CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

216660Orig1s000

CLINICAL PHARMACOLOGY REVIEW(S)

Office of Clinical Pharmacology Review

NDA Number	216660
Link to EDR	\\CDSESUB1\evsprod\NDA216660\0001
Submission Date	Oct 29, 2021
Submission Type	*505(b)(1) Priority Review*
Brand Name	RELYVRIO
Generic Name	Phenylbutyrate (PB) and taurursodiol (TUDCA)
Dosage Form and Strength	Powder for oral (b) (4) 10-g powder-filled packet containing 3 g PB and 1 g TUDCA
Route of Administration	Orally taken twice daily
Proposed Indication	Treatment of Amyotrophic lateral sclerosis (ALS)
Applicant	Amylyx Pharmaceuticals, Inc.
Associated IND	IND 129563
OCP Review Team	Xiaohan Cai, Ph.D./ Bilal AbuAsal, Ph.D. Michael Bewernitz, Ph.D./ Atul Bhattaram, Ph.D./ Sreedharan Sabarinath, Ph.D./ Ramana Uppoor, Ph.D./ Hao Zhu, Ph.D.
OCP Final Signatory	Mehul Mehta, Ph.D.

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1. EXECUTIVE SUMMARY

The applicant, Amylyx Pharmaceuticals, Inc., is seeking approval for AMX0035 (RELYVRIO) via 505(b)(1) pathway for the treatment of Amyotrophic Lateral Sclerosis (ALS) in adults. AMX0035 is a fixed dose combination (FDC) product, formulated as a powder for oral [redacted] (b) (4) and supplied as packets, each containing 3 g phenylbutyrate (PB) and 1 g taurursodiol (TUDCA). BUPHENYL® (sodium phenylbutyrate) is an approved drug product for treating patients with urea cycle disorders. URSO 250® (ursodiol), a metabolite of TUDCA, is an approved drug product for treating patients with primary biliary cholangitis. AMX0035 was administered orally (or via feeding tube) in clinical studies. The mechanism of action for AMX0035 in treating ALS is unknown.

To demonstrate efficacy, the applicant is relying on a single double-blind, placebo-controlled Phase 2 Study AMX3500 (CENTAUR study) conducted in 137 patients with ALS. The applicant reported positive results on the primary efficacy endpoint, the rate of decline in total ALSFRS-R score, in the 24-week placebo-controlled phase (p=0.034, 2.34 points difference comparing to placebo at Week 24). Further, the applicant submitted additional survival analyses and biomarker data to supplement the evaluation of the treatment effect and support mechanistic activity of AMX0035.

Biomarker data were obtained from the CENTAUR study in ALS patients and from a recent study with AMX0035 in Alzheimer's disease (AD) patients (PEGASUS). Data from CENTAUR study included neurofilament heavy chain (pNF-H), neurofilament light chain (NF-L), Chitinase-3-like protein 1 (CHI3L1 or YKL-40), and Chitinase 1 (CHIT1) in plasma samples to supplement the evaluation of the treatment effect of AMX0035. The biomarker data of pNF-H and NF-L collected in the CENTAUR study did not show statistical differences between patient groups receiving AMX0035 and placebo. Based on the descriptive data summary provided by the applicant, the plasma level of inflammatory biomarkers (YKL-40 and CHIT1) after 24-week treatment in CENTAUR study, suggested a numerical differences in change from baseline between the AMX0035 and placebo arms on YKL-40. The study in AD patients (PEGASUS) assessed 18 cerebrospinal fluid (CSF) biomarkers. In this study, AMX0035 lowered levels of CSF total tau, p-tau 181, neurogranin, and YKL-40, and raised the ratio of Aβ42/Aβ40, markers of neurodegeneration that are felt to be relevant to AD pathology. The Applicant suggested that these biomarkers may support mechanistic activity of AMX0035 in patients with ALS, because some of these CSF biomarkers like YKL-40 may be associated with ALS clinical function or survival. However, the reliability and translatability of all submitted biomarker results is not established for multiple reasons as described in section 3.3.1.

In addition to the biomarker data, the clinical pharmacology package for this NDA included in vitro drug-drug interaction studies, pharmacokinetics (PK) data collected from the Phase 2 CENTAUR study, and a Phase 1 food effect study A35-002. The population PK model was not established for one of the components (TUDCA and its metabolites) and therefore exposure-response analyses for AMX0035 for efficacy and safety were not conducted.

