SAP indicated that separate analyses of the three components of the composite survival endpoint would be done, but it gave no priority for the death alone component.
- At the Type C meeting on March 12, 2020, regarding the topline results of CENTAUR, the Division reiterated the importance of using a joint-rank analysis and questioned the results of the single trial as able to independently demonstrate substantial evidence of effectiveness. The Division recommended the Applicant begin work on a second efficacy study.
- Breakthrough Designation was denied on March 27, 2020, because the preliminary clinical data from the CENTAUR study did not clearly demonstrate a benefit over the currently approved therapies.
- On April 1, 2020, a new supplementary SAP dated March 27, 2020 was submitted for the survival analysis of the open-label extension study. The submission of this SAP occurred after the double-blind period was unblinded and after preliminary survival analyses of data from the double-blind and OLE period through September 25, 2019 had been viewed and presented at the March 12, 2020 Type C meeting.

- At the Type C Meeting on February 4, 2021, the Division reiterated that although the data are encouraging, another randomized, placebo-controlled study would likely be necessary to support a marketing application. The Applicant discussed plans for a Phase 3 study A35-004, with the possibility of an interim analysis at 24 weeks that may be able to provide independent substantiation of effectiveness to support a future NDA.
- The Division invited the Applicant to request a pre-NDA meeting on May 11, 2021 as it was determined that the claims of a survival benefit warranted a more thorough consideration of the data. At the July 15, 2021 Pre-NDA meeting, the Division inquired about the ability to submit the NDA expeditiously to allow for earlier review of the data.

3 AMX0035 PRODUCT DESCRIPTION

3.1 Product Overview

The Applicant's Position

AMX0035 is a co-formulation of two active pharmaceutical ingredients (APIs), sodium phenylbutyrate (PB) and taurursodiol (TURSO), designed to reduce neuronal death in persons with ALS by simultaneously mitigating endoplasmic reticulum stress and mitochondrial dysfunction.

AMX0035 is formulated as a powder for oral suspension supplied as sachets each containing 3 g PB and 1 g TURSO. AMX0035 was administered as an oral (or via feeding tube) product in all clinical studies.

3.2 Mechanism of Action

The Applicant's Position

ALS is a disease characterized by rapid motor neuron death in the motor cortex and corticospinal tract. A myriad of insults, including genetic and environmental, can contribute to motor neuron death in ALS mediated by multiple pathways.

The high metabolic requirement and long lifespan of neurons require healthy endoplasmic reticulum (ER) and mitochondria to maintain neuron function and survival. Many studies have implicated mitochondrial dysfunction and endoplasmic reticulum stress as key elements of ALS pathology. Markers of ER stress and the unfolded protein response have been found in post-mortem samples from people with ALS (Lautenschlaeger J et al, 2012). Additionally, postmortem changes in ER morphology have been shown in ALS samples (Oyanagi K et al., 2008). The excess accumulation of unfolded proteins and the chronic activation or dysfunction of the unfolded protein response has been shown to trigger cell death (Fribley et al., 2009; Kim R et al., 2006).

Respiratory chain deficits, markers of reactive oxygen species and reactive oxygen species-damage, and morphology deficits in the mitochondria have also been found in post-mortem spinal cord samples of people with ALS (Smith EF et al., 2019). Magnetic resonance spectroscopy studies also show dysregulation of mitochondrial markers in ALS (Sassani M et al., 2020). Mitochondrial apoptosis has been hypothesized to be one of the key pathways underlying neuronal death in ALS (Muyderman H et al., 2014).

Many studies in preclinical models of ALS, post-mortem tissue, and in people living with ALS highlight the dysfunction in the ER and mitochondria that may play a key role in ALS pathophysiology and underlie the motor neuron death that ultimately causes the disease.

Given the importance of ER and mitochondrial dysfunction in ALS, Amylyx developed AMX0035 as a combination of PB and TURSO to simultaneously mitigate ER stress and mitochondrial dysfunction. PB functions both as a chemical chaperone to stabilize protein folding and reduce ER stress and as a transcriptional regulator of antiapoptotic and

antioxidant proteins (Wiley JC et al., 2010; Zhou W, 2011). TURSO stabilizes the mitochondrial membrane by reducing the translocation of Bax, a cell death regulator, leading to improved mitochondrial function and energy production (Rodrigues CM, et al., 2003). The Sponsor has conducted multiple neurodegenerative disease models in which the combination of the two agents demonstrate effectiveness in attenuating neuronal death and other pathology associated with ALS.

The Sponsor has conducted a preclinical battery including models of endoplasmic reticulum stress, mitochondrial stress, oxidative stress, glutamate toxicity, experimental autoimmune encephalitis and ALS genetic models demonstrating effectiveness of the combination in relevant models.

Additionally, the two compounds have been studied in a number of published investigations. PB has shown efficacy in three separate mouse model investigations of the SOD1 G93A mouse model of ALS (Ryu H et al., 2005; Del signore SJ et al., 2009; Petri S et al., 2006). In Ryu H et al., PB is also shown to reduce the levels of pro-apoptotic caspases, consistent with its mechanism of action and in the additional studies histology showed a reduction in cellular death. A chemical screen additionally found PB to be a hit in reduction of toxicity in a cellular and zebrafish model of C9orf72 toxicity, the most common genetic cause of ALS (Corman, A et al., 2019).

Phenylbutyrate has been shown to improve motor function and cellular survival in the rotenone mouse model of Parkinson's disease (Inden, M et al., 2007). In this model, phenylbutyrate also showed effects on GRP78, DJ-1 and Pro-Caspase12, markers associated with ER stress (Inden, M et al., 2007). Phenylbutyrate was shown to reduce the toxicity of experimental autoimmune encephalomyelitis (EAE) in the common EAE mouse model of multiple sclerosis (Dasgupta S et al., 2003). Phenylbutyrate also reduced the progression of a mouse model of progressive supranuclear palsy (Bondulich M et al., 2016). In this model, phenylbutyrate was shown to modulate pathways of unwanted protein degradation (p62 ubiquitin proteasome system). Phenylbutyrate has additionally been studied in models of Huntington's Disease and Stroke and shown to have beneficial effects (Hogarth, P et al., 2007., Gardian, G et al., 2005., Qi, X et al., 2004). Collectively, these results show preclinical evidence of the activity of Phenylbutyrate both in ALS disease specific and non-ALS models of neurodegeneration.

TURSO has shown efficacy in a C9orf72 cellular model of ALS (Zhang et al., 2014). In this model, TURSO was also shown to reduce Caspase 3, a marker for mitochondrial apoptosis (Zhang et al., 2014). TURSO was shown to be a 'hit' in a human cellular screen designed to find potential drugs for ALS (Thams S et al., 2019). In addition, TURSO showed a reduction in muscle denervation in the SOD1 G93A mouse model of ALS (Thams S et al., 2019).

TURSO has shown dose dependent efficacy and reduction of proapoptotic caspase proteins in a model of intracerebral hemorrhage in rats (Rodrigues CM, et al., 2003). TURSO has also shown efficacy in a MPTP model of Parkinson's disease including

showing a reduction in reactive oxygen species and phosphorylated BAD, a protein involved in mitochondrial apoptosis (Castro-Caldas et al., 2012). TURSO also showed efficacy in a mouse model of Huntington's disease including showing a significant reduction in apoptosis (Keene et al., 2002).

Finally, both PB and TURSO monotherapies have been studied in pilot clinical trials in people living with ALS which have been published. PB was studied in an open label trial which demonstrated target engagement but did not have a concurrent placebo control for efficacy comparison (Cudkowicz, 2009). TURSO was studied in a small pilot trial which supported an effect on disease progression in people with ALS. This pilot trial had different inclusion criteria as Study AMX3500 (Elia, 2015) and was conducted only in Italy.

The FDA's Position:

FDA acknowledges the Applicant's rationale for the development of AMX0035, a fixed dose combination product of sodium phenylbutyrate(PB) and taurursodiol (TURSO, also known as TUDCA). Under 21 CFR 300.50 (Fixed-combination prescription drugs for humans), two or more drugs may be combined in a single dosage form if it is demonstrated that each individual component makes a contribution to the claimed effects of the fixed-combination drug.

FDA notes that the pathophysiology of ALS is unknown, but likely involves multiple complex processes and pathways. The mechanism described by the Applicant by which PB and TURSO are proposed to be therapeutic in ALS patients (i.e., endoplasmic and mitochondrial stress) is but one of a number of potential processes implicated in the pathophysiology of ALS.

The Applicant has conducted a series of in vitro and in vivo pharmacology studies to investigate the pharmacodynamic effects of PB and TURSO; however, whether AMX0035 is effective for the treatment of ALS will be determined based on the results of clinical studies.

FDA also acknowledges that PB and TURSO were both studied (as monotherapy) in patients with ALS in the two small clinical studies noted above. PB was evaluated in a 20-week open-label, dose-escalation study at doses of 9-21 g/day in 26 completers, in which the biomarker of interest, histone acetylation, was the primary endpoint. The authors hypothesized that PB would inhibit histone deacetylase, thereby leading to an increase in histone acetylation, and potential modulation of aberrant transcription that may lead to motor neuron cell death. The authors concluded that at the lowest dose of 9 g/day, PB was well tolerated, and had the desired effect of improving histone acetylation levels. No clinical outcomes were studied. Histone modification is one of the proposed epigenetic mechanisms implicated in the etiology of ALS; however, there is insufficient evidence at this time to support that histone acetylation levels are correlated with clinical symptoms in ALS.

TURSO was studied in a small, randomized study of 34 patients with ALS in Italy. Patients were treated with 1 g TURSO for 54 weeks and was well tolerated. At the end of the study, ALSFRS-R bulbar and upper limb scores were reported as improved for patients who received treatment compared to placebo.

FDA notes that both of these are very small clinical studies that were not designed to show contribution of individual components in the treatment of ALS.

3.3 Dose Selection

The Applicant's Position

Dosage in AMX3500 was selected based on three methods: pharmacokinetics modeling to determine a target dose to reach optimal concentrations based on in vitro models, allometric scaling of animal dosages to human dosages and evidence from prior clinical

investigations of PB and TURSO. Collectively, these studies supported the proposed dosage of 3 gram PB twice a day and TURSO 1g twice a day.

The FDA's Position:

FDA acknowledges the Applicant's methods for the selection of doses used in the clinical studies. The effectiveness of the selected doses will be determined based on the results of the clinical studies.

3.4 Proposed Indication and Dosing

The Applicant's Position

The proposed product labeling for AMX0035 includes the following key elements:

- AMX0035 is indicated for the treatment of ALS.
- AMX0035 should be administered prior to a meal according to the following regimen:

  Starting Dose:

  The recommended starting dose of AMX0035 is 1 sachet once daily (QD) for 21 days.

  Maintenance Dose:

  The recommended maintenance dose of AMX0035 is 1 sachet twice daily (BID), morning and evening.

4 CLINICAL EFFICACY IN STUDY AMX3500 (CENTAUR)

4.1 Study Design

The Applicant's Position

AMX3500 is a relatively large (within the field of ALS), randomized (with 2:1 ratio of AMX0035 vs placebo), double-blind, placebo-controlled study of AMX0035 for the treatment of ALS. The study was designed with investigators from centers of excellence in the field of ALS research and was conducted at 25 medical centers through the Northeast ALS Consortium (NEALS), the largest ALS research consortium in the US. The study was designed to be both a rigorous test for safety and efficacy but was also participant-friendly – this philosophy is mirrored in the FDA *Guidance for Industry: Amyotrophic Lateral Sclerosis: Developing Drugs for Treatment.* Although the final *Guidance* was published in late 2019 as the trial was reaching its conclusion, the AMX3500 study followed most of the key tenets of the *Guidance*, including:

- Randomization (2:1) that enhanced the chance of a participant receiving active treatment instead of placebo
- Standard of care ALS concomitant medications were included – participants were allowed to be on concomitant riluzole and/or edaravone as advised by the treating physician
- Inclusion of a long-term, open-label extension phase where the blind to original treatment assignment was maintained after the randomized effectiveness portion was completed
- An allowance in the open-label phase for additional prespecified effectiveness assessments (a second statistical analysis plan for the open-label phase portion)
- The use of the ALS Functional Rating Scale-Revised (ALSFRS-R) as the primary endpoint, an instrument that measures function in daily activities and the most widely used clinical scale in ALS treatment and care
- The use of a muscle strength measurement as a secondary endpoint
- The use of a respiratory function test as a secondary endpoint
- The inclusion of an assessment on mortality as a critical endpoint with assessment of mortality both inclusive and exclusive of ventilation events
- The inclusion of an exploratory biomarker (plasma-based phosphorylated neurofilament heavy chain) as a secondary endpoint

The FDA's Position:

FDA acknowledges that the CENTAUR study was aligned with many of the recommendations outlined in the FDA Guidance on ALS Drug Development. However, although the guidance does recommend the use of the ALSFRS-R as the primary endpoint, the guidance also clearly recommends an analysis method that accounts for deaths that may occur by combining survival and function in a single overall measure, such as the joint rank test.

The Applicant also notes that the blind to original treatment assignment was maintained in the open-label extension period; however, this was not clearly stated in the original protocol.

4.1.1 Study Overview

The Applicant's Position

AMX3500 was a multicenter study comprising the following two parts:

- A randomized, double-blind, placebo-controlled, parallel-group, 24-week phase (Weeks 1-24) evaluating the safety, tolerability, efficacy, PK, and biological activity of AMX0035 (referred to as the randomized controlled phase).
- A 132-week open-label phase (Weeks 24-156) to further evaluate safety and efficacy of AMX0035 in those who completed the randomized phase of the study on AMX0035 or placebo. In this phase of the study, all participants received AMX0035.

The FDA's Position:

AMX3500 (CENTAUR) was a Phase 2, randomized, double-blind, placebo-controlled study in the United States that randomized patients 2:1 to AMX0035 or placebo for 24 weeks. Patients received 1 sachet twice daily orally, or via feeding tube, as tolerated (1 sachet = 1 g taurursodiol and 3 g sodium phenylbutyrate).

Patients were then allowed to participate in an optional open-label extension study (AMX3500-OLE or CENTAUR-OLE) which followed patients for up to 132 weeks. The OLE study was primarily intended for evaluation of long-term safety.

4.1.2 Inclusion Criteria

The Applicant's Position

Study inclusion criteria were chosen based on a historical analysis of the PRO-ACT database, an open-source database of over 10,000 participants from prior ALS clinical trials and analysis of the ceftriaxone trial database and published reports from the dexpramipexole trial. The goal of the inclusion criteria was to enroll a relatively faster-

progressing trial population that would allow for an assessment of function, as measured by the ALSFRS-R, over 24 weeks. Participants had to be ≥18 and <80 years of age with a confirmed El Escorial Definite diagnosis of sporadic or familial ALS as defined by the World Federation of Neurology revised El Escorial criteria, had to be ≤18 months from first ALS symptom (i.e., muscular weakness), and had to have a slow vital capacity (SVC) ≥60% of predicted capacity for age, height, and gender.

These criteria predispose the population to a faster progression – El Escorial Definite means that participants must have upper and lower motor neuron signs of ALS in at least 3 of 4 body regions, and less than or equal to 18 months from symptom onset means that participants were early in disease course. The SVC cutoff was chosen to increase the likelihood that participants could complete the 24-week study.

The clinical trial protocol also stated that the investigator should not enroll any participant who would be unlikely to complete the 24-week study to reduce the impact of mortality on functional assessment.

Participants were also allowed to be on a stable dose of riluzole for no less than 30 days. Participants on edaravone, or planning to initiate edaravone, without restrictions, were eligible for entry into the study. This choice was made because edaravone was approved in the US just after AMX3500 trial enrollment initiated.

The FDA's Position:

FDA acknowledges that the CENTAUR study design and enrollment criteria were appropriate for a Phase 2 study in patients with ALS.

4.1.3 Primary Endpoint Selection

The Applicant's Position

The primary efficacy endpoint for the randomized, placebo-controlled phase of Study AMX3500 was the rate of decline in total ALSFRS-R score.

The Amyotrophic Lateral Sclerosis Functional Rating Scale – Revised (ALSFRS-R) is a validated measure of clinical function that is correlated with quality of life and survival in people with ALS (Cedarbaum 1999). The ALSFRS-R shows internal consistency and construct validity (Cedarbaum 1999). Initial validity was established by documenting that in people with ALS, change in ALSFRS-R scores correlated with change in muscle strength and lung function over time, and predicted survival (Cedarbaum 1999, Kaufmann 2005). With appropriate training, the ALSFRS-R can be administered with inter-rater reliability and test-retest reliability (Cedarbaum 1999).

The ALSFRS-R is a quickly administered (5 minutes) ordinal rating scale (ratings 0 to 4) of 12 functional activities relevant to ALS across four functional domains. Higher scores

indicate better performance, and the maximum score is 48 points (corresponding to normal functioning). The ALSFRS-R can be broken down into 4 domains as described below:

Bulbar • Speech • Salivation • Swallowing	**Breathing** • Dyspnea • Orthopnea • Respiratory Insufficiency
Fine Motor • Handwriting • Cutting food and handling utensils (for both individuals with and without gastrostomy) • Dressing and hygiene	**Gross Motor** • Turning in bed • Walking • Climbing stairs

The ALSFRS-R is the most widely used clinical scale in ALS treatment and care and is suggested as the preferred primary outcome in ALS trials in the FDA *Guidance For Industry*.

While formal clinical significance studies of the ALSFRS-R are somewhat lacking, a survey of 65 US ALS experts found that most experts would consider a 20% change in the rate of decline of the ALSFRS-R as when a clinically significant change starts to be noted (Castrillo-Viguera, C, 2009).

In the FDA summary basis for approval of edaravone, it was stated that each category in the ALSFRS-R appears clinically important. Additionally, as each domain spans only 5 points, even the prevention of a 1-point worsening (in one domain) would seem meaningful / important to those living with ALS.

The FDA's Position:

FDA notes that the primary endpoint, ALSFRS-R, is a clinically relevant measure of functional change in ALS and was the basis for the approval of edaravone. FDA acknowledges that ALSFRS-R was appropriate for selection as the primary endpoint of the study. However, analysis of the rate of decline of ALSFRS-R score does rely on linearity assumptions for an interpretable slope analysis (see Section 4.1.6.2).

Also, as noted above, the Division typically recommends a joint-rank analysis of the ALSFRS-R change from baseline and mortality as the primary analysis in ALS. This approach was first recommended to the Applicant in March 2016 at the pre-IND meeting and subsequently in an advice email after review of the SAP in March 2019. The Applicant responded during IND development by discussing limitations of the joint rank analysis in this context. However, FDA continues to have concerns with analyses that do not incorporate deaths and prefers an analysis that combines survival and function.

