AMX0035 FOR THE TREATMENT OF AMYOTROPHIC LATERAL SCLEROSIS (ALS)

AMYLYX BRIEFING DOCUMENT

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PERIPHERAL AND CENTRAL NERVOUS SYSTEM DRUGS ADVISORY COMMITTEE BRIEFING MATERIALS: AVAILABLE FOR PUBLIC RELEASE

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List of Abbreviations

Abbreviation	Definition
AB	Amyloid beta
AD	Alzheimer's disease
AE	Adverse event
ALS	Amyotrophic Lateral Sclerosis
ALSFRS-R	ALS Functional Rating Scale-Revised
APIs	Active pharmaceutical ingredients
APOE ε4	Apolipoprotein E gene ε4 allele
ATLIS	Accurate Test of Limb Isometric Strength
BID	Twice daily
CFR	Code of Federal Regulations
CI	Confidence interval
CNS	Central nervous system
CSF	Cerebrospinal fluid
CSR	Clinical study report
Del-FS	Change in functional score (rate of disease progression)
ELISA	Enzyme-linked immunosorbent assay
ENCALS	European Network for the Cure of ALS
EU	European Union
FABP3	Fatty acid binding protein 3
FDA	Food and Drug Administration
FDG	Fluorodeoxyglucose
FPI	First patient in
FVC	Forced vital capacity
GDS	Geriatric Depression Scale
GFAP	Glial fibrillary acidic protein
GI	Gastrointestinal
IL	Interleukin
ITT	Intention to treat
IQR	Interquartile range
IV	Intravenous
JAMA	Journal of the American Medical Association
LCM	Late-cycle Meeting
LPLV	Last Participant Last Visit
MAA	Marketing Authorisation Application
MCI	Mild cognitive impairment
MCP-1	Monocyte chemoattractant protein-1
MIND	Massachusetts General Institute for Neurodegenerative Disease
mITT	Modified intention to treat

MMP-10	Matrix metalloproteinase 10
MMRM	Mixed measures
MoCA	Montreal Cognitive Assessment
mOS	Median overall survival
MRI	Magnetic resonance imaging
NDA	New drug application
NEALS	Northeast ALS Consortium
NfL	Neurofilament light chain
NOC/c	Notice of Compliance with Conditions
24-OHC	24S-hydroxycholesterol
8-OHdG	8-hydroxy-2-deoxyguanosine
OS	Overall Survival
p-tau	Phosphorylated tau
PB	Phenylbutyrate
PDUFA	Prescription Drug User Fee Act
PET	Positron emission tomography
PRO-ACT	Pooled Resource Open-Access ALS Clinical Trials
PSM	Propensity score matching
QD	Once daily
RCP	Randomized controlled phase
RPSFTM	Rank Preserving Structural Failure Time Model
RWE	Real world evidence
SD	Standard deviation
SE	Standard error
sIR	Soluble insulin receptor
SOC	Standard of care
SVC	Slow vital capacity
TURSO	Taurursodiol
US	United States
vMRI	Volumetric
VOD	Veno-occlusive disease
YKL-40	Inflammatory glycoprotein (also known as CHI3L1)

INTRODUCTION (AMYLYX)

An Advisory Committee meeting to discuss AMX0035 for the treatment of ALS occurred on 30 March 2022. This briefing book builds upon the evidence for the effectiveness of AMX0035 and focuses on additional confirmatory evidence and supportive new analyses to further support the conclusion that AMX0035 meets the standard of substantial evidence of effectiveness (FDA 2019 Guidance).

Executive Summary

This briefing document focuses on the application of the substantial evidence of effectiveness standard (FDA 2019 Guidance) to AMX0035 for the treatment of ALS based on one adequate and well-controlled clinical study plus support from confirmatory evidence.

Confirmatory evidence can help substantiate the primary findings from a single study and may be particularly valuable in cases of serious diseases, diseases of unmet medical need, or rare diseases. ALS meets all three of these criteria.

The 2019 Substantial Evidence of Effectiveness Guidance from FDA describes both the circumstances for when confirmatory evidence may be considered in establishing effectiveness, as well as examples of confirmatory evidence. Confirmatory evidence as described in the guidance is meant to encompass additional data elements which provide further support for the primary findings of a study. As noted in the guidance, comparison to external controls, particularly in the case of a mortality benefit, could be used as confirmatory evidence.

This document starts with an assessment of CENTAUR as a single, adequate and well-controlled study. First, the CENTAUR trial was a multicenter, randomized controlled trial that met its prespecified primary outcome, and was robust across many sensitivity analyses. A review of key discussion points from the first Advisory Committee is also provided along with new supportive analyses.

Subsequently, there is strong confirmatory evidence supporting the primary finding. In CENTAUR there was a demonstrated statistically significant Overall Survival benefit in the ITT population. The robustness of the survival benefit is further supported by the following:

- A new analysis utilizing a statistical method to adjust for the effect of treatment crossover (Section 5.1.2).
- A new analysis comparing observed survival in the CENTAUR study to predicted survival using the ENCALS ALS survival prediction model derived from an ALS natural history database (Section 5.1.3).
- A new analysis comparing observed survival from CENTAUR treatment group to survival of matched treatment naïve participants from historical clinical trials of ALS (Section 5.1.4).

New biomarker evidence from the PEGASUS, Phase 2 randomized, placebo-controlled study of AMX0035 in Alzheimer's Disease is also shared, which may provide further confirmation of neurobiological activity of AMX0035 against neurodegenerative disease targets in the central nervous system (Section 7).

The CENTAUR study met the prespecified primary outcome, was robust across multiple sensitivity analyses, and is a single adequate and well-controlled study. The survival

benefit demonstrated in the ITT overall survival analysis, which is now further supported by three new sensitivity analyses including analyses using external controls, provides confirmatory evidence for the primary findings in CENTAUR. Together, this data meets the standard for substantial evidence of effectiveness.

1 BACKGROUND

1.1 ALS

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, 2017).

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

1.2 Unmet Need

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.

There are two FDA approved therapies for ALS: riluzole (approved in 1995), and edaravone (approved in 2017). The development paths, and approvals, for each product highlight examples of how the FDA has previously exercised regulatory flexibility in ALS. The following trial findings of these agents were submitted as part of their NDAs:

Riluzole:

Study 1: N=155, 90-day (3 month) difference in median time to death or tracheostomy, p=0.12 (primary outcome). Differences in function were not observed.

Study 2: N=959, 60-day (2 month) difference in median time to death or tracheostomy, p=0.076 (primary outcome). Differences in function were not observed.

Edaravone:

Study 16: N=206, 0.65-point difference on ALSFRS-R, p=0.41 (primary outcome). Significant differences were not observed secondary outcomes of strength, vital capacity or survival.

Study 19: N=137, 2.5-point benefit on the ALSFRS-R p=0.001 (primary outcome). Significant differences were not observed on secondary outcomes of strength, vital capacity or survival, but did demonstrate significant effect on an alternate functional rating scale and a patient self-reported rating scale.

An oral suspension formulation of edaravone was also recently approved in May 2022 under priority review based on results of a bioavailability study comparing the IV formulation of edaravone to the oral suspension formulation of edaravone.

1.3 Regulatory and Development History

1.3.1 FDA Interactions – post 30 March 2022 Advisory Committee

Amylyx and FDA met on 03 May 2022 for the late-cycle review meeting (LCM), which is part of the review cycle. Amylyx corresponded with FDA regarding items relevant to the LCM.

Post-LCM, Amylyx made multiple submissions to FDA which included further analyses on CENTAUR survival data and additional data evaluating a series of biomarkers derived from Study AMX8000 (PEGASUS – Phase 2 in Alzheimer's disease [AD]).

FDA convened a meeting on 01 June 2022 notifying Amylyx that these submissions warranted a Major Amendment and recommended a PDUFA extension. It was requested at this meeting that Amylyx would integrate the survival analyses and biomarker data into a single robust submission that focused on this evidence as confirmatory of the results from CENTAUR and submit this information as a major amendment to the NDA.

Amylyx submitted the requested integrated analysis document on 17 June 2022.

FDA requested a meeting with Amylyx on 01 July 2022 and notified Amylyx that the PCNSDAC would be convened again with the main purpose of discussing these new data which FDA considered important to the review of the drug.

1.3.2 Health Canada Approval and EMA Review of AMX0035

On 10 June 2022, AMX0035 (ALBRIOZA™) was approved by Health Canada for the treatment of adults living with ALS. The approval was authorized under Health Canada's Notice of Compliance with Conditions (NOC/c) policy. One of the conditions of the approval is the post-market provision of data from the ongoing Phase 3 PHOENIX trial. AMX0035 (ALBRIOZA) is currently being prescribed for the treatment of ALS in Canada.

AMX0035 is under review by European Medicines Agency based on an MAA submitted 03 January 2022. The proposed pathway is a conditional marketing authorization under which Phase 3 PHOENIX data would be required as a post marketing requirement.

2 PATHWAY TO AMX0035 APPROVAL

All drugs must meet the standard of substantial evidence of effectiveness to be approved. While this often comes from support of two adequate and well-controlled studies, substantial evidence of effectiveness may be derived by either a single very persuasive trial or a single adequate and well-controlled study plus confirmatory evidence, as outlined in the FDA 2019 Substantial Evidence of Effectiveness Guidance. Table 1 outlines the pathway to AMX0035 approval within the aforementioned regulatory guidance. Specifically, a single adequate and well-controlled study can meet the regulatory standards for approval if supported by confirmatory evidence. Confirmatory evidence as described in the guidance is meant to encompass additional data elements which provide further support for the primary findings of a study. As noted in the guidance, comparison to external controls, particularly in the case of a mortality benefit could be used as confirmatory evidence.

This submission details how the results demonstrated from the randomized CENTAUR study in people with ALS and the blinded treatment extension may be viewed as meeting the threshold for a single adequate and well-controlled trial along with confirmatory evidence of survival.

The guidance also stresses 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). As part of the guidance, more flexible statistical considerations might be acceptable, including a somewhat higher p value, if prespecified and appropriately justified in rare and fatal diseases.

This briefing document will focus on the analyses which support the conclusion that the data constitute a single adequate and well controlled study plus confirmatory evidence. Table 1 below details how the data meet the standard for substantial evidence of effectiveness.