The primary focus of this review is to evaluate the impact of the biomarker data submitted to support the registration of RELYVRIO, and to evaluate the PK and the proposed dosing recommendations based

on intrinsic and extrinsic factors. In addition, the review evaluated the provided rationale for each component of the combination in AMX0035.

1.1 Recommendations

The Office of Clinical Pharmacology has reviewed the information submitted under this NDA and there are no clinical pharmacology issues that preclude approval.

Key review issues with specific recommendations and comments are summarized below:

Review Issue	Recommendations and Comments
Pivotal or supportive evidence of effectiveness	The efficacy of AMX0035 for the treatment of ALS was evaluated in a single randomized, double-blind, placebo-controlled and open label extension (OLE) of phase 2 study (CENTAUR study) in patients with ALS. In this study a single dose level of AMX0035 was tested. The pivotal and supportive evidence of effectiveness for AMX0035 is from the CENTAUR study and from its OLE phase. The clinical pharmacology review focused on the utility of available biomarker information and explored the possibility of exposure-response (E-R) analyses. However, definitive conclusions were not possible based on available biomarker data, and E-R analyses were not possible because of the lack of PK characterization for one of the components of AMX0035 from CENTAUR study.
General dosing instructions	1 packet, containing 3 g of PB and 1g of TUDCA, should be mixed with water and administered orally or via feeding tube before a meal or snack. Recommend to dose once daily for the first 3 weeks and increase the dose to twice daily subsequently.
Dosing in patient subgroups (intrinsic and extrinsic factors)	No dose adjustment is needed for AMX0035 for the following intrinsic factors: • Mild hepatic impairment • Mild renal impairment Avoid use for the following intrinsic factors: • Moderate and severe hepatic impairment • Moderate and severe renal impairment Avoid use for the following extrinsic factors: • Concomitant use with substrate of CYP1A2, CYP2C8, CYP2B6, and CYP3A4 in which a small change in substrate plasma concentration may lead to serious toxicities or loss of efficacy. • Concomitant use with transporter inhibitors of OATP1B3

	• Concomitant use with substrates of OAT1, BCRP, and P-gP in which a small change in substrate plasma concentration may lead to serious toxicities or loss of efficacy. • Concomitant use with bile acids sequestering agents, aluminum-based antacids, inhibitors of bile acid transporters, probenecid, and HDAC inhibitors
Labeling	The review team recommends changes to the USPI to reflect the potential for AMX0035 to affect other concomitant drugs, other drugs to affect AMX0035, dose recommendation for patients with mild renal and hepatic impairment, avoid use in patients with moderate and severe renal and hepatic impairment, and description of PK (section 12.3).
Bridge between the to-be-marketed and clinical trial formulations	Although the clinical formulation was different from the to-be-marketed (TBM) formulation, the dissolution data support the bridging of the formulations. (Refer to the Integrated Quality Review, DRRTS, 4/18/2022)

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1.2 Post-Marketing Requirements and Commitments

- In vivo pharmacokinetic drug interaction study to evaluate the effect of RELYVRIO on inhibiting and/or inducing CYP2C8, CYP1A2, CYP2B6, and CYP3A4 enzymes using an appropriate probe substrate for each enzyme. We recommend evaluating these drug interactions as a single cocktail drug-drug interaction (DDI) study.
- In vivo drug interaction study to evaluate the effect of a transporter inhibitor of OATP1B3 on pharmacokinetics of RELYVRIO
- In vivo pharmacokinetic drug interaction study to evaluate the effect of RELYVRIO as an inhibitor of OAT1, BCRP, and P-gP transporters. we recommend evaluating these drug interactions as a single cocktail DDI study with an appropriate probe substrate of each transporter.
- In vivo hepatic impairment study to evaluate the effect of hepatic impairment on the exposure of sodium phenylbutyrate and taurursodiol after oral administration of RELYVRIO relative to that in subjects with normal hepatic function.
- In vivo renal impairment study to evaluate the effect of renal impairment on the exposure of sodium phenylbutyrate and taurursodiol after oral administration of RELYVRIO relative to that in subjects with normal renal function.

