AMX0035 Clinical Overview-AC Script

SLIDE 1

My name is Veneeta Tandon. I am a clinical reviewer in the Division of Neurology I for the New Drug Application for AMX0035.

SLIDE 2

This presentation includes the Agency's clinical overview of efficacy and safety of AMX0035.

SLIDE 3

AMX0035 is a fixed dose combination of 3 grams of sodium phenylbutyrate and 1 gram of taurursidiol, or commonly known as TURSO or TUDCA. AMX0035 is formulated as a powder for oral suspension packaged in sachets.

The proposed dosing regimen includes a starting dose of 1 sachet once daily for 21 days and a maintenance dose of 1 sachet twice daily.

SLIDE 4

ALS is a progressive neurodegenerative disease characterized by the death of motor neurons. While there are several mechanisms of motor neuron death in ALS patients, the Applicant stresses that dysfunction in the endoplasmic reticulum and mitochondria may play a key role motor neuron death and the pathophysiology of ALS. Phenylbutyrate is proposed to ameliorate endoplasmic reticulum stress through upregulation of chaperone proteins. TURSO is proposed to ameliorate mitochondrial stress by reducing mitochondrial permeability and increasing the apoptotic threshold of the cell. The combination of the two is postulated to have a synergistic effect that can reduce neuronal death by simultaneous inhibition of endoplasmic reticulum and mitochondrial stress

FDA notes that the pathophysiology of ALS is unknown, but likely involves multiple complex processes and pathways. The mechanism of ameliorating endoplasmic and mitochondrial stress is but one of a number of potential processes hypothesized to be involved in the pathophysiology of ALS.

SLIDE 5

The Applicant has conducted a single randomized, double-blind, controlled Phase 2 study followed by an open label extension in support of this Application.

Study AMX3500, also named CENTAUR, was a 24-week randomized, double-blind, placebo-controlled study in 137 ALS patients conducted in the United States. This double-blind study

was followed by an optional 132-week open label extension study. A total of 90 patients entered the extension phase.

SLIDE 6

A 48-week pivotal randomized, double-blind, controlled study in 600 patients is ongoing with full results anticipated in late 2023 or early 2024. The primary endpoint planned in this study will be a joint assessment of ALSFRS-R total score progression and survival over 48 weeks.

SLIDE 7

I will now give a brief overview of the CENTAUR study.

SLIDE 8

CENTAUR was a multicenter, randomized, double-blind, placebo-controlled study conducted in the United States.
A total of 137 patients were randomized 2:1 to AMX0035 or placebo, where 89 patients received AMX0035, and 48 patients received placebo for 24 weeks. Patients received 1 sachet twice daily orally, or via feeding tube, as tolerated during the maintenance phase.

The study enrolled patients 18-80 years of age with a definite diagnosis of sporadic or familial ALS who were less than or equal to 18 months since onset of their first ALS symptom. If on riluzole, patients were required to be on stable doses for at least 30 days prior to enrollment. Riluzole naïve patients were permitted in the study. Edaravone was approved in the US in May 2017, after the CENTAUR Study was initiated, and patients were allowed to initiate edaravone after study entry. Some patients who enrolled later in the study may have also been on a stable dose of edaravone at baseline.
After the completion of 24 weeks, patients could enroll in the optional, open label extension study within 4 weeks of completion of the 24-week period.

SLIDE 9

I will now discuss the clinical endpoints used in CENTAUR Study. The primary endpoint for CENTAUR was the rate of decline or the slope of change in ALS Functional Rating Scale-Revised (ALSFRS-R) scores at Week 24. The ALSFRS-R measures 12 functional activities in 4 domains including bulbar, breathing, fine, and gross motor domains. Higher scores on the ALSFRS-R indicate better performance.

FDA agrees that ALSFRS-R is an acceptable primary endpoint to measure functional change in ALS. However, rate of decline is not generally the appropriate approach to analyzing treatment

effect as it assumes that the change in ALSFRS-R is linear over time, which has not been established. Additionally, it does not account for loss of data due to death of patients during study. A joint rank analysis is recommended if there are deaths during the study. FDA had advised sponsor to use a combined analysis of function and mortality, such as the joint rank, at the pre-IND meeting in 2016 and again when reviewing the statistical analysis plan in 2019. This recommendation is included in the FDA Guidance on Drug Development in ALS.

This concern will be discussed further in the FDA Statistical presentation.

SLIDE 10

Secondary endpoints are also analyzed using the primary slope model and have similar concerns of the linearity assumption.

The key secondary endpoint in the study was the rate of change in Accurate Test for Limb Isometric Strength (referred to as ATLIS in this presentation). ATLIS is a measure of static muscle strength in each limb. ATLIS can give Total, Upper Extremity, or Lower Extremity ATLIS scores. It was not specified in the Statistical Analysis Plan which of these would be the key secondary endpoint.

The second secondary endpoint was a rate of change in plasma neurofilament heavy chain, a potential biomarker of neuronal degeneration and neuronal axonal injury at Week 24. It may be hypothesized that a therapy that shows benefit in the treatment of ALS may also decrease pNF-H levels.