Table 1 AMX0035 Data Meets the Statutory Standard for Substantial Evidence of Effectiveness

	Yes	No
Single adequate and well-controlled trial	X	
PLUS		
Confirmatory evidence		
Survival (ITT population)	X	
Supportive evidence		
Survival Comparison to Natural History or External Controls	X	
WITH TOLERANCE FOR UNCERTAINTY WHEN		
Life threatening / severely debilitating	X	
Unmet need	X	
Disease is rare	X	

3 AMX0035 PRODUCT DESCRIPTION

3.1 Product Overview

AMX0035 is a co-formulation of two active pharmaceutical ingredients (APIs), sodium phenylbutyrate (PB) and taurursodiol (TURSO), hypothesized 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 Proposed Indication and Dosing

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.

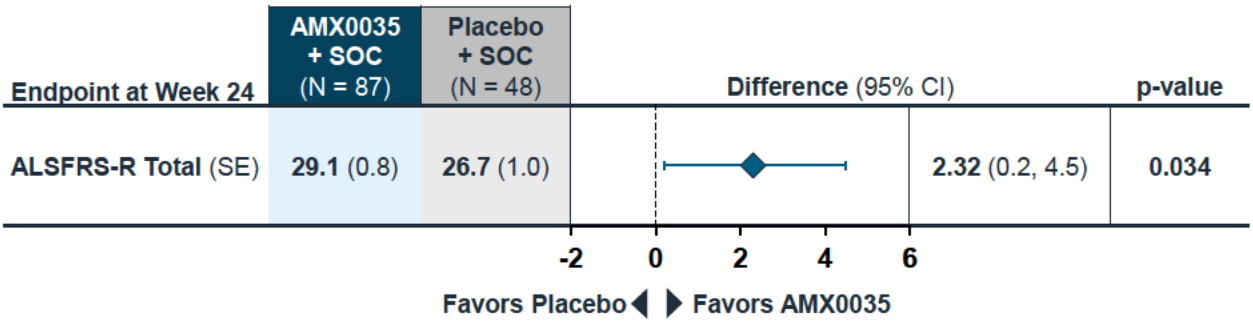
Maintenance Dose:

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

4 CLINICAL EVIDENCE – CENTAUR

CENTAUR was a 137 participant, 25-center, randomized, placebo-controlled, double-blind study in people living with ALS conducted at top ALS centers of excellence in the United States. The study met its prespecified primary outcome, a statistically significant change on the ALSFRS-R at 24 weeks (2.32-point difference at 24 weeks, p<0.05). This represents a 25% slower decline in function for the AMX0035 group compared to placebo as the monthly decline in function was -1.66 [placebo] points as compared to -1.24 [AMX0035]. The magnitude of the benefit is considered clinically meaningful based on published ALS expert assessment (Castrillo-Viguera et al., 2010).

Table 2 ALSFRS-R Total Score; Primary, Prespecified Analysis



Several sensitivity analyses support the primary findings. Amylyx presented an analysis abandoning the linearity assumption, using a traditional MMRM, and found results similar to the prespecified primary model (Week 24 ALSFRS-R difference 2.15 points, p=0.034). Amylyx has also presented multiple analyses testing for the sensitivity of the data to mid-study deaths, missing data and concomitant medication use all of which find results consistent with the primary findings.

Clinical secondary outcomes including strength as measured by ATLIS and breathing capacity as measured by the slow vital capacity trended in favor of AMX0035. No difference was seen for phosphorylated neurofilament heavy chain or neurofilament light chain levels. Time to the composite of death, permanent ventilation or hospitalization also trended in favor of AMX0035 after 24 weeks (p=0.11). However, as expected, there were few deaths in both groups after 24 weeks, given the inclusion/exclusion criteria.

4.1 New Analyses and Discussion Supporting the Robustness of CENTAUR Findings

During the 30 March 2022 Advisory Committee, the advisors and FDA brought up several discussion points which Amylyx continued to analyze. Subsequent sections summarize key data and present new analyses and discussion addressing these elements.

4.1.1. Early Randomization Error

At the beginning of CENTAUR, there was a randomization error that resulted in the initial 18 kits shipped to participants to all be AMX0035. This event was detected and corrected quickly by the appropriate unblinded staff assigned to such tasks in CENTAUR. After correction, the 2:1 AMX0035:placebo randomization ratio was maintained (next 9 kits sent were placebo). Participants, investigators, and study staff were never unblinded to this error.

To determine the potential impact of such an event, an analysis was conducted and presented at the last advisory committee in which all affected participants were removed from analysis. The primary endpoint results were consistent with the total population results (Week 24 ALSFRS-R difference 2.52 points, p=0.042).

New information on baseline characteristics participants affected compared to the overall population are contained in Table 3. Results demonstrated that both populations were balanced and the removal of the 27 participants affected by the randomization error did not impact the overall positive result.

Table 3 Patient Populations – Comparison of Overall and Those Part of the Randomization Error

	mITT Population		mITT Population with participants affected by shipping error removed	
Baseline Characteristic	**AMX0035 + SOC (N = 87)**	**Placebo + SOC (N = 48)**	**AMX0035 + SOC (N = 71)**	**Placebo + SOC (N = 39)**
Time Since ALS Diagnosis (months), mean (SD)	**5.9 (3.3)**	**6.3 (3.2)**	**5.8 (3.3)**	**6.3 (3.3)**
Time Since ALS Symptom Onset (months), mean (SD)	**13.5 (3.8)**	**13.6 (3.6)**	**13.4 (3.7)**	**13.1 (3.7)**
ALSFRS-R Total Score, mean (SD)	**35.7 (5.8)**	**36.7 (5.1)**	**35.3 (6.0)**	**36.4 (5.1)**
ATLIS Total Score (% predicted normal), mean (SD)	**57% (20.1)**	**54% (20.9)**	**54% (19.5)**	**53% (19.9)**

SVC (% predicted normal), mean (SD)	**84% (18.2)**	**84% (15.9)**	**82% (17.5)**	**83% (15.6)**
Pre-baseline ALSFRS-R slope (Del-FS), mean (SD)	**0.95 (0.4)**	**0.93 (0.6)**	**0.99 (0.4)**	**0.99 (0.6)**

A new analysis was also conducted which analyzed the groups based on the original intended randomization schedule. This as-randomized analysis was also consistent with primary findings (Week 24 ALSFRS-R difference, 2.69 points, p=0.013).

The randomization error was quickly detected and appropriately handled, has been well-examined and is confirmed to have no impact on the interpretation of the primary results findings.

4.1.2. Use of Concomitant Medications

In the United States there are two approved drugs for ALS, riluzole and edaravone. The advisors and FDA have shared interest in further understanding the potential impact of use of these drugs on the study.

Previously, time dependent and baseline covariate analyses were presented to determine if concomitant medications impacted primary findings. These analyses are statistical methods which adjust either for use vs. non-use or the total duration of use of these medications. As was previously presented, these analyses were all consistent with the primary outcome, indicating that the use of riluzole and edaravone did not impact the primary finding (Table 4 and Table 5).

Table 4 Concomitant Edaravone and Riluzole Use – Adjusted for Baseline

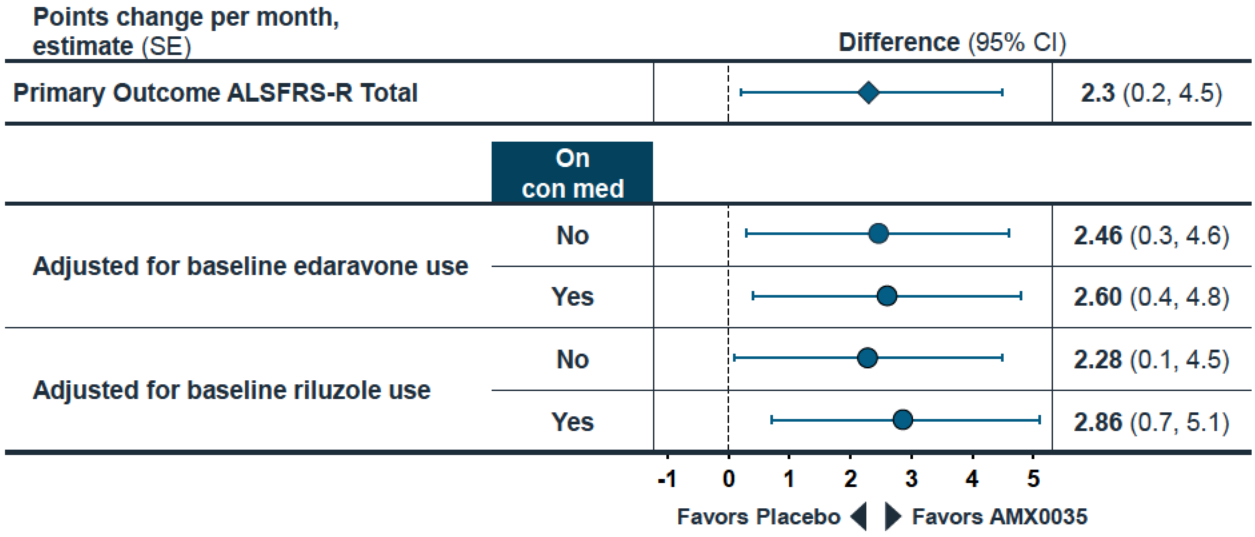
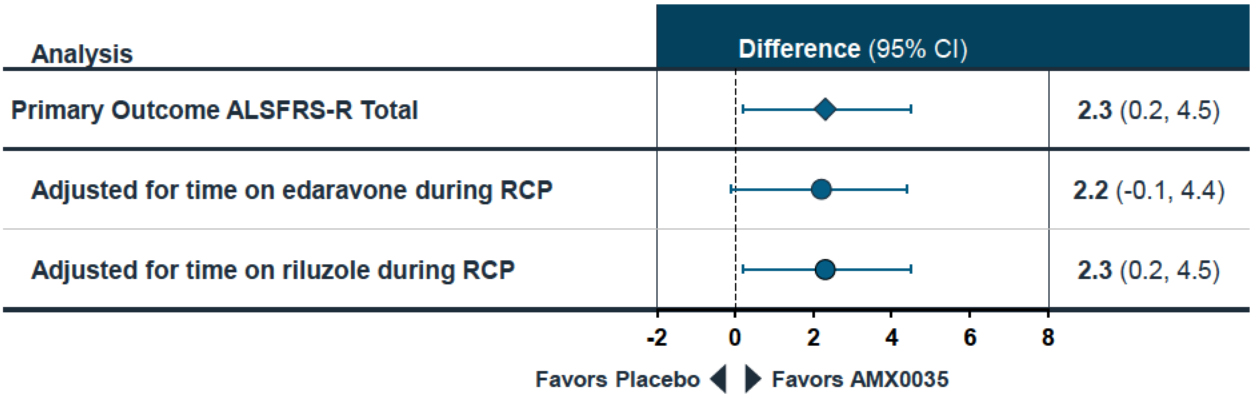


Table 5 Concomitant Edaravone and Riluzole Use – Adjusted for Time



Edaravone was approved during the CENTAUR study. As a result, there were instances of mid-study starts for edaravone. Specifically, upon analysis there was 4% of the placebo arm and 13% of the AMX0035 arm who started edaravone mid-study.