4.1.4 Secondary Endpoints

The Applicant's Position

Secondary endpoints for the randomized controlled phase (randomization through Week 24) were as follows:

- Accurate Test of Limb Isometric Strength (ATLIS) – a new strength measurement device. Given the historical challenges with measuring strength in clinical trials, Amylyx chose to pioneer this outcome for the first time in an interventional study.
- Levels of plasma Neurofilament heavy chain (pNF-H) – a biomarker that at the time of study design was thought to be a potential marker of neuronal degeneration.
- Slow Vital Capacity (SVC). SVC is the preferred method by ALS centers of excellence for measuring pulmonary capacity.
- Survival measured as the rate of deaths, hospitalizations, and tracheostomies.

AMX3500 was powered for the primary outcome but was not designed to be powered to detect changes on secondary outcomes.

The FDA's Position:

The ATLIS is a relatively new measurement of strength. FDA acknowledges that a valid measurement of muscle strength may be an appropriate endpoint in ALS for treatments intended to increase or preserve muscle strength. However, the clinical meaningfulness of differences in muscle strength should be supported by the magnitude of the effect observed (based on the mean change or on a responder analysis of patients who exceed a clinically meaningful threshold of change), or by the demonstration of the drug effect on another appropriate clinically meaningful measure of function in activities of daily living (such as the ALSFRS-R). The protocol did not specify between the three possible components of the ATLIS score (Total, Upper Extremity, or Lower Extremity) in the testing hierarchy.

Because decline in respiratory function is a direct result of the known pathophysiology of the disease, demonstration of a treatment benefit on respiratory endpoints may also provide evidence of effectiveness. SVC is an appropriate outcome measure of respiratory function in patients with ALS.

FDA does not agree with inclusion of tracheostomy or hospitalizations in the definition of survival, as there is considerable variation in clinical practice as to when to hospitalize a patient or perform a tracheostomy. Differences in standard of care by treating physicians, as well as patient preference and comfort, may influence these outcomes. For example, tracheostomies may be placed for the management of secretions, or they may be performed earlier in the disease course prior to the onset of acute respiratory insufficiency/failure in anticipation of future need for ventilatory support. FDA routinely advises sponsors against such survival definitions.

4.1.5 Open-Label Phase Endpoints

The Applicant's Position

Outcomes from the randomized controlled phase through long-term follow-up included:

1. Rate of decline in total ALSFRS-R score;
2. The impact of AMX0035 on survival, hospitalization, and tracheostomies
3. Rate of progression in ATLIS scores;
4. Rate of progression of SVC;

During long-term analysis, ALSFRS-R, SVC and ATLIS data was analyzed at Study Week 48 (Week 24 of Open-label Phase [OLP]) to provide similar duration of follow-up during the open-label and randomized phases (24 weeks in both cases). Time to event endpoints were followed from initial randomization through completion of the OLP (March 1st 2021).

Statistical treatment of these analyses and type 1 error control will be discussed in Section 4.1.6.5.

The FDA's Position:

The primary endpoint in the open-label extension study (AMX0035-OLE) was safety. Open-label efficacy analyses are often difficult to interpret.

Additionally, we note that while the assessment of AMX0035 on a composite endpoint based on survival, hospitalization, and tracheostomies was listed in the hierarchy of efficacy endpoints in the OLE study protocol, death alone was not included in this list of endpoints. Analyses of the three components of the composite survival endpoint were planned, but the death analysis was not given priority over the other two components of the composite (or the composite itself).

As noted above, the Division does not agree with the inclusion of tracheostomy or hospitalizations in the definition of survival, as there is considerable variation in clinical practice as to when to hospitalize a patient or perform a tracheostomy due to differences in standard of care by treating physicians and patient preference; tracheostomies may also be placed for the management of secretions.

FDA also notes the Applicant's comment above that the OLE study completed on March 1, 2021. It was unclear why the March 1 date was chosen for study completion, as there were two patients still receiving treatment at that time that were terminated from the study by the Sponsor. All other patients had either died, discontinued from the study, or completed the prespecified 132 weeks of treatment (see Section 4.5).

4.1.6 Statistical Analyses

4.1.6.1 Analysis Populations

The Applicant's Position

Efficacy analyses primarily used the pre-specified modified intention to treat (mITT) population; however, the intention to treat (ITT) population was also assessed to determine if results were consistent in this population. The ITT population included all participants who were randomized and received at least 1 dose of study medication. The mITT population included all participants who were randomized and received at least 1 dose of study medication and had at least 1 post-baseline total ALSFRS-R score available. Two participants in the ITT population were not included in the mITT as they dropped out of the study before their Week 3 visit. Safety Analyses used the ITT population.

The FDA's Position:

FDA acknowledges the definition of the mITT population and the ITT population for the primary analysis of Study AMX3500. FDA also notes that the mITT and ITT definition include those participants "as randomized" for the efficacy analyses, and "as treated" for the safety analyses.

4.1.6.2 Primary Efficacy Analysis for the Randomized Controlled Phase

The Applicant's Position

The primary efficacy endpoint for the randomized controlled phase of the study was rate of decline (slope of decline) in the total ALSFRS-R score. The placebo and AMX0035 groups were compared by a shared-baseline, linear mixed effects analysis. Covariates of age, rate of disease progression prior to entering the study (i.e., ΔFS [DEL-FS]), switched for DEL- of the other efficacy outcomes of interest, in those analyses, interacting with time were included in the analysis. Time was a quantitative measure in the primary analysis, with Day 1 being the baseline/randomization visit. Time for subsequent visits was the number of days since randomization. All post-baseline visits (including post-baseline unscheduled visits and assessments collected via telephone calls) were included in the efficacy analysis, even if they were categorized as the same nominal visit.

The FDA's Position:

FDA has the following comments about the primary analysis methods:

- FDA has concerns with the slope analysis because of questions regarding whether the ALSFRS-R is linear over time. With such an approach, sensitivity analyses allowing for non-linearity are important.
- FDA had also recommended the joint rank analysis of ALSFRS-R and mortality to the Applicant since some deaths were expected in the double-blind period; FDA notes that deaths may cause bias if ignored in the primary analysis.
- Analyses were conducted in the mITT population and excluded two patients who were treated with AMX0035 but died before any post-baseline ALSFRS-R measurements. FDA acknowledges that patients without any post-baseline visits have often been excluded from analyses. However, exclusion of such randomized patients can introduce bias in comparisons, such that sensitivity analyses in the ITT population are important.

4.1.6.3 Secondary Efficacy Analytical Methods

The Applicant's Position

Similar mixed effects analysis, that were used for the primary efficacy endpoint, were used for all continuous secondary efficacy endpoints: ATLIS, biomarker, and SVC.

The FDA's Position:

FDA notes that the secondary efficacy analyses have the same issue of not incorporating deaths. The use of a joint rank would be applicable to these endpoints as well.

In addition, ATLIS has three components: Total ATLIS, Upper Extremity ATLIS, and Lower Extremity ATLIS. The protocol did not pre-specify which component would be the key secondary endpoint in the hierarchy.

4.1.6.4 Time to Event Analysis Analytical Methods

Survival analyses of key progression events were performed using a Cox proportional hazards model with covariates of del-FS, baseline ALSFRS-R, and age at baseline for time to: 1) death, 2) hospitalization, 3) death or death equivalent. The median duration of survival and the associated 95% confidence interval were estimated overall using the Kaplan-Meier method. Figures for the Kaplan-Meier estimates and the Cox proportional hazard function are presented in Section 4.5.3.

The FDA's Position:

FDA notes that the protocol and SAP for the OLE included an assessment of a composite survival outcome based on survival, hospitalization, and tracheostomies in the hierarchy of efficacy endpoints, but did not include death alone in that list of endpoints. Analyses of the three components of the composite survival endpoint were planned, but the death analysis was not given priority over the other two components of the composite (or the composite itself).

FDA also notes that the protocol and SAP created before study unblinding did not specify baseline ALSFRS-R as a covariate in the survival analyses. This covariate was added in the supplementary OLE survival SAP after the initial OLE survival data had been analyzed based on an earlier event cutoff date, which was closer to the final analysis of the double-blind period (see discussion in Section 4.5.2).

4.1.6.5 Type I Error Control

The Applicant's Position

The AMX3500 study had two prespecified statistical analysis plans (SAPs) for the randomized controlled phase and open-label phase respectively, both of which were finalized prior to database lock and study unblinding. Participants and study staff were kept blinded to original treatment assignments through OLP completion on March 1st 2021.

The first SAP described the analyses to be conducted from randomization through Week 24 (the end of the randomized controlled phase). The second SAP described those

analysis to be run from initial randomization through follow up on the open-label phase. Both statistical plans prespecified a hierarchy of outcomes with type I error control at two sided α=0.05. As such, the study controlled for type I error through two statistical plans. These outcomes are shown in the table below:

Table 1: Statistical Hierarchies Specified in AMX3500

Position in Hierarchy of Efficacy Outcomes	Prespecified Hierarchy for Randomized Controlled Phase	Prespecified Hierarchy for Long Term Follow Up
1	ALSFRS-R rate of decline	ALSFRS-R rate of decline
2	ATLIS rate of decline	The impact of AMX0035 on survival, hospitalization and tracheostomies
3	pNF-H rate of decline	Upper and Lower ATLIS Scores rate of decline
4	SVC rate of decline	SVC rate of decline
5	The impact of AMX0035 on survival, hospitalization and tracheostomies	Rate of progression on ALSFRS-R subdomains
6	Pharmacokinetics of AMX0035	Rate of progression on total ATLIS score
7	Results from exploratory TSPO PET substudy presenting in a listing only	

The decision to use two prespecified hierarchies was based on the 2019 FDA Guidance for Industry in ALS which states: “Trials should include prespecified plans for a long-term, open-label extension that maintains the blind to the original treatment assignment after completion of the randomized effectiveness portion of the clinical trial. This extension should allow for additional prespecified effectiveness assessments.”

During the randomized phase, few deaths were expected because the inclusion criteria required patients to be early in disease and for investigators to only enroll those participants expected to complete 24 weeks. However, it is recommended in all ALS studies including in the *Guidance for Industry* that mortality be measured as a secondary endpoint, and it was included as the 5th outcome in the hierarchy. This data is not reported in this report as there are few events.

Pharmacokinetics were analyzed and Translocator Protein (TSPO) PET Scan results were only available in a small number of participants, so a listing was prepared and presented to FDA. Neither of these analyses are presented in this document.

Prior datasets had suggested that SVC would be unlikely to have adequate power to see treatment differences and for this reason it was placed lower in the hierarchy of secondary endpoints. Neurofilament was considered a relatively higher priority because

it was assumed that this outcome might be more sensitive and have greater statistical power.

ATLIS was a novel strength measurement device and the powering requirement for this outcome was unknown; however, the applicant assumed that it might have greater power than SVC and more meaningfulness than pNF-H and therefore prioritized it higher.

During long-term follow up, study events for mortality were the second highest outcome in the hierarchy as these were expected to accumulate during longer follow-up. ALSFRS-R subdomains are not presented in this document.

The FDA's Position:

FDA notes that the protocol and SAP for the OLE included an assessment of a composite survival outcome based on survival, hospitalization, and tracheostomies in the hierarchy of efficacy endpoints, but did not include death alone in that list of endpoints. Analyses of the three components of the composite survival endpoint were planned, but the death analysis was not given priority over the other two components of the composite (or the composite itself). The focus on death alone, and the submission of a new supplementary OLE survival SAP, occurred after preliminary survival analyses of data from the double-blind and OLE period through September 25, 2019 had been viewed and presented at the March 12, 2020 Type C meeting.

The Applicant did several survival analyses corresponding to different event cutoff dates including after the last patient last visit in the double blind period as well as after three survival data sweeps (29 Feb 2020, 20 Jul 2020, and 1 Mar 2021), creating a multiplicity of survival analyses, in addition to the multiple different survival and survival composite endpoints.

Applicant's Position:

- AMX3500 was a rigorously study designed in collaboration with leading medical centers to evaluate the effectiveness and safety of AMX0035 in addition to providing important scientific knowledge for the ALS field—including development of novel outcomes such as the ATLIS measurement device.
- The Primary Endpoint was the rate of decline in the ALSFRS-R total score, which is a standardized and validated instrument which has been used in the majority of clinical investigations of ALS.
- The study design was intended to be patient centric with 2:1 randomization, use of an open label extension, and allowance for concomitant use of riluzole and edaravone.
- The inclusion/exclusion criteria were designed based on investigation of prior clinical studies to enroll a relatively faster progressing patient population who would be able to complete at least 24-weeks of follow up.
- The study prespecified two hierarchies based on the 2019 FDA ALS guidance for industry (one for the initial 24 weeks and one during long-term follow up). The sponsor acknowledges the potential for type I error inflation with two SAPs.

The FDA's Position:

- FDA agrees with use of the ALSFRS-R as a clinically relevant primary endpoint.
- FDA acknowledges the choice of ATLIS and SVC as potentially clinically meaningful secondary endpoints.
- FDA has concerns regarding the planned primary analysis methods, including the planned use of a slope analysis when it is unclear if ALSFRS-R is linear over time (see below).
- Additionally, the primary analysis does not account for deaths, which are expected in a study of ALS, even of 24 weeks duration. If there are deaths, a joint rank analysis of function and death is recommended as the primary analysis. The Applicant was advised of this recommendation at the pre-IND meeting in 2016 and in advice on the SAP provided in 2019.
- The open-label extension study was proposed to study safety as the primary outcome. A composite survival analysis of death, hospitalizations, and death equivalent was planned in the OLE protocol and SAP, but death was not included in the list of endpoints. An analysis of death alone was planned only as one of the three separate analyses of the components of the composite with no priority over the other components or the composite itself. The focus on death alone, and the submission of a new supplementary OLE survival SAP, occurred after initial data were known.

4.2 Participant Disposition and Demographics

4.2.1 Participant Disposition and Baseline Characteristics

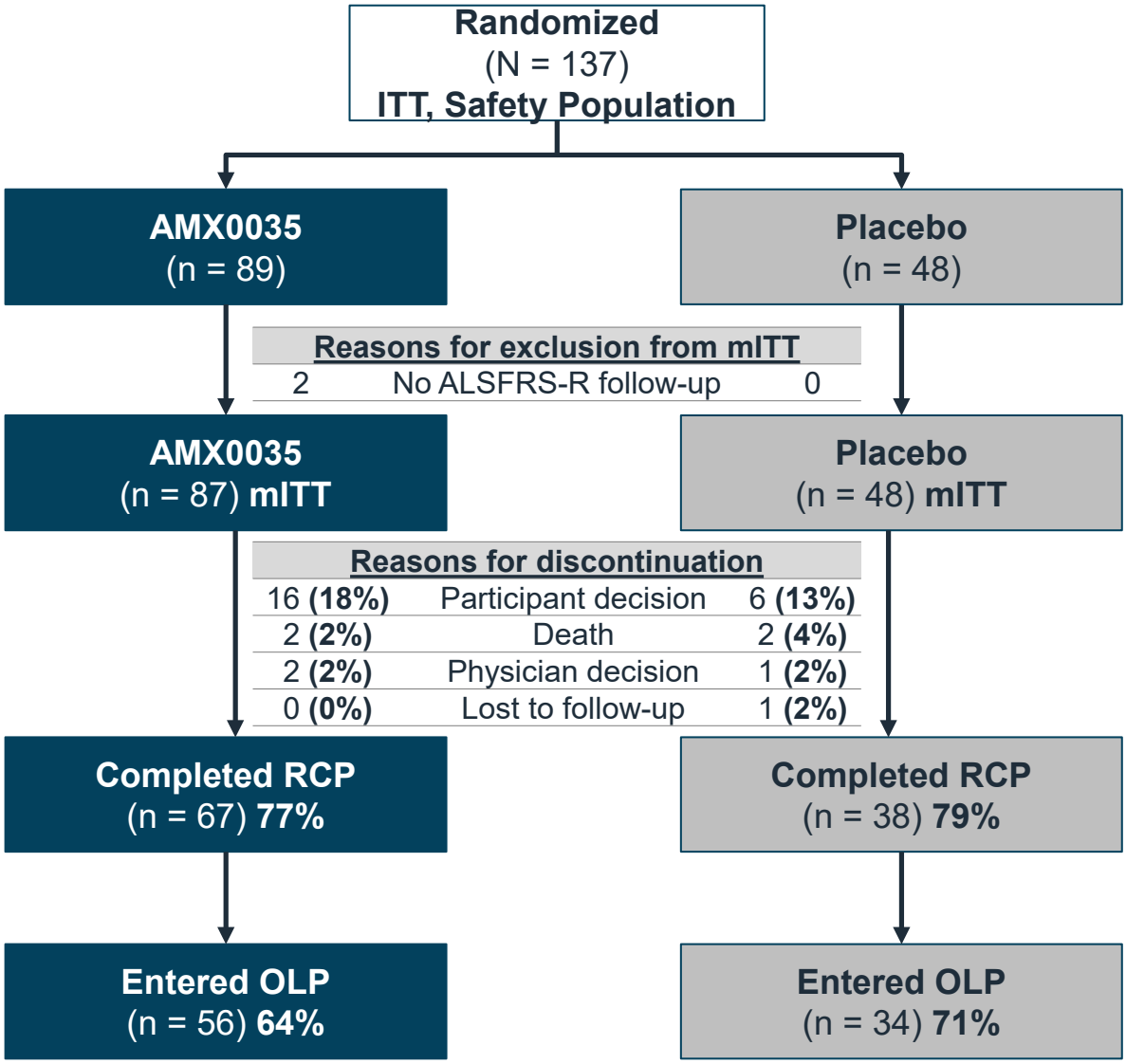
4.2.1.1 Participant Disposition

The Applicant's Position

Of the 137 participants enrolled, 105 (77%) completed the 24-week, double-blind study (Figure 1). Approximately 23% of participants prematurely discontinued from the study; the most common reason for study discontinuation was participant withdrawal. The historical dropout rate in previous ALS clinical studies is estimated at approximately 22% (Atassi, 2013).

Ninety participants continued from the randomized controlled phase into the open-label phase, 56 who were treated with AMX0035 and 34 treated with placebo. The longest follow-up (randomized controlled phase through study completion) was 42 months after randomization. The open-label phase was ended by the Sponsor on March 1st 2021 and all remaining participants were offered a transition to a less arduous protocol with the intention of compassionate continuation.

Figure 1: Participant Disposition



Abbreviations: ITT=intent to treat; mITT=modified intent to treat.
Note: Death=death or death equivalent (includes tracheostomy or permanent assisted ventilation (PAV)). Some deaths were recorded after participant withdrawal from trial and are not accounted in reason for discontinuation.
Source: Table 14.1.1 Main CSR

The FDA's Position:

FDA acknowledges the study disposition as outlined above. FDA also notes that the number of study completers for the double-blind treatment period includes patients who completed the study but had already discontinued treatment with the drug. Accounting for those additional discontinuations, only 60 patients in the AMX0035 arm (67%) and 37 patients in the placebo arm (77%) completed the study still on drug, indicating higher discontinuations in the treatment arm. FDA also notes that the 2 patients in the treatment arm who were excluded from the mITT because they did not have post-baseline ALSFRS-R measurements both died during the study.