SLIDE 11

Third in the hierarchy was a rate of change in Slow vital capacity or SVC at Week 24. SVC is a measure of respiratory function in ALS.

Survival, defined as rate of death, tracheostomy, permanent assisted ventilation, and hospitalizations at week 24 was last in the hierarchy of secondary endpoints in this study. Inclusion of tracheostomy and hospitalizations in the definition of survival is problematic as there is considerable variability 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, or in anticipation of future need for ventilatory support.

SLIDE 12

Next, I would like to give brief summary of key regulatory interactions with the Applicant regarding the efficacy analyses.

At the pre-IND meeting on March 21, 2016, the Division recommended the Applicant use a joint-rank analysis of survival and change from baseline in ALSFRS-R for the proposed CENTAUR Study.

In the initial SAP dated March 6, 2019, the Applicant proposed a slope analysis of ALSFRS-R. The Agency again recommended that "if there are deaths, then the joint rank analysis of function and survival should be the primary analysis". Given the choice to use a slope analysis which relies on linearity, FDA also recommended a backup analysis plan if change from baseline in the ALSFRS-R was non-linear over time.

The Applicant provided responses to these comments on August 26, 2019 and submitted a revised SAP on October 15, 2019. The Agency did not review the revised SAP prior to unblinding in November 2019.

Subsequently, 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 whether the results of study could serve as a single trial that is able to independently demonstrate substantial evidence of effectiveness. The Division recommended the Applicant begin work on a second efficacy study.

In April 2020, a new supplementary SAP for survival was submitted for the post hoc survival analysis of the open-label extension study, after the original study was unblinded. The submission of this SAP occurred after the double-blind period was unblinded and after preliminary survival analyses of data had been viewed and presented at the March meeting. Subsequently, in May 2020, the applicant provided topline results from an initial post hoc survival analysis based on a vital status sweep of the data, with a cut-off date of February 29, 2020.

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.

A pre-NDA meeting was held in July 2021. The Division invited the Applicant to request a pre-NDA meeting and submit the NDA expeditiously so that we could more critically evaluate the claims of the survival benefit.

SLIDE 13

In the forthcoming slides, I will discuss the efficacy results and potential concerns identified during the Agency review of the data for the controlled and open label phase of CENTAUR.

The details of the statistical analyses will be presented by Dr. Massie in the FDA Statistical Presentation.

SLIDE 14

137 randomized patients constituted the ITT population, which included all participants who were randomized and received at least 1 dose of study medication. The mITT included all participants who were randomized, dosed, and had at least 1 post-baseline efficacy assessment. Two patients in the AMX0035 arm did not have post baseline efficacy evaluations and were excluded, for a total of 135 patients in the mITT population.

20 patients discontinued from the AMX0035 treatment arm with a total of 67 patients completing the study, and 10 patients discontinued from the placebo arm, with 38 patients completing the study. Among those who completed the 24 week study, 8 patients (7 in the treatment arm and 1 in placebo) discontinued the study drug but remained in the study. Most of the discontinuations were due to patient decision.
Patient decision included discontinuation due to adverse events, disease progression, and "termination of participation". We note that there were additional deaths in the 24-week study that were not recorded as a disposition event if death was recorded after patient withdrawal from study.

SLIDE 15

There were no imbalances observed in the baseline demographic characteristics of the patients in the study.

SLIDE 16

There were a few imbalances in the baseline disease characteristics. Disease characteristics that favor the treatment arm are shown in red on this slide. This includes a higher baseline ATLIS score in the AMX0035 group which may indicate that those patients may have been stronger at baseline. On the other hand, baseline characteristics favoring the placebo arm include a higher percentage of patients with limb-onset ALS in the placebo arm and higher percentage of patients on concomitant ALS medication at baseline in the placebo arm, shown in blue in the Table. Clinical relevance of difference in family history is unclear.

No clinically meaningful difference between groups were observed in other disease characteristics that include- Time since symptom onset and ALS diagnosis, rate of ALSFRS-R decline, baseline ALSFRS-R, SVC.

FDA notes that in a small trial such as this, baseline imbalances are more likely to occur than in larger randomized controlled trials, and such imbalances are exaggerated by the 2:1 randomization. As a result, the groups may be poorly matched, baseline prognostic differences

are possible, and such differences need to be taken into account in the interpretation of the study results.

SLIDE 17

There were a few issues during the conduct of the study that are notable in our review.

We note that there was a randomization implementation error, such that the first 18 patients that is 13% of the overall sample size were assigned to drug arm because of a shipping error which resulted in the unavailability of placebo doses. The subsequent 9 patients were then all assigned to placebo. The unblinded statistician was aware of this problem and attempted to adjust the pre-planned randomization schedule to fix this problem. It is unclear the impact this may have had on the outcome of the study, but will be discussed further in the statistical presentation.

Additionally, edaravone was approved after the study was initiated, and therefore, patients were allowed to start edaravone during the study. There was an imbalance in the number of patients in each arm initiating new treatment with edaravone during the study. A higher proportion of patients started edaravone after the baseline assessment in the AMX0035 arm compared to the placebo arm. This post-baseline starting of ALS medications is a possible confounder of the primary analysis.