Now, a new analysis is presented that completely removes participants who had mid-study starts of edaravone from the primary analysis to determine if these participants impacted findings—results of this new analysis are consistent with primary findings: Week 24 ALSFRS-R difference 2.39 points, p = 0.041.

Collectively, these analyses demonstrate that use of concomitant medications does not impact the interpretation of the findings.

4.1.3. Potential for Functional Unblinding and Continued Blinding to Original Assignment During OLE Phase

There is no evidence of unblinding in CENTAUR. However, unblinding was raised as a general question, centering around the taste of study drug and the impact of GI side effects, and is discussed in more detail below.

Regarding taste, it is important to note that the placebo and AMX0035 formulations were matched for taste by an expert group.

Regarding GI side effects, Amylyx notes that the GI side effects observed in the study were generally mild and that the overall incidence of GI adverse events was similar between the AMX0035 and placebo groups, 66% and 63%, respectively. On this basis, functional unblinding due to GI side effects is unlikely.

Finally, data was collected that supports the absence of functional unblinding in CENTAUR. Participants and physicians were required to complete an exit questionnaire at the end of the double-blind phase of the CENTAUR study. As part of this exit questionnaire, they were asked to provide a guess of their treatment assignment.

For participants randomized to AMX0035, 44% correctly guessed they were on AMX0035 and 46% incorrectly guessed they were on placebo (10% of questionnaires were not completed). For their investigators, 49% correctly guessed their participant

was on AMX0035, 38% incorrectly guessed their participant was on placebo (12% of questionnaires were not completed). Neither of these results are significantly different from chance (by random chance 50% of participants and physicians would be expected to guess correctly). As such, there is no evidence participants could guess they were on active drug.

A concern was also raised whether participants remained blinded to their original assignment through the extension phase. Sites were emailed original treatment assignment information on 15 October 2021, well after the 1 March 2021 last participant last visit in the CENTAUR extension phase. As such, participants and physicians were blinded to original study arm throughout the extension phase of the study.

As discussed above, there is no evidence of unblinding in CENTAUR. Participants and physicians also remained blinded to their original assignment through the LPLV of the extension phase.

4.2. Summary of Clinical Evidence: Primary Endpoint

The CENTAUR study met the prespecified primary outcome. This result is robust through several previously presented and new sensitivity analyses. These results were also supported by trends on secondary outcomes in favor of the treatment effect.

ALS experts involved with the study have also published their opinions on the robustness of the study conduct and findings subsequent to the 30 March 2022 Advisory Committee meeting in a publication in *The Annals of Neurology* (Cudkowicz, 2022).

While the analyses show that the CENTAUR primary outcome was robust, the p value for the prespecified primary outcome of 0.034 leaves some residual uncertainty in the context of reliance on a single study to support substantial evidence of effectiveness. Therefore, confirmatory evidence is valuable to bolster confidence in the findings.

Analyses are presented in the subsequent sections which constitute confirmatory evidence and supporting analyses. These results include the following:

- Confirmatory Evidence: ITT OS analysis (Section 5.1.1)
- Analyses Supporting the ITT OS
    - ITT OS new analysis addressing crossover effects (Section 5.1.2)
    - ITT OS analysis compared to two separate external/historical controls (Section 5.1.3 and Section 5.1.4, respectively)

Biomarker data is also presented from a separate trial in Alzheimer's disease which supports the biological activity of AMX0035 against neurological markers of interest in cerebrospinal fluid samples (Section 7).

Collectively, the overall survival benefit of AMX0035 provides important confirmatory evidence for the prespecified primary outcome and new analyses to be presented

further bolster the findings on overall survival including with comparisons to external controls.

5 CONFIRMATORY EVIDENCE – ITT OVERALL SURVIVAL

5.1 Long-Term Overall Survival Evidence and Analysis

The Overall survival (OS) analysis that can serve as confirmatory evidence for the effectiveness of AMX0035 in ALS and new supporting analyses are provided below.

5.1.1 Analysis of Overall Survival in the ITT Population from a Randomized, Placebo-Controlled Design

Given that ALS is a universally fatal disease with a median overall survival of just 2 years from diagnosis (Karanevich et al., 2018, Traxinger et al., 2013) and only a small proportion (~10%) of people living with ALS may have prolonged survival, measuring survival is of particular interest to ALS physicians.

The ITT OS analysis compares survival between 2 groups – all 89 participants in the AMX0035 group and all 48 participants in the concurrent control (placebo) group. The ITT OS was therefore a placebo-controlled analysis.

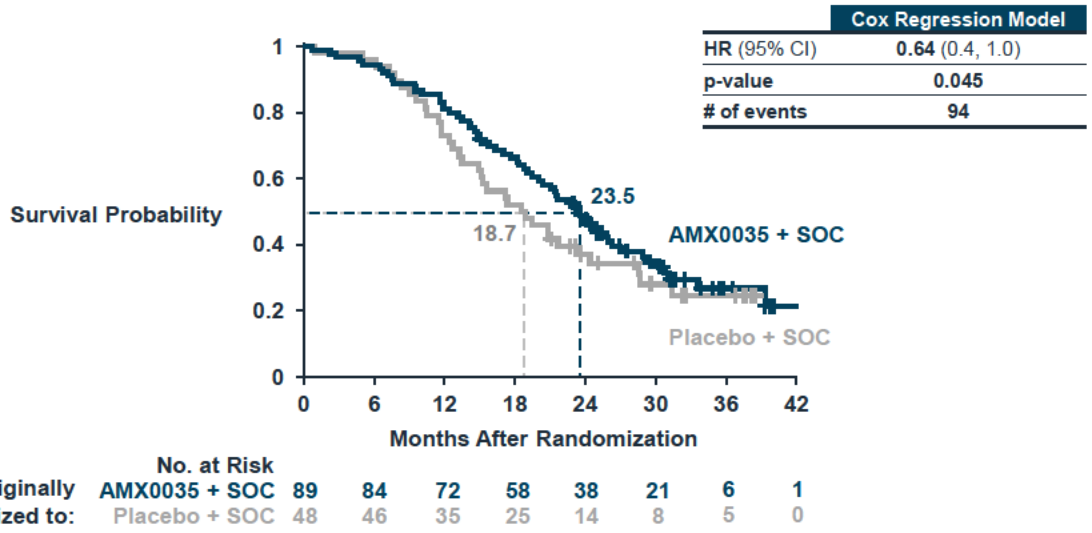
All participants were followed for overall survival until death or last day of blinded treatment extension – the survival status of 136 out of 137 participants is captured and source verified; the one participant not captured as of the cut-off date is censored as of their last clinic contact. Survival status was confirmed even on those participants who dropped out of the study through an evaluation of public records including the social security death index and state and city records.

March 1, 2021 was the last day of the blinded treatment extension (Last Participant Last Visit [LPLV]) and agreed to by the Division on August 10, 2021 as the appropriate final timepoint in the study for efficacy assessment. All survival analyses presented use March 1, 2021 as the analysis cut-off date.

All participants were followed for survival status regardless of dropout or enrollment in the extension phase. All data collected in the randomized phase through the completion of the blinded treatment extension are included in the OS analysis. In the ITT OS analysis, participants were analyzed in their assigned treatment group regardless of treatment received in treatment extension phase.

With vital status verified on 136/137 participants in the trial as of the LPLV and with 94 total deaths in this final analysis the survival data are mature for the analysis.

Figure 1 ITT Overall Survival Analysis for All Participants Randomized in CENTAUR



Cox proportional hazards model with covariates of age at randomization and pre-baseline Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised (ALSFRS-R) slope

Comparing the AMX0035 arm to the placebo arm of the study, there was a nominally significant overall survival benefit (HR=0.64, p<0.05). A 4.8-month longer median OS is observed in participants receiving AMX0035 (mOS 23.5 months) than the participants receiving placebo (mOS 18.7 months).

Similar to the primary outcome, sensitivity analyses were performed to assess the impact of concomitant use of riluzole or edaravone on the ITT overall survival results. The results remained consistent and significant when correcting for baseline use of these medications.

While the ITT OS results provide confirmatory evidence on their own, one important note is that the placebo-controlled analysis makes no adjustment for those placebo participants who crossed over to AMX0035 in the extension. In CENTAUR, the majority of placebo participants (71% [34/48]) crossed over and received AMX0035 after the 24-week randomized-controlled phase. Analyses that account for this crossover are important to estimate what the treatment benefit may have been had the placebo group remained treatment-naïve. Additionally, confirmation against external control groups can provide further confidence in the robustness of the observed ITT overall survival benefit.

Three new analyses are presented below that provide further detail for and confidence in the observed ITT overall survival benefit: (1) Rank Preserving Structural Failure Time Model (RPSFTM), a method frequently employed in oncology clinical trials to adjust for treatment crossover effect, (2) Natural History data and known prognostic factors to compare survival time versus a benchmarked, predicted control and (3) Propensity Score Matching to match against participants from control groups from historical ALS clinical trials and to estimate survival difference. These additional

analyses provide additional context and support for the survival benefit of AMX0035 in the CENTAUR study.