Overall exposure in the double-blind treatment period is summarized in the below table.

AMX3500 Overall Exposure (double-blind treatment period)

Duration of exposure (categories) n(%)	AMX0035+SOC (N=87)	Placebo +SOC (N=48)	Combined (N=135)
0 to ≤3weeks	6 (6.9)	1 (2.1)	7 (5.2)
>3 to ≤12 weeks	11 (12.6)	4 (8.3)	16 (11.8)
>12 to ≤18 weeks	4 (4.6)	3 (6.2)	7 (5.2)
>18 to ≤21 weeks	5 (5.7)	2 (4.2)	7 (5.2)
>21 to ≤ 24 weeks	22 (25.3)	17 (35.4)	39 (28.9)
>24 to ≤27 weeks	36 (41.4)	21 (43.8)	57 (42.2)
>27 to ≤33 weeks	3 (3.4)	0	3 (2.2)

FDA also notes the large number of treatment discontinuations in the study. Most patients discontinued largely related to "participant decision", some of which were also related to adverse events.

FDA notes that the active drug contains a bitter taste and causes transient gastrointestinal symptoms (i.e., diarrhea, abdominal pain) were reported most frequently in the first three weeks after initiation. Although bittering agent was added to mask the placebo in the double-blind treatment period, there were still a number of patients who discontinued early in the study (20% in the first 12 weeks), potentially due to the bitter taste and/or the GI symptoms. The potential for diarrhea and bitter taste were described to patients in the informed consent, which may have alerted patients to these symptoms and potentially could have led to functional unblinding.

The open-label phase is noted above as being ended by Sponsor on March 1, 2021 and this was the cutoff date selected by the Applicant for the survival analyses. No patients were receiving drug in the study up to this date. All participants had either completed dosing, had died, or discontinued dosing prior to this date.

4.2.1.2 Baseline Demographics and Disease Characteristics

The Applicant's Position

Baseline demographics were well-balanced between the AMX0035 and placebo groups (Table 2). There were no imbalances between the treatment groups with respect to age, gender, weight, or height at study enrollment.

Table 2: AMX3500 Demographic and General Baseline Characteristics – mITT

	AMX0035 + SOC (N=87)	Placebo + SOC (N=48)
Gender (n [%])		
Male	61 (70.1)	32 (66.7)
Female	26 (29.9)	16 (33.3)
Age at Enrollment		
Mean (SD)	57.6 (10.45)	57.3 (7.56)
Median	59.0	57.5
Race (n [%[)		
White	82 (94.3)	46 (95.8)
Asian	2 (2.3)	1 (2.1)
Black or African American	2 (2.3)	1 (2.1)
Unknown	1 (1.1%)	0
Race Group (n [%[)		
White	82 (94.3)	46 (95.8)
Other[a]	5 (5.7)	2 (4.2)
Ethnicity (n [%[)		
Hispanic or Latino	6 (6.9)	1 (2.1)
Not Hispanic or Latino	81 (93.1)	47 (97.9)
BMI at Enrollment (kg/m^2)		
Mean (SD)	26.9 (4.42)	26.4 (5.81)
Median	26.8	25.3

Abbreviations: BMI=body mass index; mITT=modified intent to treat; SOC=standard of care; Note: Percentages are based on the number of participants with non-missing data in each treatment group and overall.
[a] Other race includes Asian, Black or African American, and Unknown.
Source: Table 14.1.5 Main CSR

The FDA's Position:

FDA agrees that there were no baseline demographic differences between the AMX0035 and placebo arm populations.

Baseline disease characteristics were generally similar for participants assigned to AMX0035 and placebo (Table 3). On average, the time since onset of first symptom to randomization in AMX3500 was approximately 13.5 months. Consistent with standard of

care in the US, most participants (77.0%) were on either edaravone or riluzole at or prior to study entry. More participants in the placebo group were receiving or had received edaravone at baseline. Baseline scores for efficacy endpoints were also similar between groups, with a mean ALSFRS-R Total Score of 36.0, a mean ATLIS total score percent of predicted normal (PPN) of 55.8, and a mean SVC PPN of 83.7.

Table 3: Baseline Disease Characteristics – mITT

Statistic	AMX0035 +SOC (N=87)	Placebo + SOC (N=48)
DEL-FS		
Mean (SD)	0.953 (0.4267)	0.926 (0.6012)
Time Since Onset of ALS Diagnosis (months)		
Mean (SD)	5.9 (3.33)	6.3 (3.22)
Time Since Onset of ALS Symptoms (months)		
Mean (SD)	13.5 (3.83)	13.6 (3.64)
Use of Either Edaravone or Riluzole at or Prior to Study Entry	62 (71.3%)	42 (87.5%)
Use of Both Edaravone and Riluzole at or Prior to Study Entry	19 (21.8%)	19 (39.6%)
Use of Edaravone Only at or Prior to Study Entry	3 (3.4%)	5 (10.4%)
Use of Riluzole Only at or Prior to Study Entry	40 (46.0%)	18 (37.5%)
Use of Edaravone at or Prior to Study Entry	22 (25.3%)	24 (50%)
Use of Riluzole at or Prior to Study Entry	59 (67.8%)	37 (77.1%)
Time Since First Exposure to Edaravone at Baseline (months)		
Mean (SD)	3.5 (3.04)	3.6 (2.60)
Time Since First Exposure to Riluzole at Baseline (months)		
Mean (SD)	5.7 (3.41)	5.5 (3.28)
Family History of ALS	9 (10.3%)	7 (14.6%)
Site of Onset		
Limb	59 (67.8%)	38 (79.2%)
Bulbar	26 (29.9%)	10 (20.8%)
Other	2 (2.3%)	0 (0.0%)
SVC % Predicted		
Mean (SD)	83.6 (18.17)	83.9 (15.92)
ALSFRS-R Total		
Mean (SD)	35.7 (5.78)	36.7 (5.08)
ATLIS Lower & Upper Extremities		
Mean (SD)	56.8294 (20.08198)	53.9242 (20.94439)

Abbreviations: ALS = amyotrophic lateral sclerosis; ALSFRS-R = ALS Functional Rating Scale – Revised; ATLIS = Accurate Test of Limb Isometric Strength; mITT=modified intent to treat; SD=standard deviation; SOC=standard of care; SVC = slow vital capacity.

Source: Table 14.1.6 Main CSR

The FDA's Position:

FDA acknowledges that there were a few minor imbalances of baseline disease characteristics between the treatment groups.

Imbalances that may favor the treatment group:

- The baseline ATLIS scores in the AMX0035 treatment arm were better at baseline compared to placebo.

There were also imbalances in baseline disease characteristics that may favor the placebo arm.

- There are more patients with limb-onset ALS in the placebo arm, and more bulbar-onset patients in the treatment group. As patients with bulbar-onset ALS tend to have faster rate of disease progression than limb-onset ALS, this could lead to the potential for faster disease progression in the treatment group.
- The use of edaravone and/or riluzole at baseline slightly favors placebo; however, see additional FDA comments on the differences in concomitant medication use later in this Section.

FDA notes no clinically significant differences between treatment groups in baseline ALSFRS-R, SVC % predicted, or time since onset of diagnosis and symptoms. Without further information on the specific genetic mutations, the impact of the baseline differences in family history of ALS are unclear.

Key covariates that are known to influence survival including DEL-FS, ALSFRS-R score at baseline and age were well balanced between the two groups. While the study was generally balanced, there are some key potentially prognostic variables to highlight based on historical ALS trials:

DEL-FS

The DEL-FS (or ALSFRS-R pre-slope) is often a highly prognostic variable for in-trial ALSFRS-R progression rate (Labra, J, 2015 and Taylor, A 2016). While the groups were well-balanced, a higher DEL-FS in the AMX0035 arm would predict a slightly faster progression (0.953 vs 0.926). It should also be noted that these are both high DEL-FS values relative to other historical trials including edaravone, ceftriaxone, and dexpramipexole, and would predict a faster progressing population, as was intended with the inclusion criteria.

The FDA's Position:

It is unlikely that the small difference in the ALSFRS-R rate of decline at baseline (DEL-FS) between the treatment groups is clinically meaningful.

Bulbar versus Limb-Onset ALS

There was a slightly higher percentage of bulbar onset participants in the active group versus placebo group (29.9% vs 20.8%). Bulbar-onset ALS patients have consistently shown faster progression in clinical trial datasets, so this would predict a faster-progressing active group (Atassi, 2014).

The FDA's Position:

As noted above, FDA agrees with potential for faster disease progression in patients with bulbar-onset ALS, which includes more patients in the AMX0035 treatment arm.

Use of Riluzole and/or Edaravone

There was higher use of concomitant riluzole and/or edaravone in the placebo group versus the active group (50% edaravone use in placebo and 25.3% use in AMX0035, 77.1% riluzole use in placebo and 67.8% riluzole use in the AMX0035 group). Given that these are both FDA-approved medications indicated to slow the course of ALS progression, greater use of these concomitant medications in the placebo group would be expected to reduce disease progression. It is important to note that the study did not stratify the population based on concomitant use of these medications and edaravone was approved while the study was in progress.

The FDA's Position:

FDA also notes the lack of stratification based on concomitant use of other FDA-approved medications and is concerned with the impact that may have on interpretability of the primary analysis.

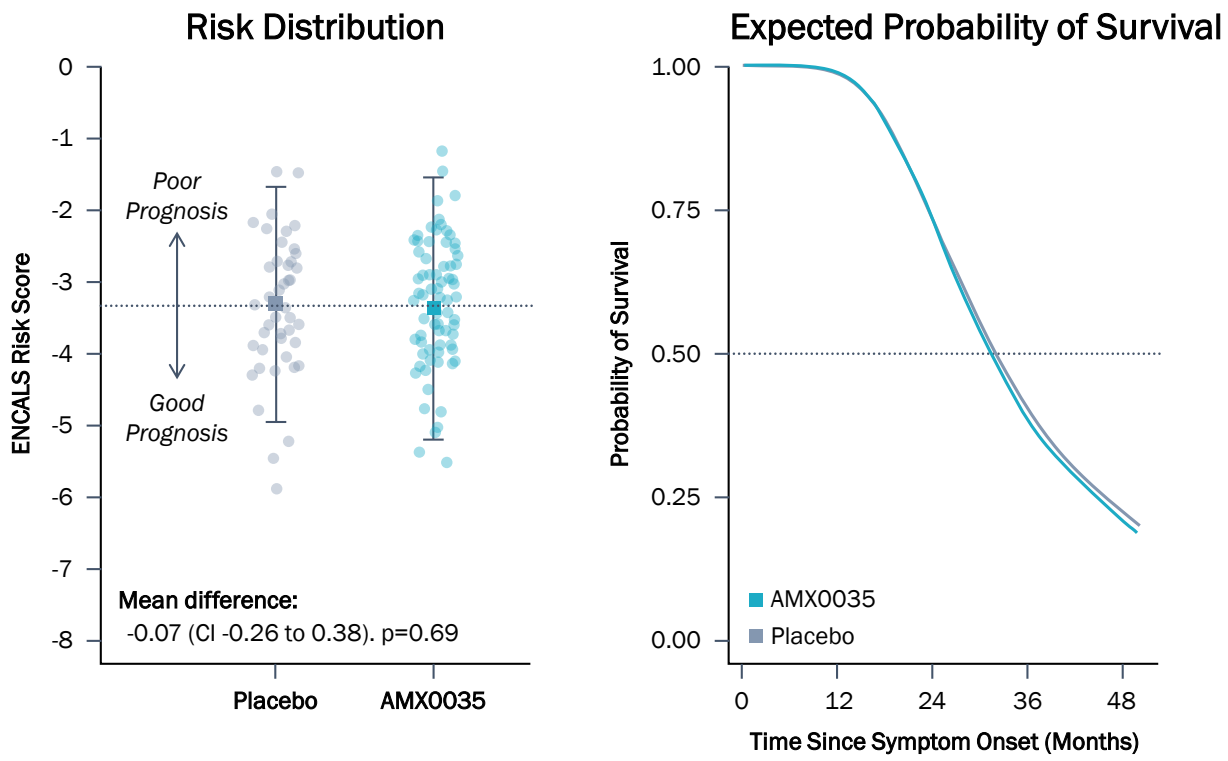
The Applicant claims above that greater use of concomitant medications in the placebo group would be expected to reduce disease progression. However, FDA notes that riluzole doses were required to be stable for 30 days prior to study entry and there was no significant imbalance in ALSFRS-R or the rate of decline in ALSFRS-R at baseline, so it is unlikely that the noted imbalance in concomitant medication use at baseline would have an impact on disease progression throughout the study.

On the other hand, edaravone was approved after the study was initiated; therefore, patients were allowed to start edaravone during the study. An imbalance in the number of patients in each arm initiating new treatment with edaravone occurred during the study. It is concerning that there was a higher proportion of patients starting edaravone or riluzole post-baseline in the AMX0035 arm (14/89 [15.7%] ITT) compared to the placebo arm (2/48 [4.2%] ITT). It is possible that baseline imbalances in background ALS therapy may have inadvertently led to a higher incidence of initiation of riluzole or edaravone post-baseline. This post-baseline starting of ALS medications more in the drug arm could have tipped the balance in the other direction and possibly confounded the primary analysis. FDA notes that data after post-baseline starting of ALS medications occurred at a higher rate in the drug arm and ALSFRS-R assessments after starting concomitant ALS medications were not censored in the primary analysis.

Predicted Risk Score

In 2018, a study was published in Lancet Neurology with a model designed and validated to determine personalized prognostic scores for individuals with ALS (Westeneng, HJ, 2018). The model uses 16 participant characteristics to predict survival. This model was assessed with the participants enrolled in the AMX0035 and placebo groups to determine if the groups were well balanced at baseline. This model found the groups to be well balanced at baseline. Figure 2 shows that the two groups were well matched at baseline with nearly identical prognostic risk scores and expected survival.

Figure 2: Prognostic Risk Scores and Predicted Survival Based on Baseline Characteristics



Applicant's Position:

- The baseline characteristics were well balanced between groups. The characteristics may have predisposed the AMX0035 group to faster progression: greater incidence of bulbar onset, slightly higher DEL-FS, and less use of concomitant ALS therapies were observed in the active group which would all pre-dispose this group to worse outcomes.
- Dropout rates were consistent with historical ALS trials.
- A survival prediction model was also conducted on the AMX0035 and placebo groups which takes into account 16 participant characteristics. The groups were again found to be well-balanced at baseline.

The FDA's Position:

The utility of the Applicant's survival prediction model is unclear. In summary, FDA notes the following regarding the baseline differences in the treatment arms:

- There were no clinically significant demographic differences between the treatment groups at baseline.
- There were some imbalances in the baseline disease characteristics between the two treatment groups; the clinical significance of these differences is unclear.
- There was a higher proportion of patients starting edaravone or riluzole post-baseline in the AMX0035 arm compared to the placebo arm, which may confound the study results and interpretation of the reported efficacy of the drug.

4.3 Randomized Controlled Phase Results

4.3.1 Randomized Controlled Phase: Primary Endpoint Results (ALSFRS-R)

The Applicant's Position

AMX0035 met the pre-specified primary endpoint, demonstrating a statistically significant (p=0.0340) slowing of disease progression as measured by the ALSFRS-R total score compared to placebo (Table 4, Figure 3) in the 24-week randomized, controlled phase.

The estimated least squares (LS) mean ALSFRS-R total score was 2.32 points higher at Week 24 compared to placebo. The primary prespecified model was a shared baseline linear mixed effects model, commonly used in ALS trials.

Table 4: ALSFRS-R Total Score at Week 24 – mITT

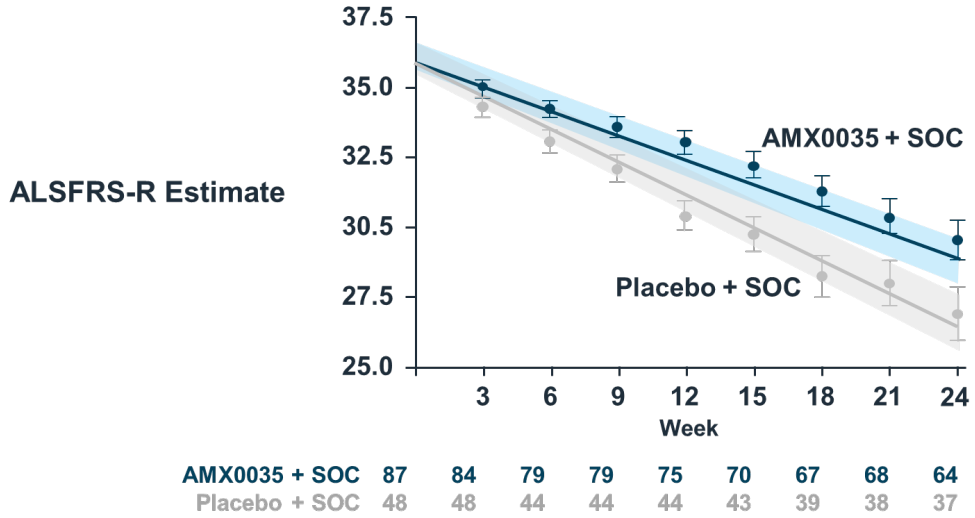
	Estimate (SE)		Estimated Difference (SE)	95% CI	p-value
	AMX0035+SOC (N=87)	Placebo+SOC (N=48)			
ALSFRS-R Total Score					
Week 24	29.06 (0.781)	26.73 (0.975)	2.32 (1.094)	0.18, 4.47	0.0340

Abbreviations: ALSFRS-R = ALS Functional Rating Scale – Revised; CI = confidence interval; ITT = intention to treat; mITT = modified intent to treat; SE = standard error; SOC = standard of care.

Source: Table 14.2.1.3 Part 1 Main CSR

Results are also demonstrated graphically below. Both groups show relatively linear decline and the groups separated by 0.42 points on the ALSFRS-R per month of treatment. This difference in slopes represents a 25.3% slowing in disease progression.

Figure 3: Estimated Rate of Decline in ALSFRS-R Total Score Over 24 Weeks – mITT



Abbreviations: ALSFRS-R = Amyotrophic Lateral Sclerosis Functional Rating Scale Revised, mITT = modified intent-to-treat.

The ALSFRS-R metric is validated and defined as the total score across the instrument. However, different patients may lose functional domains at different times, although ultimately ALS will affect the whole body: all domains will be affected as long as the patient lives long enough. Some examples of questions are shown in Figure 4.