Finally, FDA notes that the active drug contains a bitter taste and causes transient gastrointestinal symptoms, such as diarrhea and abdominal pain, that are more frequently reported in the first three weeks after initiation. Although a bittering agent was added to mask the placebo in the double-blind treatment period, there were still a number of patients who were able to correctly guess which treatment they had received on exit interviews. The potential for diarrhea and bitter taste were described in the informed consent, which may have alerted patients to these symptoms and potentially could have led to functional unblinding during the study.

These are potential review issues we have concerned in our analysis of the study results.

SLIDE 18

I will now give an overview of the Applicant's primary efficacy analysis on ALSFRS-R at week 24. The Applicant reports a statistically significant mean treatment difference of 2.32 points in favor of AMX0035 on the ALSFRS-R rate of decline between the treatment arm and placebo in the mITT population with a modest p-value of 0.034.

FDA concerns on the primary analysis are summarized in the blue box on this slide. Details of these will be discussed in the FDA Statistical presentation.

Briefly, I note that the Applicant's primary slope analysis assumes linearity of ALSFRS-R over time which is not established.

An analysis which combines function and survival, such as the joint rank, is critical to understand study results when there are deaths in the study. There were 7 deaths during the study (5 on AMX0035 and 2 on placebo). The primary analysis does not include these deaths that occurred during the study, which can confound the results of a functional analysis because there is loss of data. A joint-rank analysis was recommended to the Applicant if deaths were observed during the 24-week study.

Additionally, applicant's analysis is on the mITT population, which is generally the recommended analysis population. However, there were 2 deaths, both patients on AMX0035, that did not have post baseline ALSFRS-R scores and were therefore excluded from the analysis. This can lead to bias and sensitivity results on the ITT become more important.

Another concern is that there is considerable missing data on alive patients at week 24, in addition to the significant dropouts during the study and the 7 patients that stayed in the study but discontinued from the treatment.

SLIDE 19

I will now give an overview of the secondary endpoints of the CENTAUR study.

On the pre-specified slope analysis of rate of change of ATLIS, the Applicant reports a nominally significant treatment difference of 4.3 points in favor of AMX0035 with a p-value of 0.0420 in the Upper Limb Strength or Upper ATLIS and a non-significant difference of 2.1 and 2.8 points was observed in Lower and Total ATLIS scores. As mentioned earlier, the ATLIS scores can be reported as Upper ATLIS, Lower ATLIS, and Total ATLIS scores.

The Statistical Analysis Plan did not pre-specify which ATLIS component would be analyzed first. Only the Upper ATLIS score was nominally positive.

In addition, there are concerns on the baseline imbalances in the Total ATLIS Score which, as you can see here, is completely driven by imbalance in the Upper ATLIS score by 3.3 points that favored the AMX0035 group. These differences at baseline could have led to proportional slower decline in the AMX0035 group. These concerns suggest weak support from the key secondary endpoint, ATLIS.

SLIDE 20

Additionally, there is limited support from other secondary endpoints.
There was a statistically non-significant treatment difference of 32.7 pg/mL in favor of placebo for the rate of decline in pNF-H with a p value of 0.260.
There was a statistically non-significant treatment difference of 5% in favor of AMX0035 for rate of decline in SVC with a p-value of 0.076
There was no survival benefit observed in the first 24 weeks of the study.

These endpoints were also slope analyses and assume linearity in change and therefore have similar concerns as the primary endpoint.
The change observed in the rate of decline in pNF-H favored placebo.

The small numerical trend in rate of decline in SVC in favor of AMX0035 is not statistically significant and is not consistent with a clinically meaningful change in SVC.
There were no statistical differences between treatment groups in percentage of events for the survival outcomes including: Death events only, Death or Death Equivalents that include tracheostomy and permanent assisted ventilation, and Hospitalizations. There was only one placebo subject that had a tracheostomy with associated permanent assisted ventilation.

SLIDE 21

Summary of concerns from double-blind treatment phase include

- Modest results on primary endpoint with limited support from any secondary endpoints
- No survival benefit at 24 weeks
- Appropriateness of Applicant's primary efficacy analysis
- Baseline imbalances in disease characteristics
- Issues during study conduct including
  - Integrity of randomization
  - Imbalances in edaravone use post-baseline
  - Potential unblinding due to gastrointestinal adverse events and bitter taste of the drug

SLIDE 22

Now I will review the open-label extension study and results.

SLIDE 23

Enrollment into the open-label extension was optional after the completion of the 24-week double blind phase of the CENTAUR. Only 90 out of the 137 patients, that is 66%, enrolled in the 132-week open label extension phase named AMX3500OLE. A total of 56 of the patients randomized to AMX0035 enrolled in the extension study referred to as RA group, and 34 of placebo patients transitioned to AMX0035 in the extension study referred to as RP group. A total of 34 % of the patients did not enter the open label extension phase, including 37 % of the AMX0035 treated patients and 29% of the placebo treated patients did not enroll in the study.

Note that a higher percentage of AMX0035 treated patients did not enroll in the extension study.