5.1.2 Rank Preserving Structural Failure Time Model (RPSFTM) to account for treatment crossover

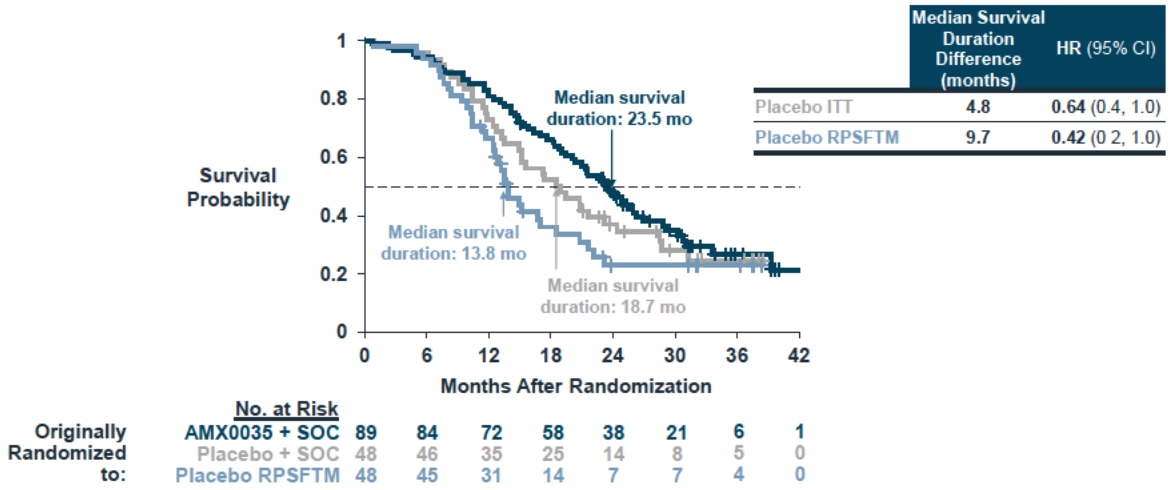
Compassionate crossover of placebo groups to active treatment is a common occurrence in other fields such as oncology which has led to the development and frequent implementation of methods that can maintain ethical clinical trial designs while providing robust estimation of survival benefits of treatments.

Rank-preserving structural failure time models (RPSFTM) have emerged as a robust method to control for the effect of crossover in OS results (Morden et al., 2011, Latimer et al., 2014, Jonsson et. al., 2014). The RPSFTM provides an estimate of the OS time for the placebo group had treatment switching not occurred and avoids potential issues of selection bias since treatment switching is often related to prognosis (Robins et al., 1991, Latimer et al., 2014). RPSFTM estimates OS measured from the time of treatment switching by applying an estimate of the benefit of the experimental treatment and assumes that the benefit of the experimental treatment is the same whether it was received from the time of randomization or only received later as a switch treatment (Bennett et al., 2018). Such models have been used in multiple publications in the *New England Journal of Medicine* (Hussain et al., 2020, Turner et al., 2018) and *Journal of the American Medical Association* (JAMA) (Zhu et al., 2021) and cited in FDA drug reviews. For example, this method was cited in Lenvatinib for the treatment of people with progressive, radioiodine-refractory differentiated thyroid cancer (206947orig1s000statr.pdf).

Other methods often used in oncology to adjust overall survival for switching, including inverse probability of censoring weighting and two-stage models, are not suitable for CENTAUR; the assumptions for these methods are not met when a large proportion of placebo participants cross over, as was the case in the CENTAUR trial.

The RPSFTM analysis of the CENTAUR data showed that the estimated difference in median survival for AMX0035 as of the Last Participant Last Visit was 9.7 months. This result compared to 4.8 months when not accounting for treatment crossover (ITT RPSFTM OS analysis; mOS = 23.5 months in AMX0035 group; mOS = 13.8 months in placebo group, 9.7-month difference, HR = 0.42, p=0.045). These findings confirm the robustness of the benefit observed in the placebo-controlled, ITT overall survival analysis and suggest if anything the treatment benefit may have been larger had the placebo group remained treatment naïve. These results have been recently published (Paganoni et al., 2022 Supplementary Appendix). These results are presented in Figure 2.

Figure 2 Rank Preserving Structural Failure Time Model Results



One limitation of the RPSFTM is that it assumes a common treatment effect (i.e., exposure-response relationship is the same, no matter what stage of disease the treatment is received). This assumption is always important to evaluate when conducting RPSFT methods. However, several factors in CENTAUR support that this assumption is reasonable: the time between randomization and crossover in CENTAUR was relatively short (only 6 months) and AMX0035 targets neuronal death, which is expected to be relevant at all stages of disease. This assumption is also often considered to be supportable in oncology trials.

The RPSFTM model finds a 9.7-month survival benefit when adjusting for treatment crossover as compared to the ITT overall survival of 4.8 months. This suggests that treatment crossover may have attenuated the observed survival differences in the ITT analysis.

5.1.3 Survival Prediction Algorithm Created from Natural History Data

Multiple prognostic factors have long been known to be predictive for survival in ALS. An effort was undertaken in Europe using over 10,000 patient records to attempt to create a survival prediction model based on prognostic factors. This model was published in Lancet Neurology (Westeneng et al., 2018).

In developing the model, the authors evaluated 16 prognostic factors in ALS and ultimately determined that 8 prognostic factors were sufficient for the model to accurately estimate survival time. These factors included bulbar versus non-bulbar onset (HR = 1.71 for bulbar onset, 95% CI = 1.63-1.79), age at onset (HR=1.03 per year of age, 95% CI = 1.03-1.03), definite versus probable or possible ALS (HR =1.47 for definite ALS, 95% CI = 1.39-1.55), diagnostic delay (HR=0·52 per month, 95% CI = 0.51-0.53), forced vital capacity (HR=0.99 per percent of normal, 95% CI = 0.99-0.99), pre-study progression rate (HR=6.33 per unit, 95% CI = 5.92-6.76), frontotemporal dementia (HR=1.34 for FTD, 95% CI = 1.20-1.50), and presence of a C9orf72 repeat expansion (HR=1.45 for C9orf72 expansion, 1.31-1.61). All prognostic factors showed

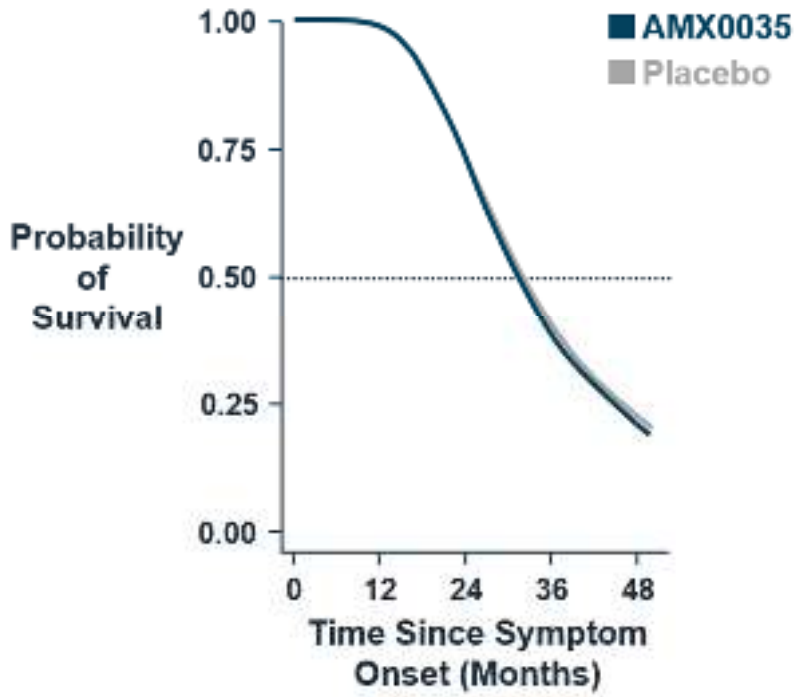
significant association with survival (p<0.0001). Collectively, with these prognostic factors, the authors created a model which could provide a reasonable estimate of survival for individual patients.

Amylyx collaborated with the originators of this model to predict treatment naïve overall survival time for each individual participant in CENTAUR. The originators of the model were blinded to treatment assignments when creating their survival estimates. Additionally, a survival estimate was calculated for the entire AMX0035 and placebo groups based on their baseline scores.

This new analysis provides two important pieces of information.

First, the model suggests that the AMX0035 and placebo groups had similar predicted survival distributions at baseline, providing evidence the two groups were well balanced at baseline in terms of prognosis. These results are presented in Figure 3.

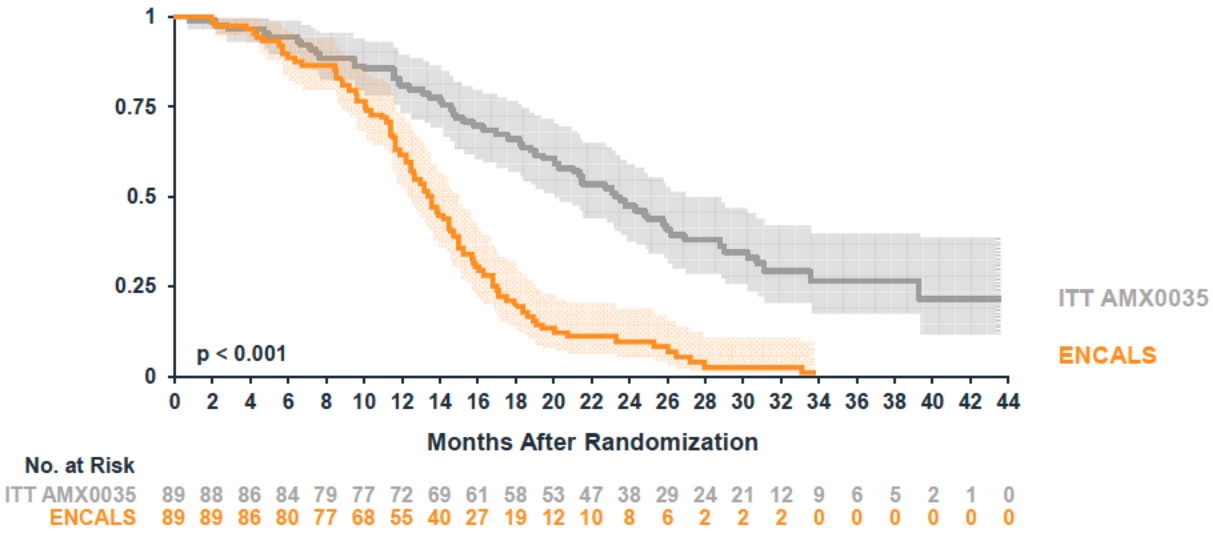
Figure 3 ENCALS Survival Prediction Model: Predicted Survival of CENTAUR AMX0035 and Placebo Groups



Second, comparing the predicted (treatment naïve) survival data generated using this model against the actual observed survival data in the AMX0035 group provides additional confirmation of the ITT overall survival benefit. In this analysis, the ITT AMX0035 group (N=89) showed a longer median OS of 9.9 months versus predicted treatment naïve median survival (mOS 23.5 months in AMX0035 group vs mOS 13.6 months from ENCALS prediction: 9.9-month median survival benefit, HR = 0.28,

p<0.0001; Figure 4). This result (9.9 months benefit) is similar to the RPSFTM findings (9.7-month benefit) supporting the robustness of this methodology.