2. SUMMARY OF CLINICAL PHARMACOLOGY ASSESSMENT

2.1 Pharmacology and Clinical Pharmacokinetics

Mechanism of Action:

The mechanism by which AMX0035 exerts its therapeutic effects in patients with ALS is unknown.

Absorption:

Following oral administration of a single dose of AMX0035 in healthy subjects under fasting conditions, PB is rapidly absorbed with a median time of 1 hour to reach the peak plasma concentration. TUDCA has a median time of 4.5 hours to reach the peak plasma concentration. A high-fat meal reduced the C_{max} and AUC of PB by 76% and 54%, respectively. For TUDCA, a high-fat meal had no effect on C_{max} and increased AUC by 39%.

Distribution:

Plasma protein binding for PB and TUDCA to human plasma protein is 82% and 98.5%, respectively.

Metabolism and Elimination:

No mass balance studies of AMX0035 have been conducted in humans to confirm the metabolic pathways and elimination routes.

Following a single dose of AMX0035, a major metabolite of PB, phenylacetic acid (PAA), was observed to have a median T_{max} of 2 hours and half-life of 0.8 hr. The mean AUC_{0-24h} of PAA was found to be 35% to that of PB under fasting condition.

Ursodeoxycholic acid (UDCA) and glycoursodeoxycholic acid (GUDCA) are considered as major metabolites of TUDCA. In CENTAUR study, significantly increased plasma concentration of UDCA and GUDCA, was observed at Week 12 and Week 24 following the multiple doses of AMX0035. In addition, the mean AUC_{0-24h} (µg*h/mL) of UDCA and GUDCA was found to be 126% and 95% to that of TUDCA following a single dose of AMX0035 under fasting condition in the phase 1 food effect study. It is unknown whether there are additional major circulating metabolites following the administration of AMX0035.

Excretion:

Following a single dose of AMX0035, the elimination half-life to PB, PAA, and TUDCA is approximately 0.5 hr, 0.8 hr, and 4 hr respectively. The elimination half-life of UDCA and GUDCA cannot be reliably estimated.

Based on USPI of BUPHENYL, an approved drug product of PB, the majority of administered sodium phenylbutyrate (~80-100%) is excreted in the urine within 24 hours as the conjugated product, phenylacetylglutamine. The excretion pathway of TUDCA is unknown.

Special Populations:
Hepatic Impairment:
A dedicated hepatic impairment study was not conducted. No dose adjustment is recommended for patients with mild hepatic impairment based on the acceptable safety profile for AMX0035 in patients with mild hepatic impairment enrolled in the CENTAUR study. The applicant conducted a population PK (PPK) approach, assessing the impact of elevated liver enzyme serum alanine aminotransferase (ALT, 50-116 U/L) on the exposure of PB and PAA, and was considered as exploratory. A PPK model for TUDCA and its metabolites has not been established in evaluating the effect of elevated ALT on the exposure of TUDCA and its metabolites due to complicated enterohepatic recirculation (please see section **4.4.1**

TUDCA, UDCA, and GUDCA for details). A dedicated hepatic impairment study evaluating the impact of hepatic impairment on the PK exposure of PB, TUDCA, and their metabolites will be requested at post-marketing setting. Currently, the use of AMX0035 in patients with moderate and severe hepatic impairment should be avoided.

Renal Impairment:

No dose adjustment is recommended for patients with mild renal impairment based on the acceptable safety profile of these patients enrolled in CENTAUR study. A dedicated renal impairment study to evaluate the impact of renal impairment on the PK of AMX0035 was not conducted. Based on PPK analyses in subjects with normal renal function as well as in subjects with mild renal impairment (60 <eGFR < 90 mL/min/1.73m^2), renal function is not a significant covariate on the PK of PB or PAA. A PPK model for TUDCA and its metabolites has not been established for evaluating the effect of renal function on the PK of TUDCA and its metabolites due to complicated enterohepatic recirculation along with the interconversion between TUDCA and UDCA (please see section **4.4.1** TUDCA, UDCA, and GUDCA for details). A dedicated renal impairment study evaluating the impact of renal impairment on the PK exposure of PB, TUDCA, and their metabolites will be requested at post-marketing setting. Currently, the use of AMX0035 in patients with moderate and severe renal impairment should be avoided.