SLIDE 24

The primary objective of this study was to assess safety with secondary objectives to assess efficacy at Week 48. Most patients discontinued from the open-label extension study at various times, with only 2 patients who completed treatment up to Week 132. This Table includes the reasons for discontinuation in the OLE.

Please also note that only 55 out of the 90 patients who enrolled in the extension study remained at Week 48, that is Week 24 of this study when the efficacy analyses were performed. This included 36 out of 56 RA patients and 19 out of 34 RP patients at week 48.

SLIDE 25

The primary endpoint for the AMX3500-OLE was safety.

ALSFRS-R, ATLIS and SVC extended slope analysis at Week 48 were the secondary endpoints in the open label extension. In addition, survival measured as rate of death, tracheostomy, permanent assisted ventilation, and hospitalizations at week 48 was an additional secondary endpoint in this study.

FDA has similar concerns on the extended slope analyses as in CENTAUR and similar concerns on inclusion of tracheostomy and hospitalization in the definition of survival due to differences in clinical practices.

SLIDE 26

I will now give an efficacy overview from the open label extension phase of CENTAUR in the subsequent slides.

SLIDE 27

The primary endpoint for the OLE phase was safety. The first efficacy endpoint was the ALSFRS-R Extended slope analysis at week 48. The Applicant reports a statistically significant extended slope analysis in favor of those randomized to AMX0035 group, referred to as the RA group with a p-vale of 0.0239.

However, we note that these open-label efficacy results on a functional endpoint are difficult to interpret. Enrollment in the OLE was optional. Only 66% of the subjects enrolled in OLE – that included 56 AMX0035-treated subjects and 34 placebo subjects. There was higher non-participation in the OLE in AMX0035 group.
There was no indication in the protocol that the blind to original treatment was to be maintained, or who among the patients, investigators, and site personnel were to remain blinded. Additionally, there is a potential for unblinding to treatment because patients may have experienced GI adverse events and bitter taste of the drug upon transition from placebo to active treatment. It is notable that 75% of patients who had received placebo in the double-blind treatment period correctly identified that they had received placebo when asked in the OLE.

There were additional discontinuations during the open-label phase with only 40% patients that remained in the study at Week 48.

Additionally, there were 23 deaths by Week 48, which are ignored in the slope analysis at Week 48.

SLIDE 28

The Applicant reports a statistically significant extended slope analysis in favor of those randomized to AMX0035 group (RA group) for upper ATLIS and SVC with a p=0.029 and 0.0372, respectively.

FDA has similar concerns regarding the interpretability of the open-label extended slope analysis for upper ATLIS and SVC at Week 48 as that for ALSFRS-R summarized in the previous slide.

SLIDE 29

I will now give an overview of the survival analyses. Overall survival analyses from the initial randomized phase compares patients randomized to AMX0035 referred to as RA group to patients randomized to placebo referred to as RP group up to the 132-week open label extension phase.

Applicant's pre-specified survival analysis included a composite time to survival event analysis including death, tracheostomy, Permanent assisted ventilation, hospitalization.

Additional pos-hoc survival analysis including time to death alone was also performed after CENTAUR study was unblinded.

SLIDE 30

I first discuss Applicant's composite survival analysis reported in the Application with the March 1, 2021 cutoff date.

The Applicant reports a statistically significant increase in the composite time to survival events (including death, tracheostomy, Permanent assisted ventilation, and hospitalization) in the RA group compared to RP group in the mITT population with a difference of 4.8 months, hazard ratio of 0.62 and p value of 0.0196.

The composite time to survival endpoint was specified in the protocol but when it would be performed was not specified.

Survival analyses were done after multiple data cutoff dates including:

25 September 2019, 29 February 2020, 20 July 2020 and 01 March 2021

Professional firm, Omnitrace, was contracted to conduct a search based on subject's family, clinic notes, CDC national death index, social security index.

SLIDE 31

I will now point out the limitations of the Applicant's composite survival analysis.

The Applicant's composite survival analysis is difficult to interpret as there were a large number of dropouts during OLE, in addition to 34% non-participation in OLE.

There are limitations of including tracheostomy and hospitalization data in a composite survival endpoint due to subjectivity involved in timing of tracheostomy placement and hospitalization due to differences in standard of care. These were not systematically collected in the OLE. Additionally, there may be missing data on tracheostomy and hospitalizations after subjects terminated from the study, which were not captured in vital status sweeps. In general, a rigorously-defined outcome of permanent-assisted ventilation would have also been acceptable to be included in the definition of survival, because there is less subjectivity regarding this definition. However, this data was not available for many patients in the vital status check.

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

Subsequently, there were several vitals status sweeps after the September 2019 initial analysis. The Vital Sweeps were completed after most subjects discontinued from the study.

There were also additional deaths that occurred after March 1 that were not counted in the reported analysis. Inclusion of these deaths changes the statistical analysis of survival, and illustrates the notion that in a small study such as this, a shift in a few deaths and the timing of the analysis can make a difference.