Figure 4 ENCALS Natural History Survival Prediction – Observed ITT AMX0035 vs. Predicted



There are potential limitations of this approach.

First, the model was developed based on data from European people with ALS and the CENTAUR trial was based in the US. However, a recent review compared demographics and characteristics of people living with ALS, and an assessment of region-specific guidelines in the US, EU and Japan (Takei K et al., 2017); there were not substantial regional differences observed with regard to patient identification, diagnosis, disease prognosis or rate of progression, nor did approaches to multidisciplinary care or symptomatic management differ significantly.

Second, the model development included people living with ALS from 1992 through 2016 (CENTAUR was conducted starting in 2017). A concern may be raised that older patient records and changes in practice could bias results. However, a recent study in Italy comparing survival for a cohort of patients between 1998-2000 and 2008-2010 found that median survival from diagnosis was 2.4 years in the 2008-2010 cohort compared to 2.2 years in the 1998-2000 cohort. As such, while improvements in care and management are likely improving prognosis in people living with ALS over time these effects are likely to be small and would not be expected to account for the larger differences observed.

The ENCALS model is a rigorously developed model that estimates survival based on well-studied prognostic factors. Applying this model to the CENTAUR survival data resulted in a large difference in survival for people randomized to AMX0035 as compared to model predictions. The findings were similar to the RPSFTM methodology supporting

the robustness of the results. Additionally, the model supports the conclusion that the two study arms were well balanced.

5.1.4 Survival – Survival Analysis Compared to Propensity Score Matched PRO-ACT Historical Control

In the ALS field, sponsors are encouraged to submit data from completed trials to a database called PRO-ACT. PRO-ACT contains over 11,000 de-identified patient records from past clinical trials. Many of the trials submitted to PRO-ACT are NEALS studies conducted with many of the same United States clinical sites as those involved with CENTAUR. In this way, a comparison to placebo participants from PRO-ACT provides an external control which is highly relevant to the CENTAUR population.

In comparing survival to the PRO-ACT population, propensity score matching (PSM) was chosen as an appropriate statistical method to compare the CENTAUR data versus this external control. PSM is an advanced method to handle comparisons to historical cohorts. Several drug reviews cite PSM-based analyses as supportive, including Crysvita® for X-linked Hypophosphatasia and Defitelio® for VOD (Jahanshahi. et al., 2021). PSM is also referred to in the FDA's RWE Framework (RWE Framework) as a way to reduce selection bias and confounding when comparing to an external control group. Studies including those in the cardiovascular setting have found PSM to perform well in estimating cohort behavior (Elze et al., 2017 and Deb et al., 2016).

The accuracy of PSM depends greatly on the prognostic variables used for matching and the similarity of the external control group as compared to the study group (Austin, 2011). In cases where the control group is not appropriate or the prognostic variables are poorly understood, this method can produce inaccurate results. In ALS, there are well-known prognostic variables for survival and the PRO-ACT provides a very similar study setting compared to CENTAUR. As such, the utilization of propensity scores is appropriate in this context.

In this analysis, PSM was conducted using the ITT AMX0035 group (n=89) and comparing to the PRO-ACT database.

The participants from the PRO-ACT historical control database were included based on the following criteria:

- Those with a baseline and at least one post-baseline ALSFRS-R score
- Those meeting major inclusion / exclusion from CENTAUR trial at Baseline in PRO-ACT
    - Between 18-80 years old
    - Definite ALS diagnosis El Escorial criteria
    - ≤18 months since ALS symptom onset
    - Predicted VC greater than 60% (FVC or SVC is used whichever is available)

- Known mortality information (either known death date, or known alive at the end of follow-up)

Propensity score caliper matching was performed on all ITT AMX0035 treated participants with a 1:1 matching ratio and a caliper width of 0.4. A comparison of the baseline covariates used in the propensity score are in Table 6 and show the groups to be generally well-balanced.

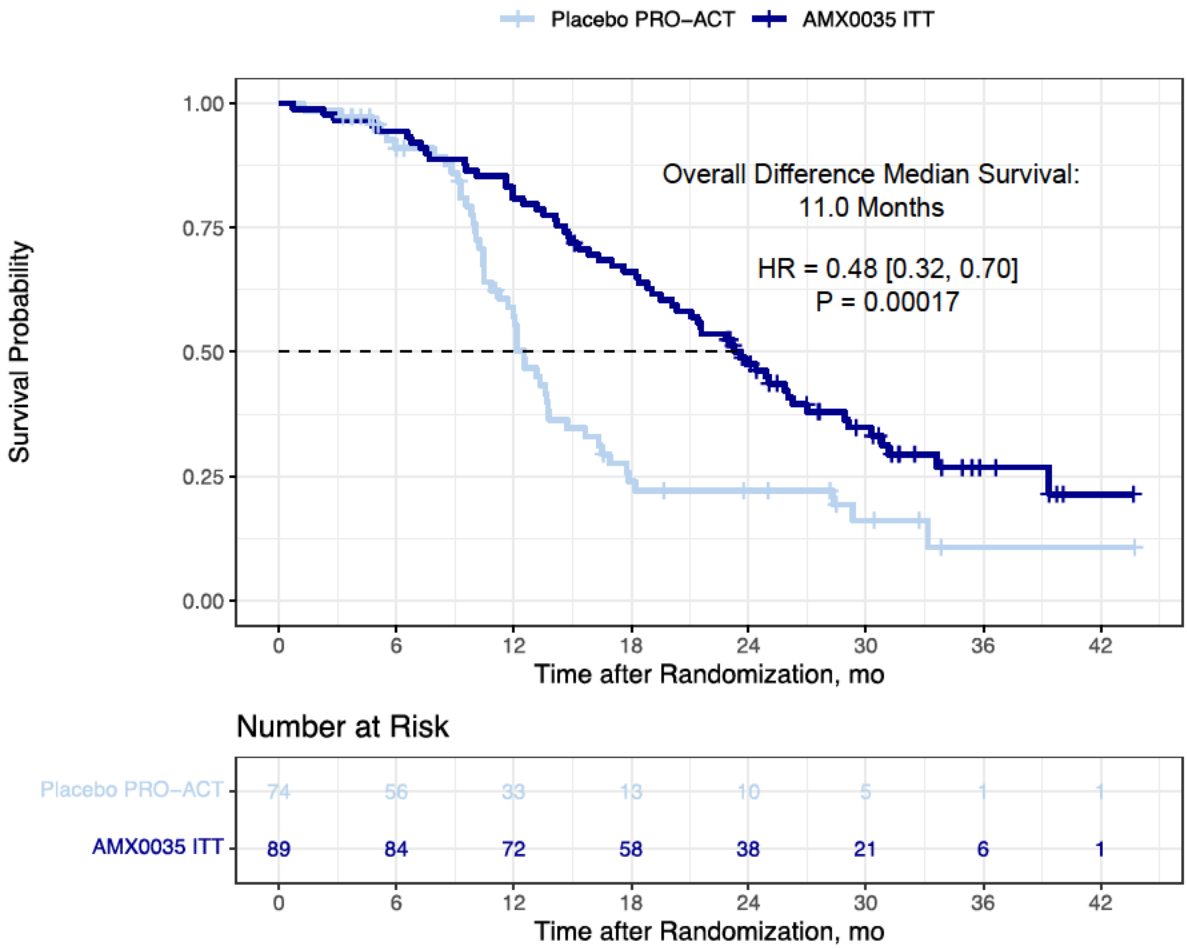
Table 6 Propensity Score Baseline Covariates – Summary

	AMX0035 ITT	**PRO-ACT Matched Set**
	N = 89	N = 74
	Median SD; (Range)	Median SD; (Range)
Time Since Onset (Months)	13.6 3.8; (3.0, 20.0)	12.3 3.4; (3.6, 17.6)
ALSFRS-R pre-baseline slope	0.96 0.42; (0.12, 1.94)	0.95 0.57; (0.14,3.14)
SVC/FVC (percent predicted)	0.83 0.19; (0.38, 1.42)	0.84 0.14; (0.6,1.31)
Age	57.9 10.6; (31, 79)	57.5 10.0; (32, 77)

A cox proportional hazards survival analysis was used to compare mortality of the ITT AMX0035-treated participants from CENTAUR with the matched PRO-ACT controls. Mortality data presented includes all participants through LPLV.

The AMX0035 propensity score analysis for Overall Survival is displayed in Figure 5. The outcome of the analysis demonstrated an 11.0-month median survival benefit for participants randomized to AMX0035 as compared to the propensity score matched population in the PRO-ACT group (mOS 23.5 months in AMX0035 group vs mOS 12.5 months from PROACT population prediction: 11.0-month median survival benefit, HR = 0.48, p=0.00017)

Figure 5 Propensity Score Analysis – Observed ITT AMX0035 vs. PRO-ACT



A limitation of PSM is that it cannot control for unobserved covariates or population differences that were not measured. While these limitations apply to this analysis, the choice of a primarily US-based clinical trial population meeting the CENTAUR inclusion/exclusion criteria as the control group, the similarities in baseline characteristics of the two comparator groups, and the fact that the chosen covariates are known to be important prognostic factors for survival in ALS reduces this concern (Berry et al., 2018).

A second limitation, similar to the ENCALS analysis, is the timeframe of the trials included in PRO-ACT. PRO-ACT contains records from 1990-2010; however, use of El Escorial information inclusion may select for more recent records since this criterion was only first published in 1994. As discussed previously, while improvements in care have occurred over time, literature suggests these effects are relatively small (0.2 years in published studies between 1998 and 2010) and would not be expected to account for the survival results observed in this analysis.