Figure 4: Clinical Meaning of ALSFRS-R Changes

	4	3	2	1	0
Speech	Normal speech	Detectable speech disturbance	Intelligible with repeating	Speech + non-vocal communication	Loss of useful speech
Walking	Normal walking	Early walking difficulties	Walk with assistance	Non-ambulatory functional movement	No purposeful leg movement
Dressing	Normal function	Independent, but with effort or less efficiency	Intermittent assistance needed	Attendant needed for self-care	Total reliance on others for self-care
Swallowing	Normal eating	Early problems; occasional choking	Dietary consistency changes	Supplemental tube feedings needed	Only enteral or parenteral feeding

The FDA's Position:

The Applicant has reported that it met the prespecified primary endpoint, demonstrating a treatment difference of 2.32 (LS mean) on the ALSFRS-R rate of decline between the treatment arm and placebo (p = 0.034).

However, the statistical evidence (p = 0.034) is not highly persuasive, and there are additional questions about the robustness of the results:

- The Applicant's primary analysis (slope analysis) assumes linearity of ALSFRS-R over time. However, linearity over time is not established for the ALSFRS-R in patients with ALS and exploratory analyses raise questions about the validity of the linearity assumption. For example, residual plots suggest issues with the model fit, and descriptive analyses of means over time (e.g., Figure 3 above) suggest a non-linear trend over time in which the separation between the data points gets smaller over time contrary to the slope model's prediction. A model with a quadratic term for time to allow more flexibility in the nature of ALSFRS-R change over time was prespecified as a backup analysis in the SAP. **Based on this analysis, the Week 24 treatment difference is estimated as 1.68 (S.E.=1.06) with a p-value of 0.1134.** The SAP included criteria under which this would become the primary analysis. Although these criteria were not met, absence of evidence of assumption violations does not imply that the assumption holds. Given the concerns around linearity, this and other analyses allowing for non-linearity are considered important sensitivity analyses.

  In summary, there are questions about the linearity of ALSFRS-R over time assumption, and the linear model appears biased based on the descriptive analyses and relative to the Applicant's prespecified quadratic backup model.

- **The analysis was conducted in the mITT population, excluding two patients who died on drug but did not have post-baseline ALSFRS-R measurements, which could lead to bias.**

- **There was considerable missing data (17% on placebo/18% on drug were alive but missing ALSFRS-R Total Score values at Week 24), and the analysis relies on unverifiable missing data assumptions.**

- **Another point of concern is the higher proportion of patients 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**). These concomitant ALS treatment intercurrent events are difficult to correct for, and the primary analysis inclusion of data after these intercurrent events could have confounded the test for treatment effect. Excluding data after the events does not address the problem, 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 have these events, which is not likely considering that they required the additional treatment.

- **There was a randomization implementation problem such that the first 18 patients (13% of the overall sample size) were assigned to the drug arm in a row, reportedly due to a shipping problem resulting in unavailability of placebo doses.** The subsequent 9 patients were then all assigned placebo. The unblinded (Data and Safety Monitoring Board (DSMB) statistician became aware of this at the first DMSB meeting and then attempted to adjust the pre-planned randomization schedule to fix this problem. The Applicant's reported analyses are for the "as-treated" groups for the first 27 patients rather than for the "as-randomized" groups.
- **This model also does not incorporate deaths in the primary analysis.** Functional endpoints can be confounded by loss of data because of patient deaths, which is why FDA recommends an analysis method that combines survival and function into a single overall measure in ALS, such as the joint rank test.

The above concerns, combined with results of some relevant sensitivity analyses (see further discussion below), demonstrate weaknesses in the statistical robustness of the treatment benefit reported by the Applicant after 24 weeks of treatment. The potentially incorrect assumptions about linearity of the functional rating scale, the ignoring of deaths in the primary analysis, and the higher percentage of patients starting intercurrent treatment for ALS, in addition to the randomization error during the study, make it challenging to interpret the positive p-value in this small sample size.

4.3.1.1 Randomized Controlled Phase: Primary Endpoint Analyzed as Change from Baseline

The Applicant's Position

The primary model used in CENTAUR was a shared baseline, linear mixed effects model. This model assumes that both the active and placebo arms start from the same baseline score and progress linearly over time.

A model was conducted in which each individual participant's change from baseline was evaluated instead of assuming a shared baseline across the study. The results from this model are presented below in Table 5.

Under this statistical model, AMX0035 showed a 30.4% slowing in disease progression and a 2.92 point least squares mean difference after 24 weeks (p=0.01).

The results on the change from baseline model demonstrate that a shared baseline assumption is not required for observation of benefit and that the differences are larger when analyzed as change from baseline.

Table 5: ALSFRS-R Total at Week 24 – Change from Baseline Analysis – mITT Population (N=135)

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					
Week 24	-9.62 (0.913)	-6.70 (0.682)	2.92 (1.134)	0.70, 5.15	0.010

The FDA's Position:

This change from baseline model was not prespecified, and like the primary analysis model, relies on a questionable linearity of ALSFRS-R over time assumption, because this particular change from baseline model still prescribes a slope model for the functional form of the trend in ALSFRS-R changes over time. The concerns regarding linearity are outlined above.

In a sensitivity analysis, FDA found that a more common model frequently used in review work, a Mean-By-Visit MMRM model of change from baseline which does not rely on a linearity assumption did not show a statistically significant treatment difference in ALSFRS-R at Week 24 (estimated difference: 1.86 [S.E.=1.04], p=0.0749).

This model included age and pre-randomization slope as covariates and interactions between pre-randomization slope and Visit, and age and Visit, as well as effects for treatment, Visit, and the treatment by Visit interaction. Note that the model did not include the baseline assessment of ALSFRS-R as the first measure of the dependent variable.

Note that this model, like the primary analysis model, was analyzed in the mITT population and also does not incorporate deaths in the analysis.

4.3.1.2 Randomized Controlled Phase: Primary Endpoint Analyzed with Addition of Non-Linear Terms

The Applicant's Position

The primary model assumes linearity. As such, an important sensitivity analysis is to determine whether non-linear effects might influence the outcome. The primary model was rerun with the addition of quadratic terms designed to capture potential non-linear effects over the 24-week study period (Table 6).

Results are consistent with the results of the primary, linear mixed effects model suggesting limited impact from non-linear terms.

Table 6: ALSFRS-R Total at Week 24 – Analysis with addition of Non-linear Terms – mITT Population (N=135)

Endpoint Time Point	Estimate (SE)		Difference		
	Placebo+SOC (N=48)	AMX0035+SOC (N=87)	Estimate (SE)	95% CI	p-value
ALSFRS-R Total					
Week 24	26.83 (0.98)	29.11 (0.78)	2.28 (1.1)	0.12, 4.44	0.039

The FDA's Position:

The model presented in the above table does not correspond to the pre-specified quadratic model. The presented model omits an individual adjustment (random effect) for the quadratic weeks term which was prespecified in the analysis plan clarifying note (November 21, 2019). This individual adjustment would be expected to be included in a quadratic extension of the slope model, given that corresponding individual adjustments (random effects) were included for the intercept and slope parameters in the model. As noted above, based on the pre-specified backup quadratic model, the Week 24 treatment difference is estimated as 1.68 (S.E.=1.06) with a p-value of 0.1134.

4.3.1.3 Randomized Controlled Phase: Primary Endpoint Analyzed to Determine Impact of Missing Data

The Applicant's Position

Approximately 23% of participants dropped out from the AMX3500 study. It is important to determine the extent to which this missing data could have impacted the primary outcome.

A Linear Mixed Model for Repeated Measures, using multiple imputation from the control arm to impute assessments missing after discontinuation of study drug was performed as a prespecified sensitivity analysis. This analysis assumes participants who discontinue medication and are no longer assessed immediately become similar to matching participants who never took active treatment, and so provides a lower bound on efficacy (control-base imputation).

In this analysis, performed in the mITT population, treatment effect sizes and p-values remained similar to the primary analysis (p=0.043, 1.87-point difference) (Table 7). This sensitivity analysis suggests that the primary results are robust to missing data and dropouts.

Table 7: Multiple Imputation to Test Missing at Random Assumption for Missing Data – mITT Population (N=135)

Endpoint Time Point	Estimate (SE)		Difference		
	Placebo+SOC (N=48)	AMX0035+SOC (N=87)	Estimate (SE)	95% CI	p-value
ALSFRS-R Total					
Week 24	27.81 (0.82)	29.68 (0.65)	1.87 (0.926)	0.06, 3.69	0.043

Abbreviations: ALSFRS-R = Amyotrophic Lateral Sclerosis Functional Rating Scale – Revised; CI = confidence interval; mITT = modified Intent-to-Treat population; SE = standard error; SOC = standard of care.

The FDA's Position:

The Applicant claims that this analysis provides a lower bound on efficacy. However, this is not true because deaths are ignored in this analysis (See Section 4.3.1.6).

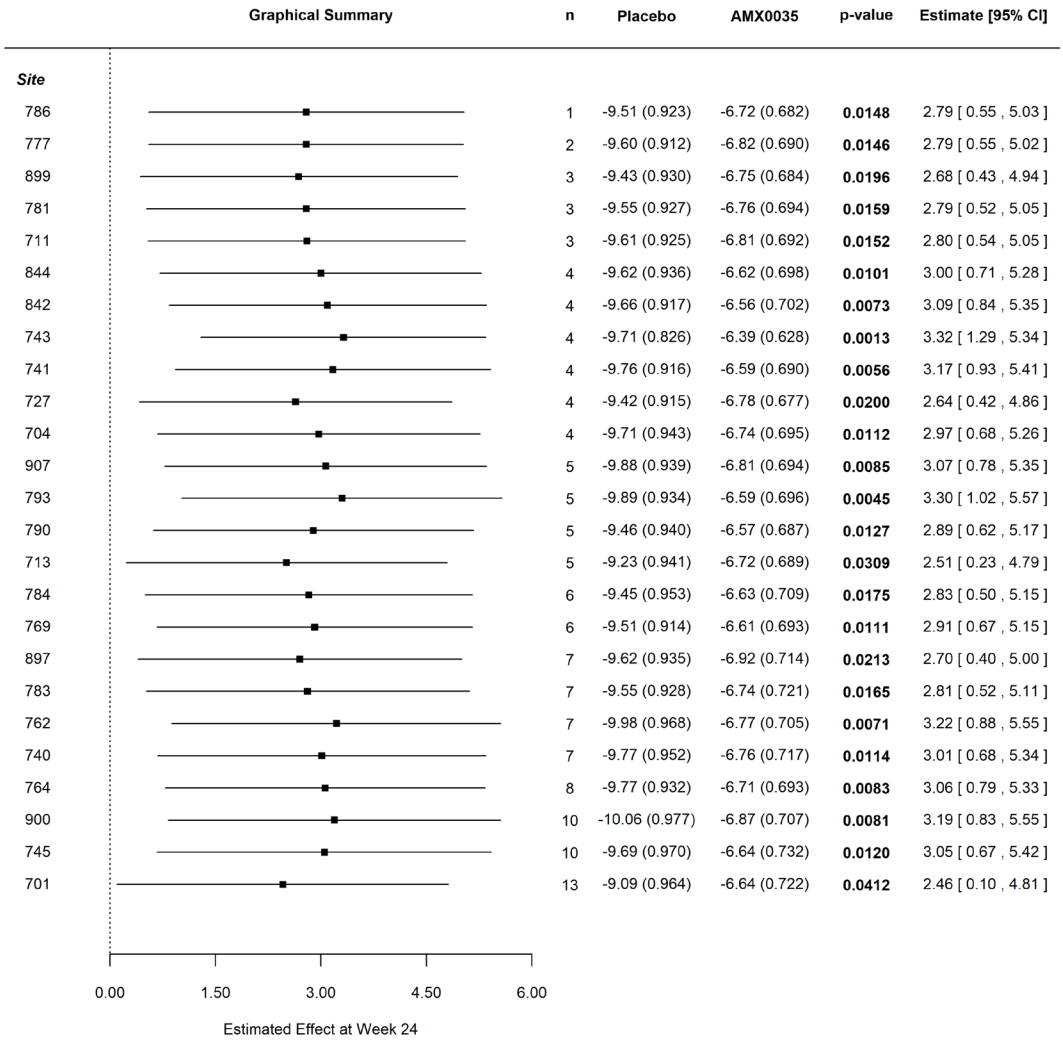
Furthermore, the prespecified quadratic model (per the November 2019 note clarifying the quadratic model) gives a lower estimated treatment difference than this (Week 24 treatment difference = 1.68), and did not reach statistical significance.

4.3.1.4 Randomized Controlled Phase: Primary Endpoint Analyzed to Determine Impact of Individual Sites

The Applicant's Position

A potential concern in any study is that individual participants or sites could drive the observed efficacy result. To test whether this could have affected results in AMX3500, the change from baseline model described above was conducted 25 times, each time with the removal of one of the sites. Change from baseline was used in this sensitivity analysis since a shared baseline assumption could potentially obscure baseline differences. This result demonstrates a highly consistent treatment effect across the study population with no individual participant or site driving the observed differences.

Figure 5: Change from Baseline Analysis of ALSFRS-R with each individual site Removed from Analysis (n is the number of participants enrolled at the site)



Site	n	Placebo	AMX0035	p-value	Estimate [95% CI]
786	1	-9.51 (0.923)	-6.72 (0.682)	**0.0148**	2.79 [0.55 , 5.03]
777	2	-9.60 (0.912)	-6.82 (0.690)	**0.0146**	2.79 [0.55 , 5.02]
899	3	-9.43 (0.930)	-6.75 (0.684)	**0.0196**	2.68 [0.43 , 4.94]
781	3	-9.55 (0.927)	-6.76 (0.694)	**0.0159**	2.79 [0.52 , 5.05]
711	3	-9.61 (0.925)	-6.81 (0.692)	**0.0152**	2.80 [0.54 , 5.05]
844	4	-9.62 (0.936)	-6.62 (0.698)	**0.0101**	3.00 [0.71 , 5.28]
842	4	-9.66 (0.917)	-6.56 (0.702)	**0.0073**	3.09 [0.84 , 5.35]
743	4	-9.71 (0.826)	-6.39 (0.628)	**0.0013**	3.32 [1.29 , 5.34]
741	4	-9.76 (0.916)	-6.59 (0.690)	**0.0056**	3.17 [0.93 , 5.41]
727	4	-9.42 (0.915)	-6.78 (0.677)	**0.0200**	2.64 [0.42 , 4.86]
704	4	-9.71 (0.943)	-6.74 (0.695)	**0.0112**	2.97 [0.68 , 5.26]
907	5	-9.88 (0.939)	-6.81 (0.694)	**0.0085**	3.07 [0.78 , 5.35]
793	5	-9.89 (0.934)	-6.59 (0.696)	**0.0045**	3.30 [1.02 , 5.57]
790	5	-9.46 (0.940)	-6.57 (0.687)	**0.0127**	2.89 [0.62 , 5.17]
713	5	-9.23 (0.941)	-6.72 (0.689)	**0.0309**	2.51 [0.23 , 4.79]
784	6	-9.45 (0.953)	-6.63 (0.709)	**0.0175**	2.83 [0.50 , 5.15]
769	6	-9.51 (0.914)	-6.61 (0.693)	**0.0111**	2.91 [0.67 , 5.15]
897	7	-9.62 (0.935)	-6.92 (0.714)	**0.0213**	2.70 [0.40 , 5.00]
783	7	-9.55 (0.928)	-6.74 (0.721)	**0.0165**	2.81 [0.52 , 5.11]
762	7	-9.98 (0.968)	-6.77 (0.705)	**0.0071**	3.22 [0.88 , 5.55]
740	7	-9.77 (0.952)	-6.76 (0.717)	**0.0114**	3.01 [0.68 , 5.34]
764	8	-9.77 (0.932)	-6.71 (0.693)	**0.0083**	3.06 [0.79 , 5.33]
900	10	-10.06 (0.977)	-6.87 (0.707)	**0.0081**	3.19 [0.83 , 5.55]
745	10	-9.69 (0.970)	-6.64 (0.732)	**0.0120**	3.05 [0.67 , 5.42]
701	13	-9.09 (0.964)	-6.64 (0.722)	**0.0412**	2.46 [0.10 , 4.81]

The FDA's Position:

The Applicant does not provide adequate justification for doing the above individual site analysis with a different model than the primary analysis model (i.e., the primary model did not use change from baseline as it included baseline ALSFRS-R as the first assessment of the modeled dependent variable).

FDA used the primary analysis model to check for impact of individual sites, and found more influential sites, some of which affected the significance of the treatment difference. In other words, the removal of a single site from the study rendered the primary analysis treatment effect no longer statistically significant [e.g., without site 701 (n=13): slope difference = -0.079; SE=0.049; p=0.1027 with a corresponding Week 24 mean difference of 1.90]. This particular site had a within site estimated treatment difference more than twice as large as the overall estimate (5.75 vs 2.32). FDA also notes that this same site had a substantive quantitative difference for time to death in the OLE phase, with a within-site hazard ratio (0.23, drug over placebo) more than two times smaller than the overall hazard ratio (0.64).

4.3.1.5 Randomized Controlled Phase: Primary Endpoint Analyzed in ITT population

The Applicant's Position

The ITT population includes two participants who dropped out of the study prior to their second visit and therefore only had a baseline ALSFRS-R score. The ALSFRS-R was analyzed as a linear mixed effects model. A result of this model is that participants with a single baseline datapoint do not contribute to the primary outcome, the slope of decline. The primary model was repeated in the ITT population and therefore returned an identical result (2.32-point difference at Week 24, p=0.034) as the mITT population.

The FDA's Position:

FDA acknowledges that the Applicant repeated the primary analysis using the ITT and returned the same result as with the mITT population.

However, FDA notes that the equivalence of the mITT and ITT analyses would not hold for the FDA recommended joint rank analysis of ALSFRS-R and survival. The Applicant's findings depend on the problematic exclusion of two randomized and dosed drug deaths who had no post-baseline ALSFRS-R assessments obtained.

4.3.1.6 Randomized Controlled Phase: Primary Endpoint Analyzed to Determine Impact of Deaths

The Applicant's Position

Two models were conducted to incorporate information on deaths into the primary outcome and thereby determine the impact of death on the primary outcome. The inclusion criteria of AMX3500 asked physicians to enroll participants who were likely to complete a 24-week study period so deaths during the initial randomized controlled phase were expected to be infrequent. Seven (7) deaths (5.1%) occurred in the randomized phase. These models designed to assess the impact of death on the primary outcome are shown below.

ALSFRS-R Primary with Adjusted Data for Deaths

In this analysis, the primary analysis was repeated using the left-censored values for all ALSFRS-R observations. All values that are censored by an intercurrent event of death and death-equivalent events were assumed to be equal to the lowest of all observed values, such that the contribution to the likelihood for each participant is the product of the density of all the observed outcomes and of the conditional distribution of the censored outcomes. The starting values for the fixed variables were the point estimates from the primary analysis. All variance parameters had a lower bound of 0.

In brief, this model was designed to adjust ALSFRS-R for patients who died towards a worse outcome.