SLIDE 32

The key concerns on the efficacy results reported by the Applicant from the open label extension include the low participation in the open label extension with only 66% of patients from the controlled study enrolling in the OLE, additional drop out of patients during the course of the open-label phase, and additional deaths during the study that were not accounted for in the functional analyses on ALSFRS-R, ATLIS, SVC that used a model similar to the primary linear slope analyses.

This renders it difficult to interpret the efficacy data on ALSFRS-R, ATLIS, SVC, and the composite survival analyses.

Of note, the ALS Guidance does recommend additional efficacy analyses can be conducted in the open-label extension. However, it is recommended that in order to make those analyses the most interpretable, sponsors should encourage participation in the OLE, have regularly scheduled visits to maintain close follow-up, and maintain the blind to original treatment, in addition to pre-specifying effectiveness assessments prior to initiation of the study, with the appropriate method for Type I error control.

SLIDE 33

The Applicant further reports post hoc survival analysis including time to death only.

The Applicant reports a statistically significant survival benefit on a supplemental time to death only analysis showing a median difference of 4.8 months, HR=0.644, p=0.0475 in the ITT population, and a median difference of 4.8 months, HR=0.62, p=0.0324 in the mITT population.

The Applicant reports the p-values from the Cox proportional hazard model instead of the pre-specified likelihood ratio test which gives a larger p-value.

Details will be discussed in the Statistical presentation, with additional reviewer analyses.

SLIDE 34

The overarching question is whether the noted survival benefit of 4.8 months is by chance alone or due to the underlying disease heterogeneity?
This is a small study which had baseline disease imbalances in the treatment groups. There was limited enrollment in the open-label extension, and time to death/survival was not prespecified endpoint, and is an exploratory analysis of the data. Therefore the borderline significant p-value is not persuasive for a treatment benefit.

Additionally, there is no correlation between duration of drug exposure and survival. There are many patients in the survival analysis who were randomized to drug based on initial randomization, but who dropped out of the study and did not take the drug for very long, but are still contributing to the overall survival benefit.

When looking at median survival in alive patients, patients on placebo who never received drug survived for a median 1295 days, and patients who received AMX0035 for > 96 weeks also had a median survival of 1237 days.

SLIDE 35

The results of the 24-week double-blind controlled CENTAUR is not persuasive for the establishment of efficacy based on a single study. This was a small study and baseline imbalances occurred; as with any small trial, an impact of these imbalances on the outcome cannot be excluded. Additionally, there are issues that have been identified with the conduct of the study, such as the randomization implementation error, which may have further exaggerated the baseline differences, and potential for some unblinding. The Agency also does not believe that the most appropriate methods were used for the statistical analyses.

Results of the primary endpoint are not robust and secondary endpoints are not generally supportive of the primary endpoint. In addition, the overall survival analysis including the 132-week open label extension phase do not provide compelling survival benefit for the reasons noted on the previous slide.

In summary, the positive findings of this small Phase 2 study do not translate to robust support for a drug effect in patients with ALS.

SLIDE 36

I will now give an overview of AMX0035 safety profile

SLIDE 37

Overall, 137 patients (including placebo patients) provided safety data in combined controlled and open label extension phase
123 patients were treated with AMX0035 for less than 6 months.
76 patients were treated with AMX0035 in the combined control and open label phase for 6 months
43 patients received AMX0035 for >1 year in the open label extension
23 patients received AMX0035 for >1.5 years in the open label extension
13 patients received AMX0035 for >2 years in the open label extension

SLIDE 38

There were no significant safety concerns with AMX0035 at proposed dose.

There were no differences in fatal or serious adverse events between AMX0035 and placebo. Most fatal or serious adverse events were secondary to complications of ALS progression and treatment procedure.

Patient discontinuations were higher in AMX0035 group of 20% compared to placebo of 10%. Discontinuations were largely driven by higher incidences of diarrhea, abdominal pain, nausea and dysgeusia in the AMX0035 group.

SLIDE 39

Common TEAEs belonged to the gastrointestinal SOC (including diarrhea, abdominal pain, nausea, salivary hypersecretion). Others common TEAES included dizziness, disease progression, respiratory tract infection, fatigue, and dyspnea.

There were no differences in laboratory abnormalities, vital signs, electrocardiograms, QTc, or suicidality between AMX0035 and placebo-treated participants.

SLIDE 40

In the open label phase, common TEAES were similar to those seen in the double-blind treatment period. Deaths and SAEs were primarily related to complications of underlying ALS or disease progression. Discontinuations were higher in the patients switching from placebo. 44% of the patients who switched from placebo to AMX0035 in the open label extension discontinued due to adverse events. This suggests a potential for functional unblinding to original treatment in this group of patients.

SLIDE 41

This slide shows the common TEAES in greater than 5% of AMX0035 treated patients and with greater than 1% difference compared to placebo. The common AEs include diarrhea, abdominal pain, nausea, respiratory tract infection, fatigue, dyspnea, salivary hypersecretion, dizziness, decreased appetite, dysarthria, proteinuria, arthralgia, and decreased weight.