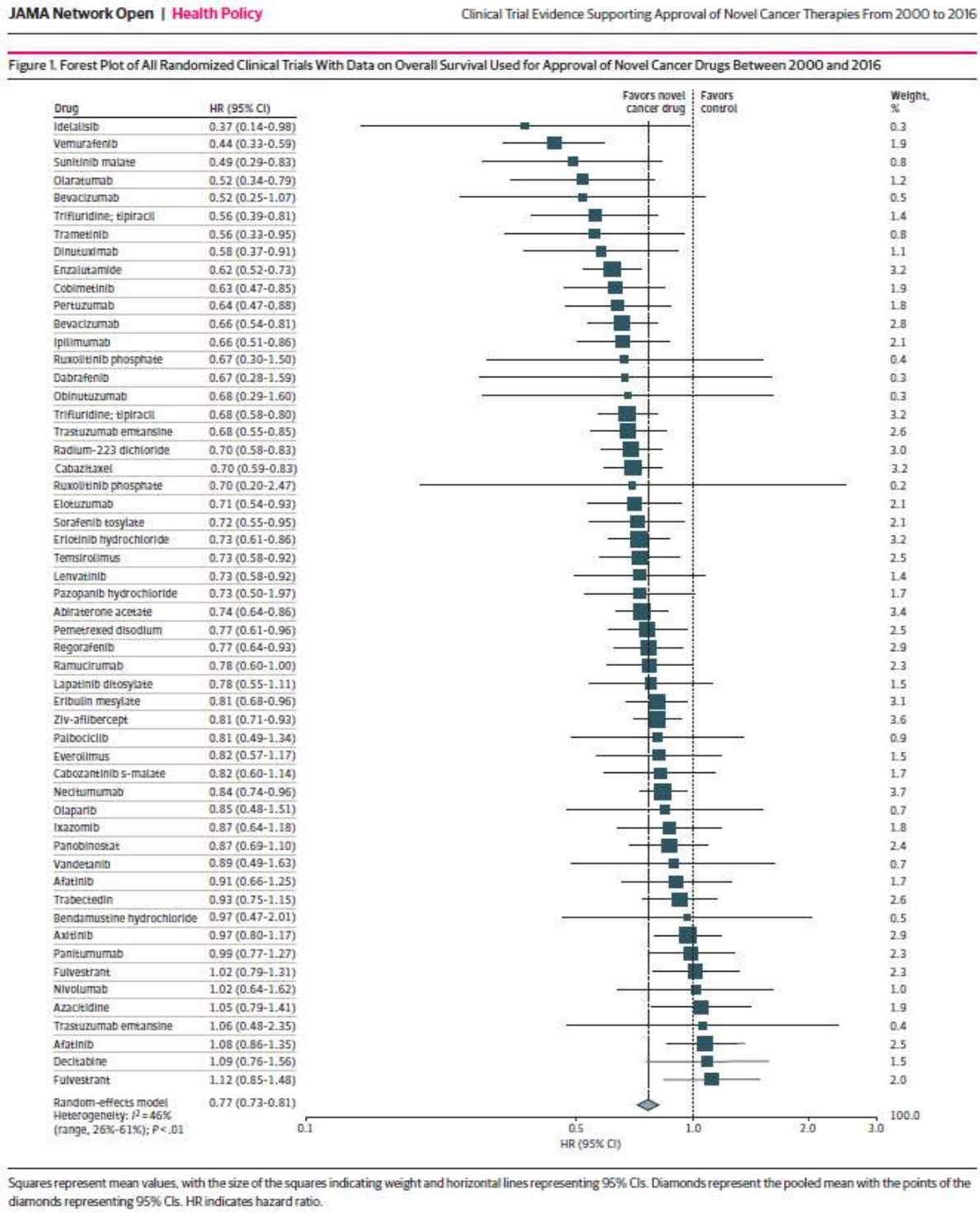
The results from the propensity score analysis (11.0-month median survival benefit) highlight a third methodology for assessing the survival benefit of AMX0035 and are consistent with the RPSFTM analysis overall survival findings (9.7-month mOS difference) and from the comparison to the ENCALS survival estimates (9.9-month mOS difference).

5.1.5 Overall Survival in Context

Survival benefits are exceedingly rare in neurology; therefore, neurology does not provide good benchmarks for magnitude and meaningfulness of survival data. As such, it is useful to compare to oncology where survival differences are frequently measured and observed. A recent review in the *Journal of the American Medical Association* (JAMA) assessed the survival benefit of all novel cancer therapies approved by FDA between 2000 and 2016.

The median overall survival benefit for approved oncology products using survival data in support of approval was 2.40 months (IQR 1.25-3.89 months). The hazard ratio and confidence intervals around the hazard ratio for these oncologic products are presented in Figure 6.

Figure 6 Overall Survival Results in Oncology Trials Leading to FDA Approval



Survival is critical in any disease and historically has been a challenging outcome to significantly change with novel drugs. The overall survival results from CENTAUR compare well to data which has supported previous therapeutic approvals (CENTAUR: ITT OS: mOS difference 4.8 month, HR = 0.64, RPSFTM: mOS difference 9.7 months,

HR = 0.42, ENCALS natural history comparison: mOS difference 9.9 months, PRO-ACT PSM comparison: mOS difference 11.0 months, HR = 0.48).

5.1.6 Survival benefit of AMX0035 provides confirmatory evidence

Death as vital status is an objective assessment that was universally captured. It is particularly clinically meaningful in a rapidly and universally fatal disease such as ALS.

In the CENTAUR study, an analysis of Overall Survival in the ITT population based on randomized, placebo-controlled study design was conducted. This analysis found a significant benefit for participants randomized to AMX0035 (mOS difference 4.8 months, HR=0.64, p=0.045). This data serves as confirmatory evidence of the benefit of treatment with AMX0035 observed on the primary outcome, a 25% slowing in the ALSFRS-R rate of decline. ITT overall survival may be considered confirmatory evidence for the below reasons:

- While changes in the ALSFRS-R are correlated with survival, they are only modestly correlated, probably because death in ALS is largely driven by respiratory failure and the ALSFRS-R assesses several other domains. In an analysis of 1034 patients from the ALS clinical trial PRO-ACT natural history database, the correlation coefficient between rate of change on the ALSFRS and survival was 0.33 (Proudfoot et al., 2016). Thus, an analysis of survival provides valuable new information, and may therefore be considered as confirmatory evidence.
- The overall survival analysis was performed as of LPLV of the blinded treatment extension, after over 3 years of follow-up, whereas the primary outcome was observed after 24 weeks. This longer follow-up also provides valuable additional information and may therefore be considered as confirmatory evidence.
- New supportive analyses have also been conducted which estimate the crossover effect and further support the robustness of ITT placebo-controlled survival findings using external controls and natural history data.
- The RPSFTM analysis estimates the treatment benefit of AMX0035 when accounting for treatment crossover to be a 9.7-month mOS survival benefit.
- The ENCALS survival prediction comparison provides a separate data source to compare with the CENTAUR study population. Comparing the CENTAUR AMX0035 arm to this new, appropriate Natural History control group further confirms the survival findings and is consistent with the RPSFTM method (9.9-month mOS benefit).
- The Propensity Score Analysis of survival comparing AMX0035-treated participants to PRO-ACT controls provided consistent results to all other survival analyses with a benefit in survival (11.0-month mOS benefit) for those participants treated with AMX0035 compared to PRO-ACT propensity score matched controls.

Each new analysis (RPSFTM, ENCALS, PRO-ACT) demonstrates similar and consistent results, as demonstrated in Table 7. These analyses also compare the data to an external control group provide new and supportive data. Most notably, the predicted survival in the control group and the estimated treatment differences are similar across the three different methodologies strengthening the robustness of this finding. Collectively, the ITT overall survival benefit provides confirmatory evidence in CENTAUR and is now supported by three separate analyses with consistent findings.

Table 7 Overall Survival – Consistency of Results by Different Methods

Analysis Method	Control Group Median OS (months)	AMX0035 Median OS (months)	Difference (months)	Hazard Ratio	p-value
ITT OS (placebo controlled)	18.7	23.5	4.8	0.64 (0.4, 1.0)	p<0.05
Methods Accounting for Treatment Crossover					
RPSFTM	13.8	23.5	9.7	0.42 (0.18, 0.99)	p<0.05*
ENCALS Natural History Comparison	13.6	23.5	9.9	0.28 (0.19, 0.41)	p<0.0001
Propensity Score Matching to PRO-ACT	12.5	23.5	11.0	0.49 (0.32, 0.70)	P<0.0002

*note that RSPFTM analyses use the p-value from the initial ITT OS analysis

6 CLINICAL SAFETY

At the 30 March 2022 Advisory Committee meeting, FDA stated they did not have significant concerns about the safety profile of AMX0035. The high-level summary of safety is as follows:

- AMX0035 is well-tolerated with a manageable safety profile.
- Majority of reported AEs were mild or moderate and not related to study treatment.
- Numerically, fewer serious adverse events were observed in the AMX0035 group as 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.
- GI events (generally nausea, diarrhea) were more frequent in the AMX0035-treated group in the first 3 weeks of treatment.
- 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.

7 ADDITIONAL INFORMATION: BIOMARKERS IN ALZHEIMER'S DISEASE

In addition to the ALS study (CENTAUR), Amylyx conducted a randomized, placebo-controlled study in 95 people with clinical Alzheimer's disease (AD) or mild cognitive impairment accompanied by biomarkers supporting AD pathology (amyloid positron emission tomography [PET], cerebrospinal fluid [CSF] AD biomarkers, fluorodeoxyglucose PET, or volumetric MRI [vMRI]), (Study AMX8000 – PEGASUS). Participants were randomized in a 3:2 fashion to receive either AMX0035 or matching placebo twice a day for 24 weeks.

In the PEGASUS trial, cerebrospinal fluid (CSF) was drawn prior to first dosing and at the 24-week visit (or early discontinuation visit, as applicable) to evaluate the effect of treatment on biomarker concentrations in the central nervous system (CNS). A series of biomarkers were prospectively identified in the study protocol and were analyzed from these CSF samples and compared between the AMX0035 and placebo arms of the study. In contrast, it should be noted that biomarkers from CENTAUR were only evaluated from plasma.

The trial was designed to incorporate broad eligibility criteria representing different stages of AD, with the goal of using the findings from this trial to inform the design of future studies of AMX0035 in AD.

Participants

The trial enrolled adults aged 55 through 89 years with a diagnosis of probable AD or mild cognitive impairment (MCI) accompanied by documented biomarkers (i.e., amyloid positron emission tomography [PET], CSF AD biomarkers [i.e., Aß$_{42}$, total tau, and p-tau], fluorodeoxyglucose [FDG]-PET, or volumetric MRI [vMRI]) supporting AD pathology.

Additional key inclusion criteria included a Montreal Cognitive Assessment (MoCA) score <8, Geriatric Depression Scale (GDS) total score <7, and not on any investigational monoclonal antibodies.

Biomarker Procedures

The biomarker core lab of the Massachusetts General Institute for Neurodegenerative Disease (MIND) has implemented a fit-for-purpose validation approach to qualify a broad array of immunoassays specifically for use in clinical trials, in both CSF and blood (Trombetta et al., 2018). The intent has been to identify the best performing biomarker assays of Amyloid-β, tau, neurodegeneration, synaptic health, inflammation, vascular injury, and other domains of interest in neurodegenerative diseases to serve as markers for both participant stratification and disease tracking in the clinical trial setting.