The difference between treatment and placebo was approximately the same in this model as in the primary analysis (2.3 points in both cases) and the p-value remained similar as well (p=0.0335 vs. p=0.0340) (Table 8). This model provides confirmation that even when death information is incorporated into the primary outcome, the results remain robust.

As discussed, since the ALSFRS-R is analyzed as a slope, the ITT population would return identical results.

Table 8: ALSFRS-R Total at Week 24 Adjusted for Deaths – mITT Population (N=135)

Endpoint Time Point	Estimate (SE)		Difference		
	Placebo+SOC (N=48)	AMX0035+SOC (N=87)	Estimate (SE)	95% CI	p-value
ALSFRS-R Total					
Week 24	26.66 (0.966)	28.99 (0.775)	2.33 (1.084)	0.18, 4.47	0.0335

The FDA's Position:

According to the Applicant's submitted analysis program to conduct this left censored analysis, 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.

Furthermore, for those patients who died before Week 18 there is no contribution to the likelihood for Week 24 in this analysis, although since they died it is known that their Week 24 ALSFRS-R score would be zero.

Additionally, the analysis does not take time to death into account, so that an earlier death could appear better than a later death, especially since ALSFRS-R tends to worsen over time due to the progressive nature of the disease.

For some deaths, the observed ALSFRS-R slope (relative to other surviving patients' slopes) gave no indication that the patient was going to die. For these reasons, the left censored analysis is biased and does not establish that the primary result is robust to deaths.

Joint-Rank Analysis of ALSFRS-R and Survival Defined as Death or Death Equivalent in the ITT population

The Applicant's Position

A joint-rank post hoc analysis was performed by ranking subjects first by time to death or death equivalent (permanent ventilation) then by change from baseline in ALSFRS-R. This type of analysis is recommended in the 2019 FDA ALS Guidance for Industry.

A regression was performed on this ranked outcome with treatment, ranked age, and ranked del-FS as terms in the regression to mirror the primary analysis as closely as possible. The p-value for treatment was used as the p-value for this analysis.

The results of this analysis were statistically significant (Table 9) and were consistent with the results of the pre-specified primary efficacy analysis.

Table 9: Joint Rank Analysis ALSFRS-R Total Score and Death or Equivalent – ITT Population (N=137)

	AMX0035+SOC Rank Estimate	Placebo+SOC Rank Estimate	Difference	p-value
Joint Rank Analysis	73.9 (3.9)	59.9 (5.3)	13.99 (6.6)	0.037

The FDA's Position:

As noted by the Applicant, the joint rank analysis was not prespecified. FDA notes that deaths did occur in the double-blind treatment period, with 5 deaths in the treatment arm and 2 deaths in the placebo arm. Two of the treatment arm deaths were excluded from the mITT because they did not have post-baseline ALSFRS-R assessments.

For the post hoc implementation of the joint rank analysis, the Applicant 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.

The Applicant also included death equivalent events (there was 1 tracheostomy/permanent ventilation event in a placebo patient in the double-blind period). The Applicant's joint rank analysis of death only for the ITT, rather than death equivalent, has a p = 0.056.

A joint rank analysis with a more appropriate method of handling missing data (multiple imputation based on a missing-at-random assumption) of ALSFRS-R and death has p=0.063 for the mITT population. FDA's multiple imputation regression model included covariates of age and pre-randomization ALSFRS-R slope and each ALSFRS-R assessment prior to the missing ALSFRS-R assessment.

For the ITT population, including the 2 deaths in the treatment arm who were dosed but had no post-baseline ALSFRS-R assessments, the joint rank analysis with a more appropriate missing data handling method (multiple imputation) has p=0.079. The joint rank analysis including death equivalents, also using the more appropriate missing data handling method, has p= 0.07.

In addition, there was a considerable amount of missing data (17% /18% of patients were alive but missing ALSFRS-R Total Score values at Week 24 on placebo/drug), and even a more appropriate missing-at-random assumption is not verifiable and may not hold. Analyses with alternative assumptions may provide less favorable results.

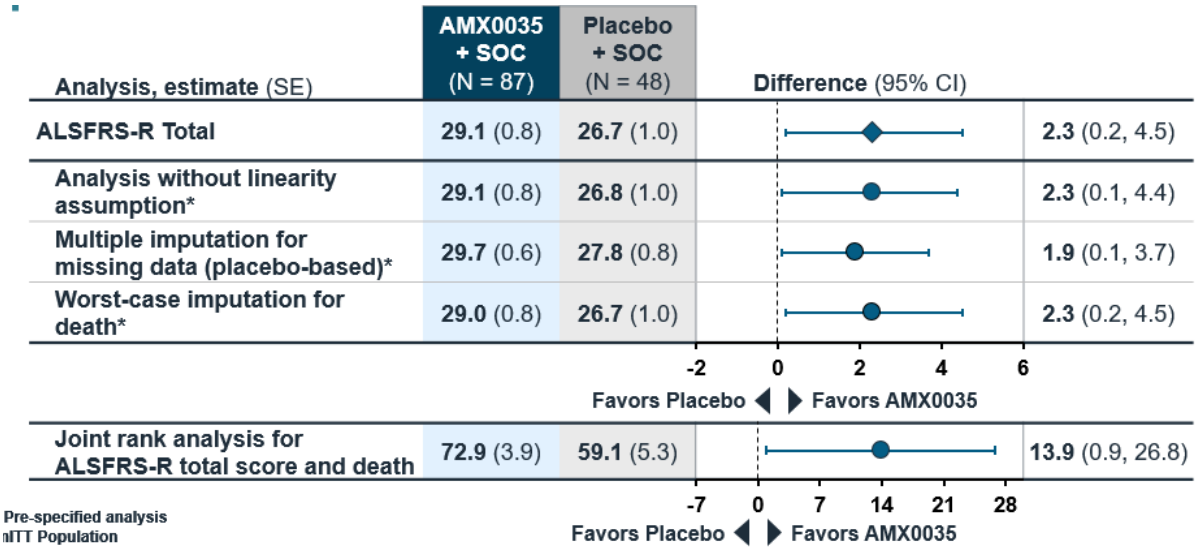
The ranking of the covariates was also not prespecified and gives slightly smaller p-values than when the covariates are not ranked. The above p-values are with the non-prespecified ranking of covariates.

4.3.1.7 Randomized Controlled Phase: Sensitivity Analyses

The Applicant's Position

In sum, the aforementioned sensitivity analyses conducted on the primary outcome are summarized in Figure 6.

Figure 6: Sensitivity Analyses to Support Primary Efficacy Endpoint Results in AMX3500



The FDA's Position:

FDA notes that in Figure 6 above, the "worst-case imputation for death" description (row 4) is potentially misleading. Worst case might be misinterpreted as meaning worse than all other surviving subjects, as deaths would be handled in a joint rank analysis; however, this analysis is better characterized as a worst (individual's) ALSFRS-R observation carried forward analysis.

As previously noted, the other sensitivity analyses that the Applicant has reported here include a model without the linear assumption analysis, which is different than that which was prespecified; the multiple imputation analysis, which ignores deaths; and the joint-rank analysis, which used the inappropriate missing data handling method of last observation carried forward in surviving non-completers. FDA's detailed positions on these analyses are provided above in the relevant Applicant sections.

4.3.2 Randomized Controlled Phase: Secondary Endpoint Results

The Applicant's Position

As stated previously, the secondary endpoints were listed in hierarchical order. The first secondary analysis was ATLIS. Because the ATLIS outcome did not reach statistical significance, all subsequent secondary outcome p values presented are nominal.

4.3.2.1 Randomized Controlled Phase: ATLIS Results

As described earlier, ATLIS was measured as a novel method to measure strength in 12 muscles. The score can be divided into upper limb strength (Upper ATLIS) and lower limb strength (Lower ATLIS), as well as Total ATLIS which is the combination of the two. The results showed a non-significant difference of 2.8 percentage points on Total ATLIS, a nominally significant treatment difference of 4.3 percentage points on Upper ATLIS, and a non-significant 2.1 percentage point difference in Lower ATLIS (Figure 7).

Figure 7: ATLIS (Percent of Normal Strength) at Week 24 – mITT

Endpoint	AMX0035 N	AMX0035 Estimate (SE)	Placebo N	Placebo Estimate (SE)	Difference (95% CI)	Nominal p-value
Total ATLIS	84	**39.1** (2.0)	47	**36.3** (2.2)	**2.8** (-0.7, 6.3)	0.1129
Upper	85	**36.6** (2.3)	47	**32.4** (2.6)	**4.3** (0.2, 8.4)	0.0420
Lower	85	**41.2** (2.4)	48	**39.1** (2.7)	**2.1** (-2.2, 6.4)	0.3424

Sources: 2.7.3 Table 12

The FDA's Position:

FDA notes that the SAP did not prespecify which ATLIS Score component (i.e., Total, Upper, or Lower) would be analyzed first, therefore creating a multiplicity concern. In addition, these analyses use the same slope model as the primary analysis, which includes similar concerns regarding the linearity assumption. FDA notes that only the Upper ATLIS score achieved nominal significance.

There is more missing data at Week 24 for ATLIS scores than for the ALSFRS-R, and deaths are again ignored in this analysis which may result in bias in the analysis.

In addition, there were some imbalances in the ATLIS score at baseline that favored the AMX0035 treatment arm by 2.9 points (See Table below). The imbalance in Total ATLIS score at baseline appears to be driven by differences in the Upper ATLIS score at baseline, which favored the AMX0035 arm by 3.3 points. A higher (better) Upper ATLIS score at baseline may have resulted in a slower decline in the AMX0035 group after 24 weeks of treatment, or could result in proportional treatment difference at Week 24, weakening the robustness of the nominal p-value of 0.0420 observed with the Upper ATLIS score only.

Baseline ATLIS Scores

	ATLIS Scores at Baseline (Mean (SD))	
	Placebo	*AMX0035*
Total ATLIS	*53.9 (20.9)*	*56.8 (20.0)*
Upper ATLIS	*51.4 (25.2)*	*54.7 (24.2)*
Lower ATLIS	*57.1 (25.8)*	*57.6 (24.8)*

FDA estimated a Week 24 difference in Upper ATLIS **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) of **2.60 (S.E.= 2.16), p=0.2319** [based on 123 subjects and 406 post-baseline ATLIS records].

FDA also notes that a composite survival endpoint was a pre-specified secondary endpoint for the double-blind period. Single and combined survival analyses over the double-blind period 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 (occurred in the same placebo subject). As shown in the Table below, while some of the analyses directionally favored AMX0035 and while the numbers of events (particularly deaths) were small, none of the analyses were statistically significant.

These survival results in the double-blind period may be relevant when considering the Applicant's survival analyses through the OLE.

Double-blind Phase Survival Analysis at 24 weeks

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: Table 14 Clinical Study Report Page 92

4.3.2.2 Randomized Controlled Phase: Biomarker Results

The Applicant's Position

Phosphorylated neurofilament heavy chain (pNF-H) was measured in plasma as a potential blood-based biomarker. In the AMX3500 study, there were no significant differences between the AMX0035 and placebo groups for the rate of change from baseline in plasma levels of pNF-H (3.58 pg/mL per month with AMX0035 and -2.34 pg/mL per month with placebo; difference, 5.93 pg/mL per month; 95% CI, -4.41 to 16.26, p=0.2601; Table 14.2.1.13 Main CSR).

The FDA's Position:

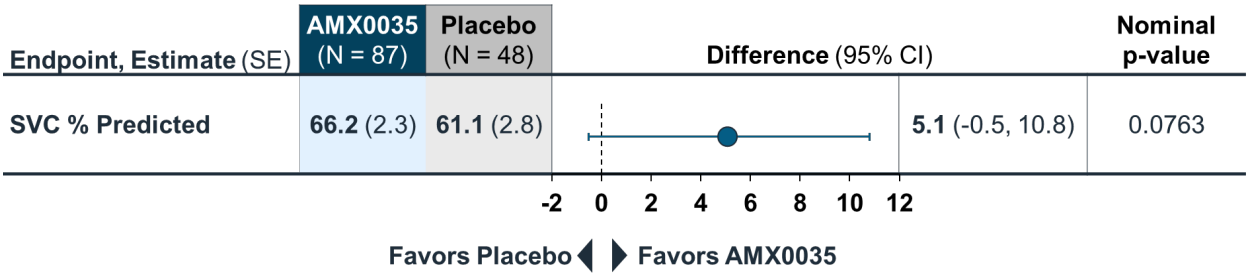
pNF-H is a marker of neuronal axonal injury and neurodegeneration. It may be hypothesized that a therapy that shows benefit in the treatment of ALS would also decrease pNF-H levels. FDA acknowledges there was not a significant difference between the rate of change from baseline in plasma levels of pNF-H and appears to numerically favor the placebo arm.

4.3.2.3 Randomized Controlled Phase: SVC Results

The Applicant's Position

Slow Vital Capacity (SVC) was utilized as a measure of breathing capacity. As shown in Figure 8, at Week 24 participants in the AMX0035 group were observed to have 66.2% of normal breathing capacity whereas those in the placebo arm were observed to have 61.1% of normal breathing capacity, a 5.1% difference. This result was not statistically significant (p=0.076).

Figure 8: SVC at Week 24 – mITT



Source: 2.7.3 Table 14

The FDA's Position:

This small numerical trend that is not nominally significant is not consistent with a meaningful benefit.

Applicant's Position on Randomized Controlled Phase Outcomes:

- AMX0035 met its prespecified primary outcome in the study's randomized controlled phase showing a statistically significant reduction in rate of progression on the ALSFRS-R.
- The ALSFRS-R rate of decline was 25.3% less in patients randomized to AMX0035 versus placebo. A 20% slowing of ALSFRS-R is considered clinically meaningful [Castrillo-Viguera 2010).
- When analyzed using individual participant change from baseline the result is more pronounced with a 30.4% slowing in the rate of decline on the ALSFRS-R, p=0.01.
- The primary outcome remained robust through multiple sensitivity analyses examining missing data, effects of individual sites, linearity, and death.
- These analyses demonstrate that the primary outcome findings in AMX3500 are robust and support the efficacy of AMX0035.
- ATLIS and SVC results were numerically consistent with the primary outcome but were not statistically significant.
- To date, no validated biomarkers exist that measure ALS disease progression. No significant differences were observed on plasma phosphorylated neurofilament heavy chain (pNfl-H). While the biomarker was chosen as a secondary endpoint based on publications of its utility as a potential marker of neurodegenerative diseases, it may not be a treatment-sensitive marker, as no effective ALS treatment has shown an ability to modulate this marker.
- The applicant believes the most important findings from the randomized phase are: (1) the study met its prespecified primary outcome (2) the primary outcome is robust through multiple sensitivity analyses and (3) ATLIS, SVC all demonstrated numerical results in favor of AMX0035 and support the strength of the primary finding.

The FDA's Position:

FDA does not agree with all of the Applicant's claims stated above. While the primary analysis was statistically significant, the statistical evidence (p = 0.034) is not persuasive and there are additional questions about the robustness of the results.

Details on FDA's position on these analyses were provided in the relevant Applicant sections. Specifically, FDA notes the following comments about the randomized, double-blind treatment phase of the study:

- A slope analysis that assumed linearity over time was used, which is not established.
- Deaths were ignored in the analyses, and an inappropriate method of handling missing data was used in the post hoc implementation of the preferred joint rank analysis.
- There is potential for functional unblinding due to bitter taste and adverse effects (i.e., GI symptoms) of the drug.
- It is also noted that post-baseline ALS medications were started at a higher rate in the treatment arm.

FDA conducted additional analyses to try to address some of the issues with the analyses conducted by the Applicant, some of which are described above. For example, the preferred joint rank analysis of ALSFRS-R and death that does not assume linearity over time, using a more appropriate method of handling missing data (multiple imputation with a missing-at-random assumption), conducted in the ITT population, has p=0.079.

4.4 Results from Long-Term Follow up: Randomized Controlled Phase Baseline through Open-Label Phase

The Applicant's Position

The results during long-term follow-up are presented below. The continuous outcomes are presented first followed by the time to event outcomes. However, the time-to-event outcome was second in the statistical hierarchy of the long-term statistical plan after the rate of decline on the ALSFRS-R.

4.4.1 Open-Label Analysis – Continuous Outcomes

4.4.1.1 ALSFRS-R Results from Randomization Up to Week 48

The rate of decline in the total ALSFRS-R was assessed using a shared-baseline, mixed-effects model in the mITT population, in the randomized and open-label phases. In this test, the same linear slope model that was used for the 24-week analysis was used for the 48-week analysis to evaluate if the treatment effect was sustained. The analysis found

that the slope difference between the 2 treatment groups (randomized to AMX0035 [RA] vs randomized to placebo [RP]) was statistically significant in favor of the RA group (Table 10).

Table 10: Extended Slope Efficacy Analysis for ALSFRS-R Total Score at Week 48 – mITT

	Estimate (SE), Points				
Analysis/Timepoint	**RA + SOC (N=87)**	**RP + SOC (N=48)**	**Estimated Difference (SE)**	**95% CI**	**p-value**
Extended Slope (48 Weeks)	21.61 (1.178)	17.38 (1.545)	4.23 (1.870)	0.56, 7.90	0.0239

Abbreviations: ALSFRS-R = ALS Functional Rating Scale – Revised; CI = confidence interval; mITT = modified intent to treat; RA = randomized to AMX3500 in the randomized controlled phase, subjects who continued in the open-label phase received AMX0035 in the OLE; RP = randomized to placebo in the randomized controlled phase, subjects who continued in the open-label phase received AMX0035 in the open-label phase; SE = standard error; SOC = standard of care.
Source: Table 14.2.1.3 OLE CSR (includes raw summary data)

The model above assumes linearity over the 48-week period and does not allow for the potential of a crossover effect. Therefore, the estimated rates of progression in the ALSFRS-R during each phase of the study (randomized and open label) using the primary MMRM model are shown in Table 11.

Table 11: Numerical ALSFRS-R Slopes at Each Study Stage by Linear Shared Baseline Model

	Study Stage	
ARM	**Randomized Controlled Phase: Weeks 0-24**	**Open-label Phase: Weeks 24-48**
AMX0035 Estimated ALFRS-R Slope (decline in points per month)	**-1.24 points per month**	**-1.26 points per month**
Placebo Estimated ALFRS-R Slope (decline in points per month)	**-1.66 points per month**	**-1.37 points per month**

Abbreviation: ALSFRS-R = ALS Functional Rating Scale – Revised.

The placebo treatment group, once crossed over to AMX0035 had a reduced numerical rate of disease progression from -1.66 points per month to -1.37 points per month, while the group originally randomized to AMX0035 maintained a very similar progression rate in both phases (1.24 points per month to 1.26 points per month).

The FDA's Position:

FDA notes that it is difficult to interpret any of the open-label efficacy data out to 48 weeks.