Sex, Age, Race, and Body Weight:

The PPK analysis showed that sex, race, or age have no significant impact on PB and PAA systemic exposure. The PPK analysis revealed that body weight had a significant effect on the exposure of PAA, but not PB. However, the clinical significance of PAA exposure change due to different body weight is not clear.

The effects of sex, race, age, or body weight on the PK exposure of TUDCA and its metabolites are unknown. A PPK model for TUDCA and its metabolites has not been established to evaluate the impact from these covariates on TUDCA and its metabolites due to complicated enterohepatic recirculation and interconversion of TUDCA and UDCA (please see section **4.4.1** TUDCA, UDCA, and GUDCA for details). Please refer to section 4.3 for details regarding the clinical PK assessments.

2.2 Dosing and Therapeutic Individualization

2.2.1 General dosing

The proposed dose for the treatment of ALS is 1 packet taken orally or via feeding tube every day for the initial three weeks. The dose will be increased to twice daily dosing of 1 packet subsequently. Patients should take AMX0035 before a snack or meal and resume normal eating after drug administration. This dosing instruction is the same as used in the Phase 2 clinical study (CENTAUR study).

2.2.2 Therapeutic individualization

Hepatic Impairment

The effect of mild hepatic impairment on the PK of AMX0035 cannot be reliably assessed. Safety profile in ALS patients with elevated ALT (50-116 U/L), categorized as mild hepatic impairment based on National

Cancer Institute Classification system (NCIc), was acceptable because no differences in adverse events (AEs) were observed when comparing to patients with normal hepatic function. Therefore, no dose adjustment is recommended for patients with mild hepatic impairment. Avoid use in moderate and severe hepatic impairment (NCIc) patients because the PK or safety data are not available in these populations.

Renal Impairment

Although mild renal impairment (60 <eGFR < 90 mL/min/1.73m^2) did not have a significant impact on the PK of PB and PAA, its effect on TUDCA, UDCA, and GUDCA PK cannot be evaluated. The safety profile in ALS patients with mild renal impairment was acceptable because no differences in AEs were observed when comparing to patients with normal renal function. Based on the acceptable safety profile, no dose adjustment is proposed for patients with mild renal impairment. Avoid use in moderate and severe renal impairment patients (Creatinine Clearance, CLcr < 60 mL/min) because the PK or safety data are not available in these populations.

Age, Body Weight, Sex, and Race:

No dose adjustment is required for patients based on sex, race, age, or body weight. The effects of age, sex, or race on PB and PAA exposure were not significant and the effects on TUDCA, UDCA, GUDCA exposure were not clear. Body weight had a significant effect on the exposure of PAA, but not PB. However, the clinical significance of PAA exposure change due to different body weight is not clear. AMX0035 was administered without regards to the covariates of age, sex, race, or body weight in CENTAUR study.

2.3 Outstanding Issues

The following clinical pharmacology studies are outstanding and will be conducted under PMR:

- A hepatic impairment study in subjects with hepatic impairment to evaluate the effect of hepatic impairment on the exposure of sodium phenylbutyrate and taurursodiol after oral administration of RELYVRIO relative to that in subjects with normal hepatic function.
- A renal impairment study in subjects with renal impairment to evaluate the effect of renal impairment on the exposure of sodium phenylbutyrate and taurursodiol after oral administration of RELYVRIO relative to that in subjects with normal renal function.
- A clinical study to assess the effect of RELYVRIO on inhibiting and/or inducing CYP2C8, CYP1A2, CYP2B6, and CYP3A4 enzymes.
- A clinical study to evaluate the effect of a transporter inhibitor of OATP1B3 on RELYVRIO pharmacokinetics
- A clinical study to evaluate the effect of RELYVRIO on inhibiting OAT1, BCRP, and P-gP transporters.

2.4 Summary of Labeling Recommendations

- Administer AMX0035 before a snack or meal.
- Dose adjustment is not required for mild hepatic/renal impairment patients or based on sex, age, race, or body weight.