Technical precision and short-term biotemporal stability within individuals were assessed for all biomarkers to be able to detect their change sensitively and

confidently with intervention over time. In this validation study MGH screened many candidate biomarker assays in two CSF samples per person collected 8 weeks apart from placebo treated participants in a clinical trial. Single and multiplex ELISAs were evaluated for those performing best in terms of sensitivity, range and linearity, and intra- and inter-assay precision.

From this analysis, 30 biomarkers across 5 major pathophysiological domains with good to excellent characteristics were identified according to technical performance and CSF analyte biotemporal stability.

Of these candidate biomarkers, the study team selected 18 for use in PEGASUS and prespecified these for analysis in the PEGASUS participant cohort across 6 pathophysiological domains:

- Core AD biomarkers $A\beta_{42/40}$, phosphorylated tau, phosphorylated tau_{181}, total tau
- Additional neurodegeneration markers NfL and fatty acid binding protein 3 (FABP3)
- Synaptic integrity: Neurogranin
- Inflammation/immune modulation markers IL-6, IL-8, IL-15, MCP-1, GFAP and YKL-40 (also known as Chintase 3-like 1)
- Neurovascular marker MMP-10
- Metabolic/oxidative stress markers 24-OHC, 8-OHdG, Leptin, and soluble Insulin Receptor.

These biomarkers were measured using a selection of previously validated single and multiplex assays (Table 8). All assays were run according to manufacturers' protocols. CSF samples were collected in low-protein binding polypropylene tubes (Sarstedt, Germany), aliquoted into 0.5 mL aliquots, and stored at -80 C. Samples were diluted according to specified recommendations when performing the assays, and sub-aliquoted to minimize freeze-thaw cycles. CSF aliquots were centrifuged at 3,000 g for 10 min at 4°C, run in duplicate, and paired CSF samples from the same individual were measured on the same plates and run blinded to treatment groups and participant demographics. Data was normalized across multiple plates using two pooled CSF controls to adjust for inter-assay variability.

Table 8 Biomarker Assays

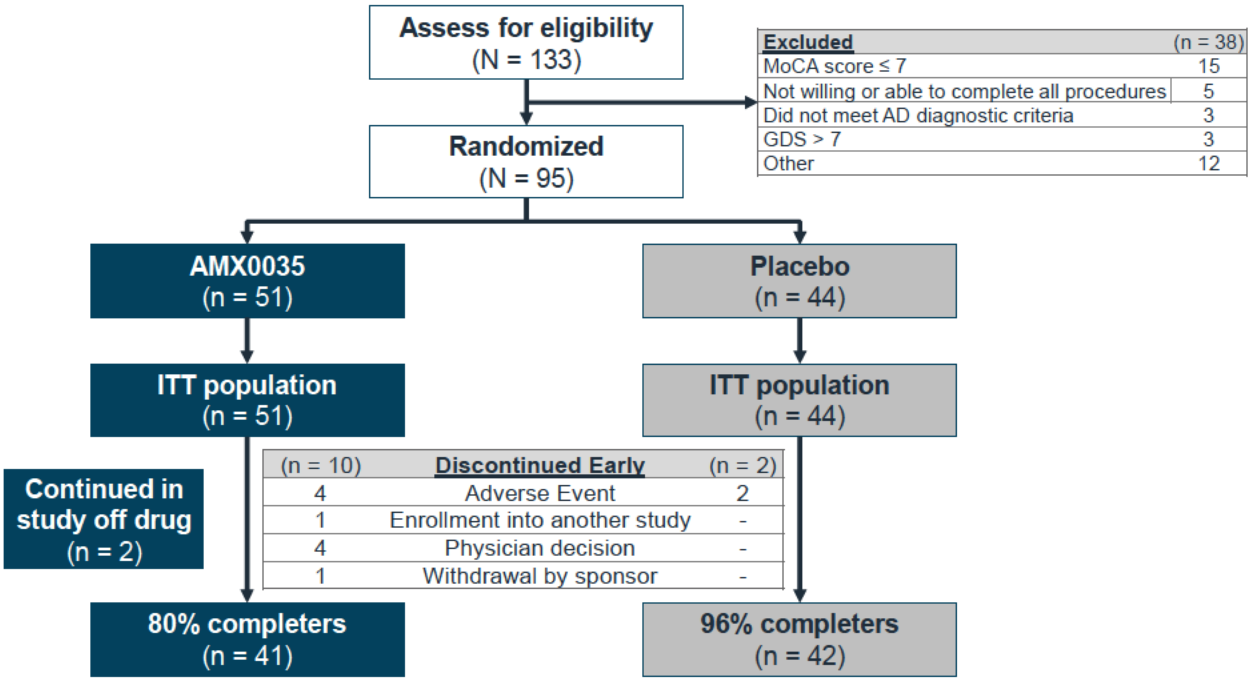
Assay Name	Biomarkers	Vendor	Catalog Number	Dilution Factor
Beta-Amyloid (1-42) Assay	Aβ42	Euroimmun	EQ 6521-9601-L	1:1
Beta-Amyloid (1-40) Assay	Aβ40	Euroimmun	EQ 6511-9601-L	1:21
Total Tau Assay	Total tau	Euroimmun	EQ 6531-9601-L	1:1
P-Tau (pT181) Assay	Phospho-tau 181	Euroimmun	EQ 6591-9601-L	1:1
Neurogranin	Neurogranin	Euroimmun	EQ 6551-9601-L	1:1
Neuro 4-Plex A	GFAP, NfL	Quanterix	102153	1:40
V-PLEX Human Leptin Kit	Leptin	MSD	K151V5D	1:2
U-PLEX Biomarker Group 1 Assay	FABP3, MMP-10, IL-8, IL-15, MCP-1,	MSD	K15067M	1:2
S-PLEX Human IL-6 Kit	IL-6	MSD	K151B3S	1:4
U-PLEX YKL-40 Assay	YKL-40	MSD	K151VLK	1:100
OxiSelect Oxidative DNA Damage ELISA	8-OHdG	Cell Biolabs, Inc.	STA-320	1:2
24(S) Hydroxycholesterol ELISA	24-OHC	Enzo LifeSciences	ADI-900-210-0001	1:2
Insulin Receptor Human ELISA	Soluble IR	BioVendor	RD1991041200R	1:2

7.1 PEGASUS Demographics and Baseline Characteristics

Trial participants

In the PEGASUS trial, a total of 95 participants were enrolled, of whom 51 were randomized to AMX0035 and 44 were randomized to placebo (Figure 7).

Figure 7 Participant Disposition



Abbreviations: AD, Alzheimer's disease; GDS, Geriatric Depression Scale; ITT, intent-to-treat; MoCA, Montreal Cognitive Assessment. *One participant discontinued due to positive COVID-19 test. The other did not provide a rationale for discontinuing study drug.

Baseline demographic and clinical characteristics are shown in Table 9. Biomarker findings were well balanced at baseline.

Table 9 Baseline Demographic and Clinical Characteristics

Characteristic	AMX0035 (n=51*)	Placebo (n=44*)
Age, mean (SD)	70.7 (7.69)	70.7 (7.30)
Male, no. (%)	27 (52.9)	25 (56.8)
White, no. (%)	49 (96.1)	43 (97.7)
APOE ε4 carrier, no. (%) 1 allele 2 alleles	37 (77.1) 29 (60.4) 8 (16.7)	27 (61.4) 18 (40.9) 9 (20.5)
CSF AB_{40}, mean (SD) pg/mL	6374.1 (2411.9)	6601.8 (2786.1)
CSF AB_{42}, mean (SD) pg/mL	330.9 (152.24)	350.7 (196.9)
CSF total tau, mean (SD) pg/mL	502.1 (172.3)	481.3 (188.8)
CSF p-tau181, mean (SD) pg/mL	117.1 (44.9)	106.9 (44.81)
CSF NfL, mean (SD) pg/mL	1321.47 (556.0)	1343.65 (733.4)
CSF GFAP, mean (SD) pg/mL	12234.1 (5932.9)	13348.7 (7902.3)
CSF Neurogranin, mean (SD) pg/mL	466.2 (176.7)	446.0 (206.2)
CSF AB 1-42/1-40 ratio mean (SD) pg/mL	0.05(0.02)	0.05 (0.02)
CSF YKL-40 mean (SD) pg/mL	223932.2 (76102.7)	229273.6 (80806.0)
CSF IL-6 mean (SD) pg/mL	1228.2 (610.2)	1163.5(423)
CSF IL-8 mean (SD) pg/mL	26.1 (6.8)	24.73 (5.8)
CSF IL-15 mean (SD) pg/mL	3.3 (1.0)	3.0 (1.0)
CSF MCP-1 mean (SD) pg/mL	246.4 (75.7)	242.6(64.6)
CSF FABP3 mean (SD) pg/mL	4059.3 (1445.1)	3926.4 (1517.5)
CSF 8-OHdG mean (SD) pg/mL	3.1 (0.7)	3.24 (1.0)
CSF 24-OHC mean (SD) pg/mL	7.38 (2.2)	7.30 (2.7)
CSF MMP-10 mean (SD) pg/mL	65.5 (35.0)	52.50(20.2)
CSF leptin mean (SD) pg/mL	124.9 (84.6)	102.81 (61.3)
CSF sIR mean (SD) pg/mL	4.4 (1.4)	4.47 (1.3)

Abbreviations: AB, amyloid beta;; *APOE* ε4, apolipoprotein E gene ε4 allele; CSF, cerebrospinal fluid; FABP3, fatty acid binding protein 3;GFAP, glial fibrillary acidic protein; IL, interleukin; MCP-1, Monocyte chemoattractant protein-1;MMP-10, matrix metalloproteinase 10;NfL, neurofilament light chain; PB and TURSO, sodium phenylbutyrate and taurursodiol; p-tau, phosphorylated tau; SD, standard deviation; sIR, soluble insulin receptor;8-OHdG,8-hydroxy-2-deoxyguanosine; 24-OHC, 24S-hydroxycholesterol

*Some participants did not complete CSF draws—numbers are included in tables provided with submission.

7.2 Biomarker Results

These full set of biomarker findings are summarized in Table 10.