SLIDE 42

This slide shows the total percentage of subjects with any gastrointestinal TEAEs over the study and shows a higher incidence in the AMX0035 arm in the first 3 weeks of the treatment compared to placebo. As previously mentioned, this also raises concern for the potential for unblinding of patients during the study, as well as upon transition to the open-label phase of the study.

Script for FDA 3_Statistical Considerations-Tristan Massie

Slide 1:

The following slides detail the statistical issues identified in the review of NDA 216660

Slide 2:

FDA guidance indicates that A Single trial (to establish effectiveness should demonstrate a “clinically meaningful and statistically very persuasive effect” Also, it should include “close scrutiny of trial conduct, including, for example, completeness of follow-up, methods of analysis, imputation of missing data, evaluation of trial endpoints, is critical” There is Uncertainty about the results from the single efficacy trial (and its OLE) of AMX0035. Therefore, the Division advised another phase 3 study was needed at (3/2020 and 2/2021 meetings) in order for the efficacy of AMX0035 to be established.

Slide 3:

The AMX3500 study was a Multi-center, randomized, double-blind, placebo-controlled, superiority study with an open label extension in adult patients with ALS . The study included two treatment groups: the AMX0035 (combination product) and placebo in a 2:1 randomization ratio, drug to placebo. Key efficacy outcomes were collected at Weeks 3, 6, 9, 12, 15, 18, and 24. The Primary Endpoint: was ALSFRS-R at Week 24

Slide 4:

The key issues with this application are first, the single study with evidence in the primary analysis that is not persuasive, with p=0.034 and a corresponding Week 24 difference of 2.32 points on the 48 point ALSFRS-R scale. Second, there are issues with study conduct and analysis assumptions, and many sensitivity analyses provide less persuasive results than primary analysis. In particular, there were issues with randomization implementation and imbalances in use of concomitant ALS medications riluzole and edaravone, Additionally, there are issues with the handling of deaths or lack thereof and missing data assumptions in the primary analysis. Also, the primary analysis assumption of linearity over time in treatment effect is questionable based on the observed data and the prespecified analysis plan. Furthermore, secondary endpoint results are not compelling. Finally, with regards to the open label extension, survival analyses for time to death alone are exploratory and not persuasive.

Slide 5:

There are two key analysis populations for the study. First, the intent-to-treat or ITT population, defined as all randomized patients who received at least one dose of study drug. Second, the modified ITT population, denoted mITT, defined as all randomized patients who received at last one dose of study drug and had at least one post-baseline ALSFRS-R assessment. The primary analysis was a mixed model with ALSFRS-R linearity (slope) assumption in the mITT population. The model fixed effects included an intercept, Week (corresponding to the slope), and interactions between the retrospective pre-randomization slope and Week as well as between patient age and Week and finally between treatment group and Week. The model also included Random effects i.e., (random adjustments) to the group intercepts and slopes for individual patients. This model assumes missing ALSFRS-R data is missing at random (including for deaths before Week 24).

Slide 6:

Here's a timeline of key events for the AMX3500 study. March 6, 2019: FDA finalized comments on the statistical analysis plan for AMX3500 which were sent to the Applicant. On October 15, 2019: a Revised, final SAP was submitted by the Applicant, On November 5, 2019: a Final separate SAP for the OLE was submitted by Applicant.The applicant reported that November 26, 2019: was the date of unblinding of the double-blind period data. On December 16, 2019: the applicant made a Press release citing positive double-blind results. On March 12, 2020: there was a Type C meeting between the Applicant and FDA. In addition to reporting top line results for the double blind period at this meeting, the applicant also reported an analysis of the survival composite endpoint as well as time to death or death equivalent for the OLE data including death or death equivalent events through September 25, 2019. On April 1, 2020: the applicant Submitted a supplemental OLE SAP for survival dated March 27, 2020. Finally, March 1, 2021: is the death event cutoff in the applicant's final Survival status sweep informing the current OLE survival analyses.

Slide 7:

Noteable FDA comments sent to the applicant during the IND stage regarding the statistical analysis plan (SAP) included: the Need to specify the estimand for the primary analysis including how to handle intercurrent events such as death. This included a recommendation for a joint rank analysis of function and survival being the primary analysis. The Importance of backup/sensitivity analyses for missing data and linearity assumptions was also conveyed.The Applicant provided responses to these comments on August 26, 2019 (including disagreeing with the joint rank analysis as primary) and later a revised SAP which was received on October 15, 2019.

Slide 8:

There was a randomization implementation issue in the AMX3500 study. In particular, the first 18 patients in a row, reportedly all received drug, due to a shipping problem resulting in unavailability of placebo doses at sites. The Unblinded Data Monitoring Committee statistician noticed this at the first meeting of the study DMC and made changes to adjust the randomization, including the next 9 patients in a row all receiving placebo. The Applicant has reported as-treated results for those affected by this shipping issue, not as-randomized results. This weakens the integrity of the results slightly as the validity of the analysis rests on using the as randomized intent-to-treat assignments. The applicants sensitivity analyses excluding the patients affected by this randomization/shipping issue have slightly less favorable pvalues, with the open label time to death analysis losing nominal significance.