There was no indication in the original OLE study protocol that the blind was to be maintained to treatment in the double-blind period. Additionally, FDA notes that the active drug contains a bitter taste and causes transient gastrointestinal symptoms (i.e., diarrhea) which were reported more frequently in the first three weeks after initiation. The solution was re-formulated for the open-label extension phase to reduce the bitter taste of the drug; however there were still a number of patients who discontinued in the open-label extension phase, potentially due to the bitter taste and/or the GI symptoms. It is unclear what role, if any, the bitter taste and GI adverse events may have had in potential unblinding of patients to the treatment previously received in the double-blind treatment period.

FDA acknowledges that participation in the OLE study was not mandatory, and also notes that participation in the OLE may have been affected by outcomes in the double-blind phase of the study; therefore, these treatment groups may not be comparable in important demographics or disease characteristics which makes it unreliable to draw any conclusions on the basis of these comparisons. Given the significant number of patients who did not enroll in the OLE and the many patients who dropped out of the OLE study, it is difficult to interpret the functional endpoints at 48 weeks.

FDA notes that only 66% of the patients from the double-blind phase of the study enrolled in the OLE. The above table (Table 10) is misleading regarding the number of patients in the extended slope analysis at 48 weeks because it lists the total number of patients who initiated the double-blind study and not the number of patients who enrolled in the OLE. Of the 34 patients initially randomized to placebo (RP group) who enrolled in the OLE, only 19 patients remained in the study at Week 48. Of the 56 patients initially randomized to AMX0035 (RA group) who enrolled in the OLE, 36 patients had week 48 data on the ALSFRS-R (i.e., 55 out of the 135 mITT patients remained in the study at Week 48).

The duration of exposure for all patients in the OLE phase of the study are summarized in the below table.

AMX00356 Overall Exposure in OLE Phase

Duration of exposure (categories) n (%)	RA+SOC N=56	RP +SOC N=34	Combined OLE N=90
0 to ≤3weeks	2 (3.6)	5 (14.7)	7 (7.8)
>3 to ≤12 weeks	8 (14.3)	8 (23.5)	16 (17.8)
12 to ≤18 weeks	3 (5.3)	5 (14.7)	8 (8.8)
>18 to ≤21 weeks	4 (7.1)	0	4 (4.4)
>21 to ≤ 24 weeks	2 (3.6)	1 (2.9)	3 (3.3)
>24 to ≤27 weeks	8 (14.3)	1 (2.9)	9 (10)
>27 to ≤33 weeks	5 (8.9)	0	5 (5.5)
>33 to ≤48 weeks	7 (12.5)	4 (11.8)	11 (12.2)
>48 to ≤72 weeks	7 (12.5)	5 (14.7)	12 (13.3)
>72 to ≤ 96 weeks	3 (5.3)	2 (5.8)	5 (5.5)
> 96 weeks	7 (12.5)	3 (8.8)	10 (11.11)

RA = randomized to AMX3500 in double-blind; RP = randomized to placebo in double-blind

In addition, all of the aforementioned statistical concerns regarding the linearity assumption associated with the slope analyses of the CENTAUR Study also apply to the extended slope analyses from OLE Study. There was also significant attrition of patients due to patient discontinuations from the study throughout the 24 weeks of the OLE (48 weeks overall).

Furthermore, by Week 48 (Day 336), there were 23 deaths in the study (10 (21%) in RP group and 13 (15%) in RA group), which are ignored in this efficacy analysis; therefore, the analysis is likely biased. The joint rank analysis of this data at 48 weeks does not seem to reach significance (note that the joint rank is not slope based).

Given the high proportion of deaths, the missing data from discontinuations, and the other concerns noted above, the Applicant's extended slope analysis of the ALSFRS-R to Week 48 is not an interpretable analysis and is not conclusive of any treatment benefit.

4.4.1.2 Open-Label Phase: ATLIS Results

The Applicant's Position

The analysis comparing the difference in the slope (i.e., from the randomized controlled phase baseline through Week 48 overall) between the 2 treatment groups (RA vs RP) was statistically significant for the upper ATLIS (p=0.029) in favor of the RA group (Table 12) and for total ATLIS (p=0.05). Results on lower ATLIS were not significant, but were numerically in favor of AMX0035 treatment (p=0.23).

Table 12: Extended Slope Efficacy Analysis for Upper and Lower ATLIS Scores over 48 Weeks – mITT

Endpoint/ Timepoint	Estimate (SE), Points		Difference		
	RA + SOC (N=87)	RP + SOC (N=48)	Difference (SE)	95% CI	p-value
Extended Slope ATLIS Upper limb (48 Weeks)	19.83 (2.591)	12.06 (3.283)	7.77 (3.550)	0.80, 14.75	0.029
Extended Slope ATLIS Lower limb (48 Weeks)	25.24 (2.893)	20.48 (3.653)	4.76 (3.923)	-2.95, 12.47	0.23
Extended Slope ATLIS Total (48 Weeks)	22.84 (2.37)	16.65 (2.97)	6.19 (3.15)	-0.01, 12.38	0.050

Abbreviations: ATLIS = Accurate Test of Limb Isometric Strength; CI = confidence interval; mITT = modified intent to treat; RA = randomized to AMX3500 in the randomized controlled phase, participants who continued in the open-label phase received AMX0035 in the open-label phase; RP = randomized to placebo in the randomized controlled phase, participants who continued in the open-label phase received AMX0035 in the open-label phase; SE = standard error; SOC = standard of care.
Source: Table 14.2.1.5 OLE CSR Upper ATLIS (includes raw summary data)
Table 14.2.1.7 OLE CSR Lower ATLIS (includes raw summary data)

The FDA's Position:

FDA notes that the above concerns regarding the ALSFRS-R extended slope efficacy analysis also apply to the lack of interpretability of the extended slope analysis of the ATLIS scores at 48 weeks.

4.4.1.3 Open-Label Phase: SVC Results

The Applicant's Position

The analysis comparing the difference in the slope from the randomized controlled phase baseline through Week 48 overall between the 2 treatment groups (RA vs RP) was nominally significant for the SVC results (p=0.0372) in favor of the RA group (Table 14.2.1.11 OLE CSR).

Table 13: Extended Slope Efficacy Analysis for SVC – mITT

Analysis/ Timepoint	Estimate (SE), Points		Difference		
	RP + SOC (N=48)	RA + SOC (N=87)	Difference (SE)	95% CI	p-value
Extended Slope SVC (48 Weeks)	37.85 (4.427)	48.52 (3.356)	10.66 (5.103)	0.63, 20.69	0.0372

Abbreviations: CI = confidence interval; RA=randomized to AMX3500 in the main phase, subjects who continued in OLE received AMX0035 in the OLE; RP=randomized to placebo in the main phase, subjects who continued in OLE received AMX0035 in the OLE; SE = standard error; SOC = standard of care; SVC = slow vital capacity.

Source: Table 14.2.1.11 (includes raw summary data); note that results based on all available data are presented in Table 14.2.1.11.00 (includes raw summary data).

The FDA's Position:

FDA notes that the above concerns regarding the ALSFRS-R extended slope efficacy analysis also apply to the lack of interpretability of the extended slope analysis of the SVC scores at 48 weeks.

Applicant's Position:

- Participants originally randomized to AMX0035 showed sustained benefit of treatment across ALSFRS-R, ATLIS, and SVC over 48 weeks
- There appeared to be some evidence of crossover benefit as measured by the ALSFRS-R but this is hard to interpret for two reasons: (1) this analysis is a single crossover and participants are at different stages of disease during the initial 24 weeks and following 24 weeks and (2) there is substantial dropout during the open-label phase (as it continues out to over 3+ years) so results may be affected by missing data.

The FDA's Position:

FDA notes that the open-label extended slope analyses through Week 48 are not interpretable for the following reasons:

- Open-label nature of the extension study
- Potential for unblinding to original treatment due to bitter taste and adverse gastrointestinal effects of the drug
- High number of patients who did not continue into the OLE
- Substantial patient drop-out during the OLE
- Use of a slope analysis
- Ignoring of deaths in the analyses

4.5 Key Study Events including Overall Survival Through Week 132

The Applicant's Position

Key study events including death, tracheostomy, permanent assisted ventilation and hospitalization in the mITT population was listed as the second efficacy outcome in the open-label phase SAP. It should be noted that all of these analyses are analyzed comparing the group originally randomized to AMX0035 versus originally randomized to placebo. Events are included from initial randomization into AMX3500 through the completion date of the open-label phase (March 1st 2021). Because the majority of participants in the placebo group crossed over to active treatment 24 weeks after randomization, these results may be conservative and the delayed use of AMX0035 in the majority of the placebo group may have attenuated treatment differences.

4.5.1 Prespecified Time-to-event Outcome: Time to First Hospitalization, Death, or Death Equivalent

The composite time-to-event outcome of time to first death, hospitalization or permanent ventilation was analyzed through the end of the open-label phase on March 1, 2021. The results (Table 17) showed that there was a statistically significant increase in time to these key study events in the group originally randomized to active (RA) versus placebo (RP) (difference=4.8 months; HR=0.62, p=0.02) (Figure 9).

While death events were able to be collected even for participants who dropped out of the study (Section 4.5.2), hospitalizations and tracheostomies may not be collected after dropout. As such, there is some risk of missing data when hospitalizations and tracheostomies are included in the composite analysis.

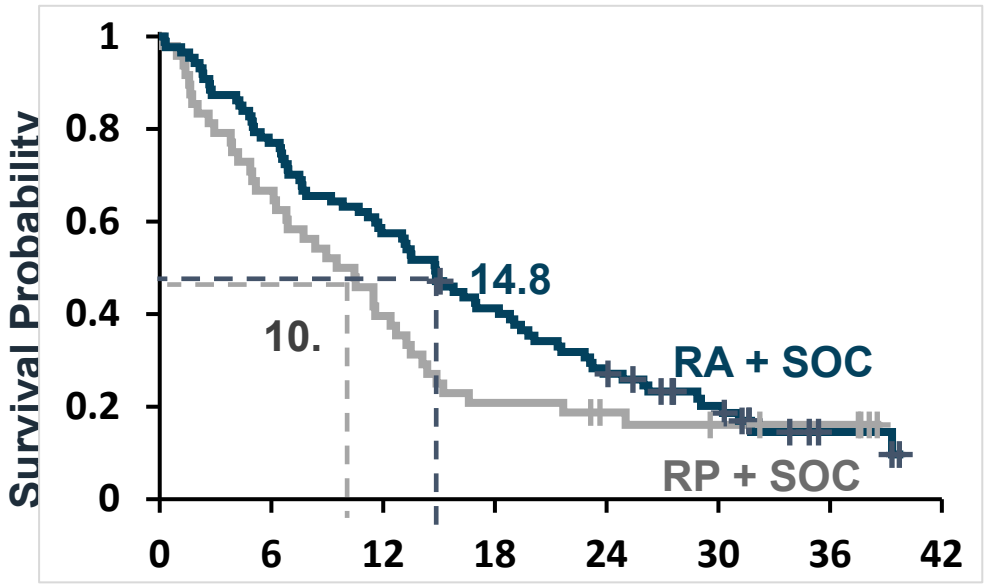
Additional discussion on the methodology of collecting death events and a discussion of ITT overall survival is included in Section 4.5.2.

Table 14: Key Study Events: Death, Tracheostomy, and First Hospitalization

Population and Outcome	Median Estimate (Months) RA+SOC	Median Estimate (Months) RP+SOC	Hazard Ratio [95%CI]	P-Value
mITT	**N=87**	**N=48**	**-**	**-**
Time to First Hospitalization, Death, or Death Equivalent	**14.8**	**10.0**	**0.615 [0.408, 0.925]**	**0.0196**

Abbreviations: CI = confidence interval; mITT=modified intent to treat.

Figure 9: Hospitalization / Death or Equivalent Survival Estimates – mITT



No. at Risk								
RA +SOC	87	67	50	35	23	13	3	0
RP + SOC	48	32	19	10	7	5	4	0

The FDA's Position:

FDA notes that the above composite survival analysis is difficult, if not impossible, to interpret given the number of dropouts during the OLE study. We have previously discussed the limitations of using tracheostomy and hospitalizations as an efficacy outcome measure.

Additionally, much of the survival data were collected through multiple vital status searches for death, including death records, obituaries, etc.; therefore, there is only limited 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. As outcomes such as tracheostomy and hospitalizations were not systematically collected in the OLE study, their inclusion as "death equivalents" in a composite survival analysis does not allow for reliable interpretation of these results.

Due to significant loss to follow-up on tracheostomy and permanent assisted ventilation events as noted above, the protocol-specified composite survival endpoint is very difficult to interpret reliably.

4.5.2 ITT Overall Survival Results

The Applicant's Position

While the mITT population was pre-specified in both SAPs as the analysis population, ITT is generally considered more appropriate in overall survival analyses in order to account for all death events in the study. Therefore, the ITT population was analyzed for overall survival and is presented below.

Survival analyses presented are analyzed from initial randomization into the AMX3500 trial through the final cutoff date of March 1st 2021 (Figure 10). An interim survival data readout using a cutoff date of July 20, 2020 was previously published (Paganoni, 2021). Overall survival was defined as all-cause mortality.

Survival analyses can often suffer from loss to follow up or missing death dates from participants. However, the survival data presented here has minimal missing data: the vital status and date of death were confirmed for 136 out of 137 participants randomized into the AMX3500 study as of the March 1st 2021 cutoff. Survival status was confirmed even on those participants who dropped out of the study through an evaluation of public records of deaths including the social security death index and state and city records.

The number of events included in analysis at each cutoff is presented in Table 15 together with the number of participants who were alive at the cutoff date. These participants who were still alive were administratively censored as of the cutoff date. All censoring, save one participant, was due to patients being alive at the data cutoff date.

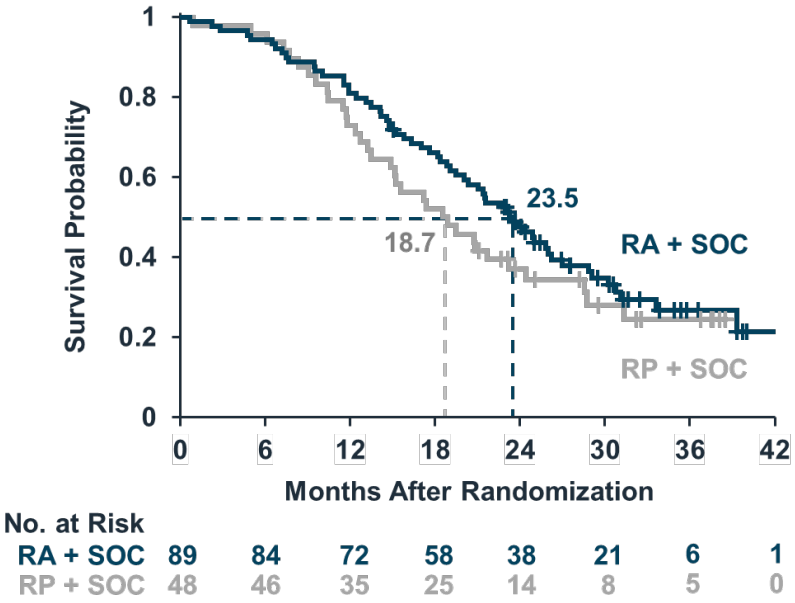
Table 15: Death Events – ITT Population

Cutoff Date	Death Events	Number of Participants alive/in follow-up as of cutoff date	Number of Participants lost to follow-up Prior to Cutoff Date
July 20th 2020	72	64	1
March 1st 2021	94	42	1

Note: Numbers may differ slightly with published literature as that information was based on interim data.

Overall survival in the ITT population is presented in Figure 10.

Figure 10 Survival Estimates – ITT



In this ITT OS analysis, patients randomized to AMX0035 led to a statistically significant survival benefit (median difference 4.8 months, HR=0.62, p=0.03).

While there are many ways to analyze the survival data given the extended treatment in the OLE, the most rigorous analysis is to measure overall survival in the entire ITT population, ignoring entry into the OLE and simply analyzing each treatment arm from initial randomization through the survival cutoff date of March 1st 2021.

It is possible this analysis underestimates the treatment benefit, given that the majority of placebo participants do receive delayed active treatment after 24 weeks. However, this analytical method preserves the randomization and is the most rigorous method of analyzing overall survival.

The FDA's Position:

FDA notes that while the OLE protocol and SAP included a composite endpoint based on survival, hospitalization, and tracheostomies in the hierarchy of efficacy endpoints, death alone was not included in this list of endpoints. Analyses of the three components of the composite survival endpoint were planned, but the death analysis was not given priority over the other two components of the composite (or the composite itself). The focus on death alone, and the submission of a new supplementary OLE survival SAP, occurred after preliminary survival analyses of data from the double-blind and OLE period had been viewed. The new SAP also added baseline ALSFRS-R as an additional covariate in the survival analyses (the results reported here in Table 15 are based on this model).

The Applicant had previously reported to the FDA at the Type C meeting in March 2020 that adding the baseline ALSFRS-R as a covariate reduced the p-value for the analysis of time to death or death equivalent from p=0.0621 (prespecified covariates of age and del-fs), to p=0.0380 with baseline ALSFRS-R as an additional covariate, based on the event cutoff after first unblinding of the study. This post hoc analysis may have influenced the subsequent decision to include baseline ALSFRS-R as an additional covariate in the new supplementary SAP for survival.

FDA notes that Figure 10 above demonstrates Overall Survival in the ITT. However, in the text following the figure, the Applicant has presented the ITT Overall Survival hazard ratio of 0.62, p= 0.03. Based on FDA review of the AMX0035OLE clinical study report, these values presented are from the Overall Survival analysis in the mITT population, not the ITT population. The Applicant's analysis of ITT Overall Survival, as depicted in Figure 10, produces a hazard ratio of 0.64, with a p-value of 0.0475.

Based on the supplementary SAP for survival analysis of time to death alone in the ITT population, with the prespecified likelihood ratio test, the p-value is 0.0518, with a corresponding hazard ratio of 0.64. Without the baseline ALSFRS-R specified as an additional covariate in the supplementary SAP for survival, the time to death p-value is 0.0453, also with a hazard ratio of 0.64.

Furthermore, there were 5 additional deaths captured with event dates after March 1, 2021, that were administratively censored due to lack of uniform follow up after March 1, 2021. However, if these deaths are included, the ITT time to death result hazard ratio increases to 0.70 with p=0.1109.

We also note that the analyses of the multiplicity-adjusted secondary survival endpoint in the double-blind period did not provide evidence of effects.

FDA also notes that many patients discontinued from the study due to ALS progression; therefore, it is unsurprising that survival was greater for patients who stayed in the study longer. However, the following table illustrates that some individual patients in the study who did not receive drug (randomized to placebo, no enrollment into OLE) survived equally as long as the longest surviving patients who received drug in the double-blind treatment phase. It is unclear how much of the survival benefit is by chance alone or disease heterogeneity, rather than by an ostensible effect of the drug.