- Avoid use in moderate and severe hepatic/renal impairment patients.
- Avoid use with substrate of CYP1A2, CYP2C8, CYP2B6, CYP3A4, OAT1, BCRP, and P-gP where a small change in substrate plasma concentration may lead to serious toxicities or loss of efficacy.
- Avoid use with transporter inhibitors of OATP1B3.
- Avoid use with bile acids sequestering agents, aluminum-based antacids, inhibitors of bile acid transporters, probenecid, and HDAC inhibitors.
- In vitro studies showed that AMX0035 induce CYP1A2, CYP2B6, and CYP3A4 at clinically relevant concentration.
- In vitro studies showed that AMX0035 inhibit CYP2C8 and CYP2B6 at clinically relevant concentration.
- In vitro studies showed that AMX0035 is a substrate of OATP1B3, MATE2-K, OAT3, and BSEP.
- In vitro studies showed that AMX0035 inhibited OAT1, P-gP, and BCRP at clinically relevant concentration.
- The relationship of age, sex, and race on AMX0035 is unknown.

3. COMPREHENSIVE CLINICAL PHARMACOLOGY REVIEW

3.1 Overview of the Product and Regulatory Background

AMX0035 drug product is a 10 g packet containing 3 g of PB and 1 g of TUDCA as the active ingredients. Sodium PB is an approved drug in the US for treating urea cycle disorders and is marketed in the US as Buphenyl™.

The clinical development program to demonstrate the safety/efficacy for AMX0035 consisted of one Phase 2 study (CENTAUR study) and an open label extension in patients with ALS and one Phase 1 study (Study A35-002) conducted in healthy subjects.

On March 30, 2022, a Peripheral and Central Nervous System (PCNS) Drugs Advisory Committee (AC) Meeting was held to discuss whether the data submitted by the applicant is adequate to establish the effectiveness for AMX0035 in the treatment of ALS. The committee members voted on the question “Do the data from the single randomized, controlled trial and the open-label extension study support a conclusion that sodium phenylbutyrate/taurursodiol is effective in the treatment of patients with amyotrophic lateral sclerosis (ALS)?”.

In June 2022, the applicant submitted additional data as confirmatory evidence to supplement the evaluation of the treatment effect and support mechanistic activity of AMX0035. These submitted data included biomarker data from a different study (Study PEGASUS) to support mechanistic activity of AMX0035, a responder analysis based on the ALSFRS-R of the proportion of patients in CENTAUR study who had an unusually strong response, and additional post-hoc long-term survival analyses.

In August 2022, the applicant submitted descriptive summary statistics for additional plasma biomarkers, Chitinase-3-like protein 1 (CHI3L1 or YKL-40) and Chitinase 1 (CHIT1), from CENTAUR study.

On September 7, 2022, an additional PCNS AC Meeting was held to continue discussion of the application in the context of the additional analyses and data submitted by the applicant. The

committee members voted on the question "Considering the new information submitted and the information presented at the March 30, 2022, PCNS meeting, is the available evidence of effectiveness sufficient to support approval of sodium phenylbutyrate/taurursodiol (AMX0035) for the treatment of patients with ALS?".

It is also noted that AMX0035 is recently approved in Canada through a conditional approval pathway. The applicant has initiated study A35-004 (PHOENIX), a Phase 3, randomized, placebo-controlled trial of AMX0035 in patients with ALS. The primary objective of the trial will be to assess AMX0035 compared to placebo on the change from baseline of ALSFRS-R. The trial is expected to complete in late 2023 or early 2024.