Slide 9:

This slide details the treatment group imbalances in use of concomitant ALS medications Edaravone and Riluzole observed for the study. At baseline there was a higher proportion of the placebo group using edaravone 50% vs. 25% as well as a higher proportion of the placebo group using riluzole 77% placebo vs. 68% for drug at baseline. On the other hand, post-baseline initiation of the ALS medications Riluzole and Edaravone was higher in the drug group, 16% for drug vs. 4% for placebo. This excess of ALS treatment intercurrent events in the drug arm may affect the interpretation of study results ,I.e., whether the treatment group difference is only due to the experimental treatment.

Slide 10:

The applicant's primary analysis did not account for deaths in the first 24 weeks which occurred at a 5.6% rate in the drug group and 4.2% in placebo. This creates a potential for corresponding bias in the primary analysis. It is more appropriate to combine survival and function, considering death as unfavorable outcome, such as with a joint rank analysis The MITT population used for the applicant's primary analysis excluded all patients without post-baseline visits , thus excluding 2 deaths on drug occurring prior to post-baseline visits. Therefore, sensitivity analyses in the ITT population are particularly important.

Slide 11:

There was considerable missing ALSFRS-R data at Week 24 in the study, 17.4% for placebo and 17.9% for drug among those who survived to Week 24. The applicant's primary analysis relied on a missing-at-random assumption for this missing data. The applicant's sensitivity joint rank analysis for which no details were prespecified in the analysis plan relied on a last observation carried forward method for handling missing data in survivors. LOCF relies on an unrealistic assumption of no worsening after dropout, which is especially unrealistic in a progressive disease like ALS, and LOCF also does not appropriately capture statistical uncertainty in missing values. The FDA reviewer used a missing at random based multiple imputation approach in this reviewer's implementation of the joint rank analysis. Multiple imputation captures some of the uncertainty in missing values but this analysis still involved the strong and unverifiable missing at random assumption.

Slide 12:

As shown here in the table of joint rank analysis results The FDA analysis incorporating deaths via joint rank test provides less persuasive evidence. The FDA analysis included the 2 ITT deaths not included in the mITT population and used multiple imputation under a missing at random assumption for missing data rather than last observation carried forward which is only valid under a more restrictive missing completely at random assumption. The applicant's implementation of the joint rank also ranked the covariates of age and pre-randomization slope in the analysis of covariance of the joint ranks used to determine the p-value. This ranking of covariates was not prespecified as no details of the sponsor's joint rank implementation were and the FDA reviewer noted that analyses without ranking these covariates tended to produce slightly higher p-values, but for consistency the FDA reviewer's reported analysis also ranked covariates. Note that the Applicant's alternative prespecified sensitivity analysis for deaths (left censored slope analysis) is not shown here because it is problematic and thus inconclusive, as was detailed in the briefing package.

Slide 13:

This slide shows that Quadratic and mean-per-visit repeated measures models suggest potential non-linearity of ALSFRS-R over time and optimistic bias at Week 24 for the primary slope model. The slope model undershoots the placebo means-per visit in the beginning and middle of follow-up in the double blind period and overshoots the placebo mean at Week 24. Residual plots used for model diagnostics, not shown here, also suggested potential non-linearity of the ALSFRS-R and inferior model fit for the slope model.

Slide 14:

The table in this slide assesses sensitivity to the linearity assumption underlying the applicants primary analysis. It shows that Sensitivity analyses allowing for non-linearity provide less persuasive evidence. In IND comments provided to the sponsor on the SAP the FDA had indicated that linearity should be assessed in a prespecified objective way and there should be a backup analysis for nonlinearity but that the slope models ignoring of deaths can cause bias and so the joint rank should be primary if there were deaths. The sponsor presented results for a different quadratic model in the study report and AC briefing package shown in the first row here which has a more favorable result than the prespecified quadratic model shown in the second row but the former is post-hoc so unreliable. There is a fairly big difference in the p-values of the post hoc and prespecified quadratic models p=.0385 for the post hoc model and p=.1134 for the prespecified one. Neither of these quadratic models allowed the quadratic term to vary by treatment group which may be unrealistic. Therefore, the FDA reviewer extended the prespecified model to allow the quadratic term to vary by treatment group and the result is shown in the third row with a p-value of .0644. None of these quadratic models is ideal in general for nonlinearity situations which is why the FDA neurology statistical team usually recommends a mean-per-visit repeated measures model in order to get an unbiased estimate of the treatment difference at the last visit while avoiding a questionable linearity assumption. The result for this non-linear compatibile model is shown in row 4 to have an estimated Week 24 treatment difference of 1.86 with a p-value of .0749.

Slide 15:

Secondary endpoint results in study AMX3500 are not persuasive. The first key secondary, ATLIS, a measure of strength has 3 possible summaries of interest and the SAP was not clear on which was primary. Only the Upper ATLIS component is nominally significant, with a p-value of 0.042. The total which would be the most likely primary summary is not nominally significant. The ATLIS analyses also ignore deaths and have slightly more missing data at Week 24 than for the ALSFRS-R.. The rest of the key secondary endpoints shown in the prespecified order of priority were not nominally significant. These include Slow Vital Capacity, a neurofilament biomarker pNF-H, and the composite survival endpoint, i.e., time to first event of hospitalization, tracheostomy, or death.