Time on AMX0035 and Median Survival in Alive Patients (cut off March 1, 2021)

Duration (weeks)	N	Analysis Day (median)
0	3	1295
>0 - ≤ 24 wks	8	1119
>24 - ≤ 48 wk	8	1000
>48 - ≤ 72 wk	9	874
>72 - ≤96 wk	7	917
>96 wks	8	1237

The estimated 12-month, 15-month, 18-month, 21-month, and 24-month survival probabilities are also presented below in Table 16. The group originally randomized to AMX0035 had a higher estimated probability of survival.

Table 16 Survival Probabilities

	Survival Probability				
Time from Randomization	**12 months**	**15 months**	**18 months**	**21 months**	**24 months**
Treatment	**% surviving** (95% CI)	**% surviving** (95% CI)	**% surviving** (95% CI)	**% surviving** (95% CI)	**% surviving** (95% CI)
AMX0035	**80.90** (71.09, 87.66)	**71.91** (61.33, 80.06)	**66.20** (55.35, 75.01)	**58.21** (47.24, 67.68)	**47.62** (36.84, 57.62)
Placebo	**72.92** (57.97, 83.28)	**62.50** (47.28, 74.46)	**52.08** (37.20, 65.03)	**41.67** (27.72, 55.03)	**37.01** (23.53, 50.51)

The FDA's Position:

FDA is unclear as to why the Applicant has presented the above survival probability at the selected times in Table 16. The proposed plan intended to compare survival between the treatment groups over the entire follow-up period, and not at any prespecified times.

4.5.3 Overall Survival Results—Subgroups Analysis Based on Enrollment into Open Label Phase

The Applicant's Position

As discussed, it is possible that the ITT overall survival results underestimate the treatment benefit due to the crossover of some members of the placebo arm to drug after 24 weeks. To analyze this, a descriptive analysis is presented below which breaks the population into subgroups based on whether or not participants enrolled into the open label extension.

These groups were analyzed for survival in a Cox proportional hazards model. Covariate adjustments expected to be predictive for survival including age, baseline ALSFRS-R and pre-randomization progression rate (del-FS score) were included in the statistical model with the goal of reducing potential bias from baseline differences between groups. Median survival is presented from the Cox model to allow for adjustment for these important covariates.

This analysis should be considered descriptive and exploratory only as subgroups have the potential to introduce bias into survival analysis. In the descriptive analysis, participants who received longer exposure to AMX0035 were observed to have substantially longer survival.

Table 17: Relationship Between Duration of Exposure to AMX0035 and Time to Death

Randomization Group	Enrollment in the Open-label Phase	N		
			Mean Exposure to AMX0035 (Months)	Median Survival [95% CI] (Months)
Active	Yes	56	15.6	29.1 [24.4-not estimable]
Placebo	Yes	34	7.5	20.8 [17.2-27.0]

Active	No	33	2.7	17.4 [14.6-22.8]
Placebo	No	14	0	15.2 [12.4-24.9]

CI = confidence interval.
Source: Table 14.2.4.32

The FDA's Position:

FDA acknowledges that participation in the OLE study was optional and may have been affected by outcomes in the double-blind phase of the study; therefore, these treatment groups may not be comparable, which makes it unreliable to draw any conclusions on the basis of these comparisons.

4.5.4 Additional Key Study Event Outcomes

The Applicant's Position

The additional prespecified key study event outcomes from the OLE SAP are presented below in Table 18. All time to event analyses were found to be statistically significant in favor of the group originally randomized to active treatment. Hazard ratios were similar between all survival analyses. Results were similar in the ITT population.

Table 18: Key Study Events: Death, Tracheostomy, and First Hospitalization

	March 1, 2021 Data Cutoff			
	Median Survival Estimate (Months)			
Population and Outcome	**RA+SOC**	**RP+SOC**	**Hazard Ratio [95%CI]**	**P-Value**
mITT	**N=87**	**N=48**	**-**	**-**
Time to First Hospitalization	31.8	14.1	0.595 [0.355, 0.996]	0.0482
Time to Death	23.5	18.7	0.619 [0.399, 0.960]	0.0324
Time to Death or Death Equivalent	23.5	17.9	0.597 [0.387, 0.923]	0.0203

Abbreviations: CI = confidence interval; mITT=modified intent to treat.

The FDA's Position:

The time to death analysis in Table 18 above uses the mITT population, which excludes two drug arm deaths because they had no post-baseline ALSFRS-R assessments; however, they were randomized and dosed. The Applicant's same analysis done on the ITT population is shown in the table below.

The other rows in Table 18 may be unreliable given the high proportion of patients (34%) not participating in the OLE, as well as the significant loss to follow-up on hospitalization and death equivalent events.

Key Study Events: Death, Tracheostomy, and First Hospitalization (ITT)

	March 1, 2021 Data Cutoff			
	Median Survival Estimate (Months)		Hazard Ratio [95%CI]	P-Value
Population and Outcome	**RA+SOC**	**RP+SOC**		
ITT	**N=87**	**N=48**	**-**	**-**
Time to First Hospitalization	31.8	14.1	0.605 [0.362, 1.011]	0.0552
Time to Death	23.5	18.7	0.644 [0.416, 0.995]	0.0475
Time to Death or Death Equivalent	23.2	17.9	0.621 [0.403, 0.957]	0.0308

Source: Study AMX3500 OLECSR Page 61

Applicant's Position:

- Participants randomized to AMX0035 demonstrated longer overall survival from initial study baseline in an analysis of the full ITT population with mature follow up and nearly no missing data.
- The study met its prespecified mITT key study events outcome over long-term follow-up
- Results were consistent across composite analyses with permanent ventilation and hospitalization. Hazard ratios are generally consistent across outcomes which supports the robustness of the survival finding.

- The overall survival results are additionally notable considering that the majority of participants in the placebo group had the opportunity to receive delayed exposure to AMX0035 24-weeks after randomization. Placebo crossover may be expected to attenuate differences between the study groups.
- In the context of a positive primary endpoint, the ITT overall survival results with a substantial effect (as measured by the hazard ratio ~0.6) provides important confirmatory evidence of a treatment benefit.

The FDA's Position:

- The apparent survival benefit on time to death has a modest nominal p-value (0.0475) that is not persuasive, especially considering the exploratory nature of the analysis (see below).
- There was a significant proportion of the ITT population who did not participate in the OLE (**34% OLE non-participation [29% placebo and 37% AMX0035]).**
- Vital status sweeps were conducted to obtain death rates; however, due to the large number of dropouts, there is only limited information regarding clinical care after discontinuation from the study, including the possibility of tracheostomy, hospitalizations, and/or other experimental treatments that could potentially affect survival. Therefore, data on the composite survival endpoint are difficult to interpret.
- Analyses of efficacy in OLE periods are typically considered exploratory in nature. Furthermore, while the OLE protocol and SAP included a composite endpoint based on survival, hospitalization, and tracheostomies in the hierarchy of efficacy endpoints, death alone was not included in this list of endpoints. Analyses of the three components of the composite survival endpoint were planned, but the death analysis was not given any priority over the other two components of the composite (or the composite itself). The focus on this endpoint, and the submission of a new supplementary OLE SAP for survival, occurred after preliminary survival analyses. Some alternative analyses of time to death provide less convincing results.
- The overall lack of statistical persuasiveness of the survival benefit, as well as the lack of replication of the results raises concern that the modest survival benefit seen may potentially be due to underlying disease heterogeneity rather than an effect of the drug.

4.6 Correlation and Collective Strength of Evidence

The Applicant's Position

ALSFRS-R, ATLIS total score, SVC and overall survival time are only modestly correlated in ALS. These correlations are generally around or below 20% (Table 19). As such, each of these outcomes provide *independent* information on the treatment benefit.

Table 19: Correlation of Measures of ALS Progression

	ALSFRS-R	SVC	ATLIS	Overall Survival
ALSFRS-R		13%	27%	19%
SVC			5%	16%
ATLIS				20%
Overall Survival				

Bayesian Hierarchical Analysis was conducted to evaluate the chance that the effects across ALSFRS-R, SVC, ATLIS and overall survival could occur by chance alone. This analysis attempted to remove any correlated elements of the outcomes and evaluate the independent contribution from each outcome. The analysis determined that the likelihood of a type I error with the concordant effect observed on ALSFRS-R, SVC, ATLIS and Overall Survival was minimal ($p<0.001$).

Applicant's Position

- The Bayesian hierarchical analysis, while exploratory, attempts to quantify the overall strength of evidence in a quantitative rather than qualitative fashion
- This analysis suggests a very low likelihood of a chance outcome in AMX3500. Ultimately, this finding suggests that seeing consistent effects across all these outcomes with an ineffective therapy would be extremely unlikely.

The FDA's Position:

FDA has major concerns with this Bayesian analysis and does not believe that any weight should be put on these calculations in considering the evidence of effectiveness. FDA concerns with the analysis include, but are not limited to the following:

- This analysis is post hoc with emphasis on a selected set of endpoints that were determined after seeing the trial results (e.g., the biomarker endpoint was higher in the hierarchy than survival but is omitted so this does not respect the prespecified hierarchy), and there was no plan to collectively examine these selected endpoints.
- This calculated "likelihood of a type I error" is paradoxical; it decreases as more endpoints are added, even if the estimated treatment effect for an added endpoint is zero or in the wrong direction.
- The analysis does not give the primary endpoint due prominence that is required per the prespecified study objective and multiple testing approach, and also may not capture false positives among the rest of the multiple endpoints that were prespecified for testing.
- Calculation of such a post hoc defined p-value or false positive probability is inadequate for quantifying the strength of evidence of this trial. The strength of evidence depends on many important factors, such as the clinical relevance of the endpoints and estimated effects, the quality of trial conduct (e.g., issues with randomization and dropout), and the sensitivity of results to violations in assumptions or limitations of the data.

4.7 Ongoing Studies

The Applicant's Position

Amylyx is committed to continued study of AMX0035 allowing us to provide physicians and their patients with the most robust and informative information possible on this therapy and we continue to study AMX0035 in multiple studies.

Table 20 lists the ongoing clinical studies being conducted by Amylyx. These studies are to further supplement our current efficacy and safety knowledge.

Table 20 Ongoing Clinical Studies

Study	Summary of objectives
A35-003	Compassionate use protocol – extended exposure to AMX0035.
A35-004	Phase III, multicenter, placebo-controlled Study in up to 600 (recruitment primarily in Europe) people living with ALS
A35-005	PK / PD Study in people living with ALS

Study	Summary of objectives
A35-006	Provide expanded access to AMX0035 for the treatment of people living with ALS who are ineligible for other clinical studies.

Applicant's Position:

- The applicant is committed to continuing to invest in ALS research and in learning about AMX0035. In line with this, the applicant is diligently conducting an additional randomized placebo-controlled study primarily in Europe. This study is already actively recruiting participants.
- The applicant estimates this study, which follows participants for 48 weeks, will read out in 2024. The applicant is using all appropriate means to complete this study as expeditiously as possible.
- AMX3500 met its prespecified primary outcome and additionally demonstrated a survival benefit in the ITT population with hazard ratio of approximately 0.6. Multiple sensitivity analyses confirmed the robustness of the primary outcome (ALSFRS-R). Additional secondary outcomes including SVC and ATLIS showed consistent effects with the ALSFRS-R results in both the randomized phase and long-term follow up. The overall evidence suggests a very low likelihood of a false positive result.
- In the context of a rare and rapidly fatal disease, it is not appropriate to delay a therapy for approximately 2 years which has demonstrated a benefit on function and survival. The evidence currently accumulated supports the utilization of this therapy for people with ALS. Additional evidence will continue to be generated in the future which can guide clinical and regulatory decision making as it becomes available.

The FDA's Position:

The Agency acknowledges the continued need for treatments for patients with ALS that are safe and effective.

In summary, FDA notes the following concerns regarding the ability of the available evidence presented by the Applicant to serve as a single study (or single study plus confirmatory evidence) to establish effectiveness of AMX0035 in the treatment of ALS.

- The results from Study AMX3500 on the primary endpoint are borderline statistically significant and may not be sufficiently persuasive to allow an effectiveness determination based on a single study.

- There are issues with study conduct and analysis assumptions, such that the results are not robust. Some of the analysis issues were raised with the Applicant in IND correspondence and have been communicated in the ALS guidance.

- Issues include the integrity of randomization, handling of deaths in the primary analysis, missing data assumptions, and assumptions of linearity over time in the treatment effect. Many sensitivity analyses to address these issues provide less persuasive results than the primary analysis. Also, a sensitivity analysis with a more plausible missing-not-at-random assumption may provide less favorable results.

- The secondary endpoint results are not compelling or supportive of the primary endpoint.

- The OLE survival results are not persuasive. There was no evidence of effects on survival (or survival-related endpoints) in prespecified, multiplicity-adjusted analyses of the 24- week double-blind period, the nominal p-values from the OLE survival analyses are borderline, and there are challenges with interpreting the OLE survival analysis results due to issues such as the open-label design and the exploratory nature of the analyses.

5 CLINICAL SAFETY

5.1 Treatment Exposure

5.1.1 Adults with ALS (AMX3500)

The Applicant's Position

Exposure to study drug in the randomized controlled phase is shown in Table 21.

Table 21: Extent of Exposure to Study Medication, Safety Population (Study AMX3500)

Parameter	Randomized Controlled Phase	
	AMX0035 (N=89)	Placebo (N=48)
Duration of Exposure (weeks)		
Mean (SD)	19.7 (7.89)	21.5 (5.82)
Median	23.9	23.9
Min, Max	0.6, 31.6	1.0, 25.9
Number of Participants (n [%])		
Increased Dose to 2 Sachets	79 (88.8)	45 (93.8)

Abbreviations: SD = standard deviation; SE = standard error
Source: AMX3500 CSR, Table 14.1.2

The FDA's Position:

FDA acknowledges the total extent of exposure to the drug for patients in the 24-week double-blind, randomized controlled study.

5.2 Overview of Adverse Events

The Applicant's Position

AEs were reported for 132 of 137 (96.4%) participants during the randomized controlled phase of the study, with similar incidences across the 2 treatment groups (Table 22). The number of participants with at least 1 adverse event (AE) in both the AMX0035 and placebo treatment groups were similar. In both groups, the majority of AEs were assessed as nonserious and mild or moderate in severity.

Serious adverse events (SAEs) were reported in a lower proportion of participants in the AMX0035 group (12.4%) compared with the placebo group (16.7%); this difference was largely driven by a lower incidence of respiratory events in the AMX0035 group compared with the placebo group.

Seven participants died during the randomized controlled phase of the study, and the incidences were similar between AMX0035 (5.6%) and placebo (4.2%). Cause of death for most of these participants was consistent with manifestations or complications of ALS, and none of the deaths was assessed as study medication related.

Table 22: Overall Summary of Adverse Events, Safety Population (Study AMX3500)

	Randomized Controlled Phase	
Assessment	**AMX0035 (N=89) n (%)**	**Placebo (N=48) n (%)**
Participants With at Least 1 AE	86 (96.6)	46 (95.8)
Participants With at Least 1 Severe AE	17 (19.1)	11 (22.9)
Participants With at Least 1 SAE	11 (12.4)	8 (16.7)
Participants With at Least 1 Fatal AE	5 (5.6)	2 (4.2)
Participants Who Discontinued Study Drug Due to AE	18 (20.2)	5 (10.4)

Abbreviations: AE = adverse event; SAE = serious adverse event
Source: AMX3500 CSR Table 14.3.1.1 and Table 14.3.1.2, and SCS Table T14.3.1.1 and T14.3.1.2

The safety profile in the open-label phase, with all participants administered AMX0035, was consistent with the AMX0035 randomized phase but is not presented in this document.

The FDA's Position:

FDA acknowledges that the overall incidence of AEs, SAEs, and deaths were similar among patients receiving AMX0035 and patients receiving placebo. However, approximately twice as many patients on treatment discontinued the study drug due to an AE (20.2%) than those patients receiving placebo (10.4%).

5.3 Common Adverse Events

The Applicant's Position

Overall, the common AEs observed during both the 24-week placebo-controlled randomized controlled phase and the additional 24-week open-label phase were largely consistent with typical symptoms related to natural ALS progression (e.g., muscular weakness, falls, constipation, sialorrhea, respiratory complications) or the known safety profile of the active pharmaceutical ingredients of AMX0035 under study (e.g., gastrointestinal complaints, including nausea and diarrhea) (Table 23).

Table 23: Adverse Events Occurring in ≥10% of Participants in Either Group, Safety Population (Study AMX3500)

MedDRA SOC Preferred Term	Randomized Controlled Phase	
	AMX0035 (N=89) n (%)	Placebo (N=48) n (%)
Participants With at Least 1 AE (n [%])	**86 (96.6)**	**46 (95.8)**
Fall	25 (28.1)	18 (37.5)
Diarrhoea	19 (21.3)	8 (16.7)
Muscular weakness	18 (20.2)	9 (18.8)
Nausea	16 (18.0)	6 (12.5)
Headache	13 (14.6)	11 (22.9)
Constipation	12 (13.5)	12 (25.0)
Viral upper respiratory tract infection	10 (11.2)	2 (4.2)
Salivary hypersecretion	10 (11.2)	1 (2.1)
Dyspnoea	9 (10.1)	4 (8.3)
Dizziness	9 (10.1)	2 (4.2)
Neck pain	2 (2.2)	5 (10.4)

Abbreviations: MedDRA = Medical Dictionary for Regulatory Activities; SOC = system organ class
Percentages are based on the number of participants in each treatment group.
Source: AMX3500 CSR Table 14.3.1.3 and Table 14.3.1.4; SCS Table T14.3.1.3 and T14.3.1.4.

The FDA's Position:

FDA notes the TEAEs that occurred in ≥10% of the participants and ≥1% more than placebo were diarrhea, muscular weakness, nausea, viral respiratory tract infection, salivary hypersecretion, dyspnea, dizziness, abdominal pain, and fatigue.

FDA conducted an independent analysis of TEAEs that occurred in Study AMX3500. In addition to the common TEAEs noted above in Table 23, abdominal pain and fatigue were also noted to occur in ≥10% of the participants in the FDA analysis.

To avoid missing any potential safety signals due to splitting of preferred terms, FDA grouped together some of the TEAEs, including the following terms: abdominal discomfort, abdominal pain, abdominal upper pain, and abdominal distension were combined into "Abdominal Pain" and asthenia, fatigue, and malaise were combined into "Fatigue", which is likely the cause of the discrepancy between the FDA analysis and the Applicant's summary table of common TEAEs.

Additionally, the exact percentages of incidence of other TEAEs may vary because of recoding of other similar preferred terms.

FDA agrees with the Applicant's conclusion that there were no significant safety findings of concern in the treatment group compared to the placebo arm.