Table 10 Change from Baseline in CSF Biomarkers after 24 weeks

		Change from Baseline at Week 24			
		AMX0035	**Placebo**	**AMX0035 and Placebo LSMEAN Difference (95% CI)**	**p-value**
Neurodegeneration* (pg/mL)	Total Tau	-64.93	8.82	-73.74 (-106.84, -40.65)	<0.0001
	Phosphorylated Tau (pTau 181)	-14.63	-0.27	-14.36 (-21.51, -7.21)	0.0002
	FABP3	-344.62	102.90	-447.52 (-684.59, -210.45)	0.0004
	Neurofilament Light Chain (NfL)	169.48	63.61	105.87 (-119.74, 331.47)	0.35
Synaptic Function	Neurogranin (pg/mL)	-81.19	-8.34	-72.85 (-110.81, -34.89)	0.0003
Inflammation (pg/mL)	YKL-40	-14635.39	1507.88	-16143.27 (-26995.89, -5290.65)	0.004
	IL-15	-0.02	0.25	-0.28 (-0.49, -0.06)	0.01
	IL-6	644.38	565.93	78.45 (-1042.50, 1199.40)	0.89
	IL-8	1.54	1.17	0.37 (-4.37, 5.11)	0.88
	GFAP	821.68	488.15	333.53 (-2080.17, 2747.22)	0.78
	MCP-1	-1.97	-0.79	-1.18 (-21.15, 18.79)	0.91
Core AD pathology	AB_{42} / AB_{40} ratio	0.0039	-0.0051	0.0090 (0.0029, 0.0151)	0.005
	AB_{42} (pg/mL)	-8.09	-41.46	33.37 (-38.37, 105.11)	0.36
	AB_{40} (pg/mL)	-752.70	-754.81	2.11 (-1007.67, 1011.88)	1.0
Metabolism / Oxidative Stress (pg/mL)	8-OHdG	0.31	-0.13	0.44 (0.13, 0.74)	0.006
	24-OHC	-0.20	-0.07	-0.13 (-0.67, 0.41)	0.63
	Leptin	0.45	4.53	-4.09 (-25.71, 17.54)	0.71

		Change from Baseline at Week 24			
		AMX0035	**Placebo**	**AMX0035 and Placebo LSMEAN Difference (95% CI)**	**p-value**
	sIR	-0.04	-0.19	0.15 (-0.25, 0.55)	0.47
Neurovascular	MMP-10 (pg/mL)	-3.13	-0.92	-2.21 (-8.48, 4.05)	0.48

*Tau and Phosphorylated Tau may also be considered Core AD Pathology

Relevance to ALS

Although the biomarker results of the PEGASUS study are of particular interest to AD as the study was conducted in the AD population, the results on several of these markers also provides potential research insights into ALS, especially as CSF was not collected in the CENTAUR study. CSF total tau levels are markedly increased in both AD and ALS (Wattmo et al., 2020, Scarafino et al., 2018). A 2018 study was conducted involving 85 participants with ALS, 30 participants with ALS-mimicking diseases, and 51 participants with other non-neurodegenerative diseases. In this study, a higher level of total tau was found in CSF in ALS cases compared to ALS-mimicking diseases ($p=0.006$) and other non-neurodegenerative diseases ($p<0.001$). Additionally, CSF levels of total tau correlated with historical rate of disease progression since first symptom (ΔFS score) at the time of spinal tap ($r=0.257$, $p=0.02$) and respiratory function loss as measured by SNIP ($r=0.315$, $p=0.03$). This suggests that total tau could be an important biomarker in ALS (Scarafino et al., 2018).

Phosphorylated tau (pTau 181) has also been shown to be elevated in people with ALS ($p<0.0001$) and to correlate with motor neuron loss ($p=0.017$). Ptau181 levels have also shown correlation with ALSFRS-R scores ($p=0.01$). Ptau181 may additionally be an important biomarker in ALS (Cousins et al., 2022, Scarafino et al., 2018).

YKL-40 (also known as CHI3L1) also has evidence supporting its relevance in ALS. CSF samples from people with ALS have shown elevation in YKL40 compared to age matched controls ($p=0.045$). YKL40 has been associated with inflammatory processes in neurodegenerative diseases (Llorens et al., 2017). YKL-40 shows a correlation with ALSFRS-R progression as measured by ALSFRS-R slope ($p<0.001$) (Andres-Benito et al., 2018).

These results from the PEGASUS study demonstrate that treatment with AMX0035 can improve several CSF biomarkers of neurodegeneration, synaptic function, and inflammation in a neurodegenerative disease. Although the study was conducted in AD, evidence of effect on these biomarkers provides support for the mechanistic activity of AMX0035 against several pathways and potential markers of relevance in ALS.

Results from PEGASUS demonstrate for the first time that treatment with AMX0035 improves several CSF biomarkers in a neurodegenerative condition over a period of 24 weeks. This study supports the clinical mechanistic activity of AMX0035 in the CNS.

8 PHOENIX – ONGOING PHASE III STUDY IN ALS

A35-004 (PHOENIX) is an ongoing Phase III, randomized, double-blind, placebo-controlled, multicenter trial in up to 600 participants to evaluate the safety and efficacy of AMX0035 versus placebo for 48-week treatment of people living with ALS. Amylyx is fully committed to the rapid and robust completion of this study which will provide critical information to clinicians and those living with ALS. Amylyx also committed to the completion of this study as part of the approval of AMX0035 by Health Canada.

At the 30 March 2022 Advisory Committee meeting, Amylyx shared that approximately 150 participants had been recruited into PHOENIX (there are now >350 participants as of 05 August 2022 and Amylyx will update recruitment numbers for the 07 September 2022 meeting).

The primary objective of PHOENIX is to assess the impact of AMX0035 treatment compared to placebo on disease progression over 48 weeks based on change from Baseline of ALSFRS-R and survival.

Additionally, an open-label extension (A35-011) will allow previous PHOENIX study participants (continued) access to AMX0035 for an additional 108 weeks (156 weeks treatment) as well as provide additional evaluation of longitudinal safety and efficacy of AMX0035 treatment.

The PHOENIX study and the open-label extension are intended to further confirm the robust findings from CENTAUR; however, as outlined in Table 11, a final CSR would not be available for submission until mid-to-late 2024.

AMX0035 met its primary outcome in a placebo-controlled study and has shown survival benefits now further strengthened by new analyses. Amylyx has already committed to providing the PHOENIX data in the post-marketing setting in Canada and would make the same commitment in the United States.

Table 11 Key Dates and Milestones for PHOENIX

MILESTONES	DATE
FPI (Randomized)	28 Oct 2021 (Actual)
US last consented (US recruitment terminated based on ongoing NDA process)	04 Mar 2022 (Actual)
Completion of Recruitment – Target	Early 2023 (Projected)
Last participant Visit 48-week treatment – Target	Late 2023/Early 2024 (Projected)
Database lock for 48-week treatment – Target	Early/Mid 2024 (Projected)
Top-line data – Target	~Mid 2024 (Projected)
Final CSR – Target	Mid/Late 2024 (Projected)

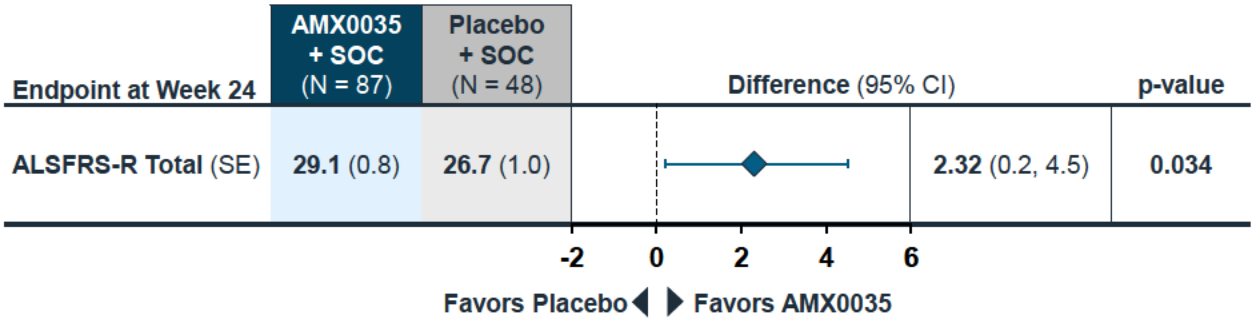
CSR, clinical study report; FPI, first participant in; NDA, new drug application

9 CONCLUSIONS

Novel therapies can be approved on the basis of a single positive study combined with confirmatory evidence. Amylyx believes the CENTAUR study data presented meets this standard.

The CENTAUR study met its prespecified primary outcome at 24 weeks, and AMX0035 demonstrated a robust and clinically meaningful change on the most widely used scale in ALS, the ALSFRS-R (Table 12).

Table 12 ALSFRS-R Total Score; Primary, Prespecified Analysis



A placebo-controlled analysis of survival in the ITT population found a benefit on survival which may be considered confirmatory evidence. This data is now further strengthened by new analyses as follows (Table 13):

- The survival result when accounting for the placebo crossover to active drug was larger and estimated at a 9.7-month median survival benefit (RPSFTM).
- Comparing the OS benefit versus two external controls using two different statistical methodologies provides further evidence of benefit. Importantly, these two new methodologies show concurrence in their estimates of Overall Survival benefit compared to the RPSFTM analysis.
- In the context of prior survival findings that have supported drug approvals, the survival results compare favorably.
- New evidence has also been provided supporting the neurobiological effect of AMX0035.

Table 13 Overall Survival – Consistency of Results by Different Methods

Analysis Method	Control Group Median OS (months)	AMX0035 Median OS (months)	Difference (months)	Hazard Ratio	p-value
ITT OS (placebo controlled)	18.7	23.5	4.8	0.64 (0.4, 1.0)	p<0.05
Analyses Supporting the Findings on ITT Overall Survival					
RPSFTM	13.8	23.5	9.7	0.42 (0.18, 0.99)	p<0.05*
ENCALS Natural History Comparison	13.6	23.5	9.9	0.28 (0.19, 0.41)	p<0.0001
Propensity Score Matching to PRO-ACT	12.5	23.5	11.0	0.49 (0.32, 0.70)	P<0.0002

*note that RSPFTM analyses use the p-value from the initial ITT OS analysis

The placebo-controlled survival benefit in the ITT population provides important confirmatory evidence for the clinical benefit of AMX0035 and new analyses, including comparisons to external controls, confirm and strengthen this finding.

CENTAUR is a single adequate and well controlled study with confirmatory evidence that meets the appropriate standards for approval especially in the context of a rare and fatal disease such as ALS.

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