Slide 16:

Turning to the up to 132 week Open Label extension study. The primary objective was to evaluate safety. Here are it's protocol specified efficacy endpoints. They are ALSFRS-R rate of decline, A Composite survival endpoint of time to first hospitalization, tracheostomy, or death, Upper and lower ATLIS scores rate of decline, Rate of progression on ALSFRS-R subdomains, and Rate of progression on total ATLIS score. Time to death alone was not specifically included in the list of efficacy outcomes or objectives. Analysis of time to death alone was included in a description of analyses of components of the composite survival endpoint, but was not given priority relative to the other two components hospitalization or tracheostomy, or relative to the composite itself. The prespecified Composite survival endpoint analysis was to be based on a Cox proportional hazards regression with age and pre-randomization slope as covariates.

Slide 17:

The primary objective of the open label extension of AMX3500 study was safety followed by the objective of investigating progression on ALSFRS-R, time to the composite event of hospitalization, tracheostomy or death, progression on the ATLIS function measure, and progression on Slow Vital Capacity. Results for all endpoints except death are very difficult to interpret due to substantial dropout and missing data and many deaths. In particular, Only 66% of patients entered the OLE, Only ~40% have Week 48 ALSFRS-R measurements, and there is 15-20% mortality by Week 48 which is ignored in the applicant's analysis. The linearity assumption for these endpoints over time over a longer period is yet another limitation.

Slide 18:

The supplemental open label extension statistical analysis plan for survival was drafted after the sponsor had already analyzed survival data from the open label extension after the last patient last visit in the double blind period. This supplemental analysis plan shifted the focus from the survival composite of hospitalization, tracheostomy, or death which was listed as an objective in the OLE protocol to the endpoint of time to death alone which had not been specifically listed as an objective in the protocol. This Supplemental SAP specified a Cox proportional hazards regression of time to death alone with age, baseline ALSFRS-R, and pre-randomization slope as covariates

Slide 19:
The figure here shows Kaplan Meier estimates of overall survival, based on time to death only, through the OLE. Note that there was no-rerandomization for the OLE and a moderate proportion of 35% did not participate, but that vital status as of March 1, 2021 was obtained for all but one randomized patient. It is important to note that The original placebo group continuers into the OLE were switched to AMX treatment in the open label extension. Using the supplemental SAP methods The covariate adjusted hazard ratio between the two groups was estimated as .64 with a 95% confidence interval from 0.42 to 1.00 based on the final vital status search's death event cutoff of March 1, 2021.

Slide 20:

The OLE time to death alone results are not persuasive for the following reasons. The time to death alone Analysis is exploratory on many levels Including: OLE periods typically focus on safety; with efficacy analyses being exploratory, Time to death alone was not included in the planned OLE endpoint hierarchy which makes it exploratory The Focus on death alone and submission of the supplemental OLE survival SAP occurred after unblinding of the double-blind period and the preliminary analysis of survival data. Also, there were Multiple survival data sweeps, resulting in multiple analyses. There was No evidence of drug effect on death or composite survival endpoint in the double-blind period. In summary, the Evidence for an effect on time to death is not compelling: with the final nominal p-value essentially 0.05 based on the supplemental SAP methods.

Slide 21:

FDA has concerns with the applicant's post-hoc Bayesian analysis and believes this analysis is inappropriate and misleading for the following reasons. The Analysis is post hoc with emphasis on a selected set of endpoints determined after seeing the trial results (e.g., the biomarker endpoint was higher in the hierarchy than survival but is omitted in this analysis) There was No prespecified plan to

collectively examine these selected endpoints. Such a Calculated “error” as this decreases as more endpoints are added, even if the estimated treatment effect for an added endpoint is zero or in the wrong direction. This Analysis does not give the primary endpoint due prominence and also may not capture the relevant false positives among the other endpoints prespecified for testing or in the setting of a hypothesis test involving multiple endpoints together. This calculation is inadequate for quantifying the strength of evidence, as this depends on many factors, such as clinical relevance of endpoints and effects, quality of trial conduct, sensitivity to violations in assumptions or limitations of data.

Slide 22:

In summary, A Single trial (to establish effectiveness should demonstrate a “clinically meaningful and statistically very persuasive effect. There is uncertainty about persuasiveness of results from the single efficacy trial (and its OLE) of AMX0035. In particular, the Primary analysis results are not highly persuasive since there are Issues with randomization implementation, imbalances in use of riluzole and edaravone, handling of deaths and missing data and the questionable validity of the primary analysis assumption of linearity of ALSFRS-r over time. These issues weaken the reliability of the study results. and in fact sensitivity analysis results are less favorable in some cases and cannot address all issues . Furthermore, Secondary endpoint results are not compelling. Finally, Open Label Extension survival analyses are exploratory and/or not persuasive, such exploratory analyses should be interpreted with caution. Thank you for your interest and attention!

Slide 23: (End slide no content (other than logo) or notes)