5.4 Adverse Events ≥ Grade 3

The Applicant's Position

Adverse events ≥ Grade 3 (i.e., severe intensity) were generally isolated occurrences in individual participants and consistent with the manifestations and complications of underlying ALS (e.g., respiratory failure, falls) or the known safety profile of PB and/or TURSO (e.g., gastrointestinal complaints of diarrhea) (Table 24).

Table 24: Severe Adverse Events Occurring in More Than 1 Participant, Safety Population (Study AMX3500)

Preferred Term	Randomized Controlled Phase	
	AMX0035 (N=89) n (%)	Placebo (N=48) n (%)
Participants with at least 1 severe AE	17 (19.1)	11 (22.9)
Respiratory failure	2 (2.2)	3 (6.3)
Diarrhoea	1 (1.1)	1 (2.1)
Bacteraemia	1 (1.1)	1 (2.1)
Fall	1 (1.1)	1 (2.1)
Device dislocation	1 (1.1)	1 (2.1)

Abbreviations: MedDRA = Medical Dictionary for Regulatory Activities; SOC = system organ class
Percentages are based on the number of participants in each treatment group.
Source: AMX3500 CSR Table 14.3.1.7; SCS Table T14.3.1.7.

The FDA's Position:

FDA review of the severe TEAEs did not identify any safety signals of concern.

5.5 Serious Adverse Events

The Applicant's Position

The reported SAEs in the ALS clinical development program were generally isolated occurrences in individual participants that were consistent with the manifestations and complications of underlying ALS (Table 25).

In the randomized controlled phase, most SAEs were single events with the only SAEs that occurred in more than 1 participant being respiratory failure, bacteremia and nephrolithiasis.

Table 25: Serious Adverse Events, Safety Population (Study AMX3500)

Preferred Term	Randomized Controlled Phase	
	AMX0035 (N=89) n (%)	Placebo (N=48) n (%)
Participants with at least 1 SAE	11 (12.4)	8 (16.7)
Respiratory failure	2 (2.2)	3 (6.3)
Dyspnoea	1 (1.1)	0
Pulmonary embolism	0	1 (2.1)
Respiratory arrest[a]	1 (1.1)	0
Bacteraemia	1 (1.1)	1 (2.1)
Catheter site infection	0	1 (2.1)
Pneumonia	1 (1.1)	0
Implant site cellulitis	1 (1.1)	0
Pneumonia respiratory syncytial viral	1 (1.1)	0
Pelvic fracture	0	1 (2.1)
Skull fracture	1 (1.1)	0
Stoma site haemorrhage	1 (1.1)	0
Subdural haematoma	1 (1.1)	0
Diverticular perforation	1 (1.1)	0
Pneumoperitoneum	1 (1.1)	0
Nephrolithiasis	1 (1.1)	1 (2.1)
Vision blurred	1 (1.1)	0
Device dislocation	0	1 (2.1)

Abbreviations: MedDRA = Medical Dictionary for Regulatory Activities; SAE(s) = serious adverse events; SOC = system organ class
Percentages are based on the number of participants in each treatment group.
a. Respiratory arrest was reported as secondary to aspiration; the event was fatal.
Source: AMX3500 CSR Table 14.3.1.6, Part 1, and SCS Table T14.3.1.6 Part 1

The FDA's Position:

FDA review of the serious adverse events (SAEs) did not identify any safety signals of concern.

FDA agrees with the Applicant's conclusion that the majority of the SAEs noted in the study were consistent with complications of ALS and not drug-related.

5.6 Deaths

The Applicant's Position

Seven participants died during the randomized controlled phase of the study, 5 participants (5.6%) in the AMX0035 group and 2 participants (4.2%) in the placebo group (Table 26). The majority of deaths in the randomized controlled phase were from respiratory failure/arrest (3 participants in the AMX0035 and 2 participants in the placebo group). Other causes of death in the AMX0035 group included subdural hematoma and diverticular perforation in one participant each. None of the deaths was assessed as study medication related by the Investigator.

Table 26: Adverse Events with Fatal Outcomes, Safety Population (Study AMX3500)

MedDRA SOC Preferred Term	Randomized Controlled Phase	
	AMX0035 (N=89) n (%)	Placebo (N=48) n (%)
Participants with a fatal AE	5 (5.6)	2 (4.2)
Respiratory failure	2 (2.2)	2 (4.2)
Respiratory arrest	1 (1.1)	0
Diverticular perforation	1 (1.1)	0
Subdural haematoma	1 (1.1)	0

Abbreviations: MedDRA = Medical Dictionary for Regulatory Activities; SOC = system organ class; AE = adverse event
Source: AMX3500 CSR Table 14.3.1.9; SCS Table T14.3.1.9.

The FDA's Position:

FDA reviewed the narratives of the reported deaths during the study; the majority of the deaths appear to be largely related to ALS progression and not secondary to treatment. A single patient died from diverticular perforation, which could not be ruled out as potentially related to use of the medication; however, that patient only received 5 doses of the drug, so it seemed less likely to be drug-related.

Fifteen additional deaths were reported during the open-label phase of the study. The causes of death during the open-label phase were respiratory failure (10 participants), disease progression (2 participants), and 1 participant each for pneumonia aspiration, amyotrophic lateral sclerosis, and cardiac arrest.

Overall, a review of the deaths does not identify any safety signals of concern attributable to treatment with AMX0035.

5.7 Summary of Other Adverse Events

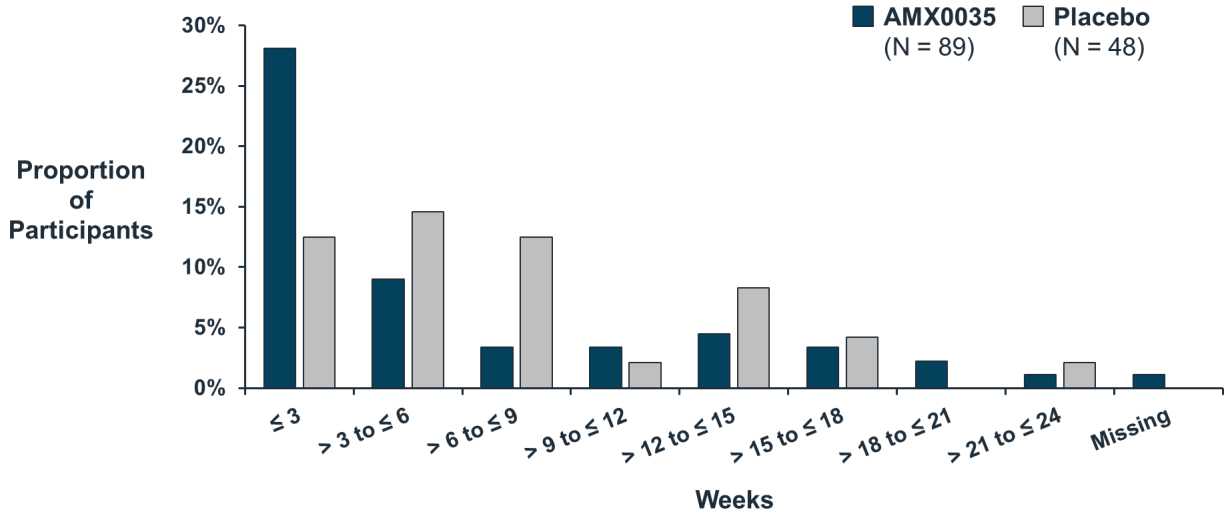
The Applicant's Position

Gastrointestinal Events:

The most common adverse events in Study AMX3500 were reported in the SOC category of gastrointestinal disorders among 65% (89/137) of participants: 66.3% (59/89) in the AMX0035 group and 62.5% (30/48) in the placebo group. The most frequent AE by preferred term (PT) was diarrhea in 21.3% (19/89) in the AMX0035 group and 16.7% (8/48) in the placebo group followed by the AE (PT) of nausea in 18% (16/89) of participants in the AMX0035 group compared with 12.5% (6/48) in the placebo group in the randomized controlled phase. These events were generally mild or moderate in severity.

Gastrointestinal adverse events were reported more frequently in the AMX0035 group than in the placebo group during the first 3 weeks, with nausea, diarrhea, and abdominal pain accounting for most of the events; thereafter, these events were reported less frequently in the AMX0035 group than in the placebo group for the remainder of the trial (Figure 11).

Figure 11: Incidence of Gastrointestinal Adverse Events by Trial Week: Safety Population



The observed adverse events (i.e., gastrointestinal complaints, including abdominal pain/discomfort, diarrhea, and nausea) are consistent with the known safety profile of the active pharmaceutical ingredients of AMX0035.

To help mitigate these symptoms, AMX0035 is administered once a day for the first three weeks of treatment.

The FDA's Position:

FDA notes the increase in gastrointestinal (GI) adverse events during the initial 3 weeks of treatment. The acute onset and transient nature of the GI symptoms raise concern for the potential for unblinding of patients during the study, as well as upon transition to the open-label phase of the study.

GI adverse events were listed in the informed consent form, which may have alerted patients to the treatment they were receiving. FDA notes that in the first three weeks of the double-blind study, 32.6% of patients in the treatment arm and 20% of patients in the placebo arm reported GI adverse events.

Psychiatric Events:

People living with ALS are known to be at higher risk for depression (Roos 2016). Review of AEs in the Psychiatric Disorders SOC in the Study AMX3500 (randomized controlled phase) suggest treatment with AMX0035 does not contribute to worsening of depression symptoms or suicidality that can be associated with ALS. Depression was similar between groups as 2 (2.2%) of AMX0035-treated participants and 1 (2.1%) placebo-treated participant reported depression during the randomized controlled phase.

In addition to a higher risk for depression, a higher risk for suicidality is also a known risk for people living with ALS; therefore, Columbia-Suicide Severity Rating Scale (C-SSRS) questionnaires were administered during the randomized controlled phase. Over the course of the randomized controlled phase, responses on C-SSRS questionnaires trended similarly in the AMX0035 and placebo groups. At Baseline, the C-SSRS questionnaire found ≥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. One subject each in the AMX0035 and placebo group also reported experiencing suicidal behavior. After initiation of treatment, the percentage of subjects reporting suicidal ideation decreased in both treatment groups, with no subject reporting suicidal behavior in either treatment group by Week 12.

Cardiac Events:

During the randomized (2:1 AMX0035 to placebo), placebo-controlled phase, there were numerically more cardiac events in the AMX0035 group versus the placebo group (7 versus 0 respectively); however, this difference was not statistically significant.

Specifically, of the 89 participants treated with AMX0035, 2 (2.2%) events of atrial fibrillation were noted, 2 (2.2%) events of palpitations, 1 (1.1%) event of atrioventricular block, 1 (1.1%) event of left bundle branch block, and 1 (1.1%) event of tachycardia were reported.

One of the two events of atrial fibrillation occurred after respiratory arrest and cardiac resuscitation so would most likely be related to these events, and the other event occurred in an elderly participant, with high BMI and hypertension, all significant risk factors for atrial fibrillation. The investigators did not believe these events were related to AMX0035.

The other events (palpitations, AV block, left branch bundle block and tachycardia) were of low clinical significance.

All of these events were reviewed by two independent cardiologists who both determined that there was not significant evidence of cardiac risk for AMX0035. A detailed review of cardiac events included a review of participant medical history, concomitant medications, study medication administration history, results of all recorded ECGs, and narratives, concluded that the incidence of cardiac events in this randomized controlled phase in 137 ALS participants was low and unlikely to be treatment related.

The FDA's Position:

FDA agrees with the Applicant's above assessment of the gastrointestinal, psychiatric, and cardiac events that occurred during the study and finds no significant safety concerns related to the use of the drug in this population.

5.8 Summary of Other Safety Areas of Interest

The Applicant's Position

Overall, in Study AMX3500, there were no clinically relevant trends for changes over time in either treatment group in respiratory rate, systolic/diastolic blood pressure, heart rate, or body temperature.

In addition, in Study AMX3500, there were no statistically significant differences between treatment groups with respect to the proportions of subjects with abnormalities in the various physical body systems (including neurological) examined. Physical examination results were reflective of the relatively older adult ALS population studied, and there were no unexpected trends in physical examination findings observed over the course of study.

5.9 Safety Summary and Conclusion

The Applicant's Position

Overall, single sachets of AMX0035 (comprised of 3 g PB and 1 g TURSO) administered up to twice daily were well-tolerated in the ALS population in AMX3500.

The AE profiles for the AMX0035 and placebo treatment groups were similar. The incidence of AEs was similar between treatment groups. In both groups, the majority of AEs were assessed as nonserious, mild or moderate in severity.

Overall, clinical safety laboratory results, vital signs, electrocardiograms (ECGs), physical (including neurological) examination findings did not find trends of clinical significance.

Applicant's Position – Safety:

- AMX0035 is well-tolerated with a favorable safety profile.
- Serious reactions were rare, and common AEs were mild or moderate and manageable.
- Numerically fewer serious adverse events were observed in the AMX0035 group as compared with the placebo group.
- GI events (generally nausea, diarrhea) were more frequent in the AMX0035-treated group in the first 3 weeks of treatment. Constipation was more frequent in the placebo-treated group.
- While there were numerically more cardiac events in the AMX0035 arm, a detailed review by two independent cardiologists of individual events determined that most events were of limited clinical significance or had a likely alternative cause and were unlikely to be treatment related.

The FDA's Position:

- 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.
- The number of participants 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.
- Many of the 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.
- GI-related TEAEs occurred in the first 3 weeks of treatment with concern for potential unblinding of patients.
- Overall, AMX0035 appears generally safe and well-tolerated.

6 CONCLUSIONS

6.1 Benefits

The Applicant's Position

Amylyx designed AMX0035, a combination of PB and TURSO, to simultaneously target the ER stress and mitochondrial dysfunction involved in ALS, thereby reducing or preventing downstream cell death. The administration of AMX0035 orally (or via feeding tube) allows for individuals to take the medicine themselves and avoid additional intravenous or other routes of administration that further impair their quality of life.

AMX0035 met its prespecified primary outcome in a randomized, placebo-controlled study in people with ALS. Specifically, AMX0035, over 24 weeks, demonstrated clinically meaningful as well as statistically significant benefits in ALSFRS-R (primary efficacy endpoint) compared with placebo. The ALSFRS-R result was consistent across multiple sensitivity analyses. Overall survival, ventilation, and hospitalization were assessed over long-term follow-up and demonstrated significant improvement in participants treated with AMX0035. Furthermore, the benefit of AMX0035 was maintained during a 48-week analysis of ALSFRS-R and additional secondary outcomes showed consistent findings at week 24 and 48.

Importantly, the positive effect of AMX0035 on slowing disease progression and improving survival time was conserved in pre-planned sensitivity analyses that corrected for the effects of concomitant use of ALS standard of care (i.e., riluzole and/or edaravone) in which 77% of participants were taking riluzole and/or edaravone.

6.2 Risks

The Applicant's Position

The main side effects noted with AMX0035 administration were gastrointestinal (e.g., nausea and diarrhea). After 2-3 weeks of AMX0035 administration, the GI AEs generally subsided. Other adverse events did not show clear or consistent trends attributable to AMX0035.

Overall, there is no evidence that AMX0035 resulted in an increase in clinically significant safety events or increased safety risk in this participant population.

6.3 Benefit-Risk Summary

The Applicant's Position

There remains a critical need for a new treatment for people living with ALS who face a rapid onset of morbidity and mortality despite currently approved therapies. Amylyx designed AMX0035 as a combination of sodium phenylbutyrate and taurursodiol to simultaneously target the ER stress and mitochondrial dysfunction involved in ALS, thereby reducing or preventing downstream neuron death. The administration of

AMX0035 orally (or via feeding tube) allows for individuals to take the medicine themselves and avoid additional intravenous or other routes of administration that further impair their quality of life.

Analyses of AMX0035's efficacy over 24 weeks demonstrated clinically meaningful slowing of ALS (function, strength and breathing) as well as statistically significant benefits in ALSFRS-R compared with placebo (both combined with standard of care). Results were consistent across multiple outcomes and analyses. Furthermore, the benefit of AMX0035 was maintained during a 48-week analysis of ALSFRS-R and additional secondary outcomes. Additionally, overall survival, ventilation, and hospitalization were assessed over long-term follow-up and demonstrated significant improvement in participants treated with AMX0035.

AMX0035 is well tolerated with a favorable safety profile. The main side effects noted with AMX0035 administration were nausea and diarrhea, which generally subsided after 2-3 weeks of AMX0035 treatment. There was not substantial evidence of any significant safety liability with AMX0035 administration.

The Sponsor continues to study AMX0035 including in a randomized, placebo-controlled study primarily conducted in Europe which is already recruiting and will provide further experience with AMX0035 in the post-marketing setting.

Data demonstrate that AMX0035 has a favorable benefit-risk profile which supports current utilization of this treatment for this debilitating and rapidly fatal disease. AMX0035 is the first therapeutic to show a benefit on both survival and function in ALS. In the context of a rare, rapidly progressing and life-threatening disease with high unmet medical need, the efficacy and safety demonstrated in CENTAUR support approval of AMX0035 for the treatment of ALS.

The FDA's Position:

- FDA acknowledges the ongoing unmet need for patients living with ALS.
- FDA notes that although the Applicant claims the CENTAUR study met its prespecified primary endpoint, the study demonstrated only a modest p-value using non-preferred analysis methods that ignore the loss of data due to patient deaths during the study and relied on a questionable linearity assumption of the ALSFRS-R over time. There was also a moderate proportion of missing data and a randomization implementation problem such that the first 18 patients in a row received the drug, which reduce the persuasiveness of the study.
- Additional concerns regarding potential for functional unblinding due to bitter taste and GI adverse events as well as post-baseline initiation of concomitant FDA-approved treatments in this small sample size further weaken the statistical robustness of the treatment benefit reported by the Applicant after 24 weeks of treatment.
- The secondary endpoints are not supportive of any benefit seen on the primary endpoint.
- The open-label results for the composite survival endpoint are not persuasive due to the small number of patients continuing into the OLE, as well as large number of drop-outs with loss to follow-up during the OLE. Survival data collected through a vital status sweep with limited information regarding the clinical care 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.
- The open-label results for survival alone are not persuasive. There was no evidence of effects on survival (or survival-related endpoints) in pre-specified, multiplicity-adjusted analyses of the 24- week double-blind period, the nominal p-values from the OLE survival analyses are borderline, and there are challenges with interpreting the OLE survival analysis results due to issues such as the open-label design and the exploratory nature of the analyses.
- The FDA draft guidance on substantial evidence states: "Reliance on a single, large, multicenter trial to establish effectiveness should generally be limited to situations in which the trial has demonstrated a clinically meaningful and statistically very persuasive effect on mortality." In this single trial, there are questions about the statistical persuasiveness of the results for the variety of reasons stated above and earlier in the document.
- FDA acknowledges that AMX0035 appears to be relatively safe and well-tolerated; the most common adverse events were gastrointestinal events (i.e., nausea and diarrhea) that tended to improve after a few weeks of treatment.

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