3.2 General Pharmacology and Pharmacokinetic Characteristics

Pharmacology	
Mechanism of Action	The mechanism by which AMX0035 exerts its therapeutic effects in treating ALS is unknown.
Active Moieties	PB and TUDCA are considered as the active moieties. The pharmacological activity of PAA, UDCA, and GUDCA, as the major metabolites of PB and TUDCA, was not evaluated and is unknown.
QT Prolongation	No large mean increases (i.e., >20 msec) in the QTcF interval was detected in this QT assessment of AMX0035 at the therapeutic dose. Potential effects of parent drugs and metabolites on QTc at higher exposure are unknown because high clinical exposure scenario was not identified in the clinical program (See QT-IRT Review dated 02/22/2022).
General Information	
Bioanalysis	The concentrations of PB, PAA, TUDCA, UDCA, and GUDCA in human plasma were measured using two validated LC-MS/MS methods. Approximately 50% samples from the Phase 2 CENTAUR study were stored beyond established long-term stability duration for PB, PAA, and TUDCA. Therefore, only the results for PB, PAA, and TUDCA from samples analyzed within the established stability duration were considered reliable and used in subsequent data analysis. Please refer to Section 4.1 for details.
Healthy Volunteers vs. Patients	For PB and PAA, there was no significant difference in PK between ALS patients and healthy subjects. A PPK model for TUDCA and its metabolites was not established. Thus, a comparison between ALS patients and healthy subjects is not established.
Dose Proportionality	Dose proportionality was not evaluated.
Accumulation	For PB and its metabolite PAA, no accumulation is expected after repeated once (QD) or twice daily (BID) dosing. For TUDCA and its metabolites, the accumulation is unknown for QD or BID dosing.
Variability	For PB, the coefficient of variation (CV%) was approximately 28% for C_{max} and 34% for AUC_{0-24h}. For TUDCA, the CV% was approximately 52% for C_{max} and 60% for AUC_{0-24h}.
Absorption	

Bioavailability	The absolute oral bioavailability of PB or TUDCA was not determined.
T_{max}	The median T_{max} for PB and TUDCA is 0.5 hr and 4.5 hr under fasting condition
Food effect (high-fat meal) GMR relative to fasted state	For PB, a high-fat meal lowered the C_{max} by 76% and AUC by 54%. For TUDCA, a high-fat meal did not change C_{max} but increased AUC_{0-24h} by 39%.
Distribution	
Volume of Distribution	9.3 for PB and 1630 L for TUDCA
Plasma Protein Binding	82% for PB and 98.5% for TUDCA
Substrate transporter systems	The following information was concluded from in vitro studies: • AMX0035 is a substrate of OATP1B3, MATE2-K, OAT3, and BSEP. • AMX0035 inhibited OAT1, P-gP, and BRCP at clinically relevant concentration.
Elimination	
Mean Terminal Elimination half-life	Approximately 0.5 hr for PB and 4 hr for TUDCA
Metabolism	
Primary metabolic pathway(s)	AMX0035 was not metabolized by major CYP enzymes from in vitro studies. • PAA was found to be a major metabolite of PB • UDCA and GUDCA were found as major metabolites of TUDCA. The disposition of TUDCA may involve enterohepatic recirculation and its metabolites UDCA and GUDCA.
Inhibitor/Inducer (in vitro)	• AMX0035 has inhibition potential on CYP 2B6 and CYP2C8 at clinically relevant concentrations. • AMX0035 has induction potential on CYP1A2, CYP2B6 and CYP3A4.
Excretion	
Primary excretion pathways	Human mass balance study has not been conducted to confirm the excretion pathways of AMX0035. Based on USPI of BUPHENYL, an approved drug product of PB, the majority of administered sodium phenylbutyrate (~80-100%) is excreted in the urine within 24 hours as the conjugated product, phenylacetylglutamine.

3.3 Clinical Pharmacology Review Questions

3.3.1 To what extent does the available clinical pharmacology information provide pivotal or supportive evidence of effectiveness?

The pivotal evidence of efficacy came from a single Phase 2 CENTAUR study in 137 patients with ALS. The only clinical pharmacology information submitted to support assessment of AMX0035's effectiveness in treating ALS were the biomarker data.

Phase 2 Pivotal CENTAUR Study and Additional Survival Analysis

CENTAUR study consisted of a 24-week, randomized, double-blind (DB), placebo controlled main phase and an open-label phase up to an additional 132 weeks in participants with ALS. During the DB phase of the study, the dose was initiated at one packet daily and then increased to one packet BID dosing for 89% (N=79) of the patients receiving AMX0035. The primary efficacy endpoint for the placebo-controlled main phase of CENTAUR study was the rate (slope) of decline over time in total Amyotrophic Lateral Sclerosis Functional Rating Scale – Revised (ALSFRS-R) score.

The applicant's primary analysis on the rate of decline in total ALSFRS-R score used a shared-baseline and linear mixed-effects model in the mITT population and the applicant reported the following findings:

- AMX0035 met the primary efficacy endpoint and demonstrated a statistically significant slowing of disease progression as measured by the ALSFRS-R total score compared to placebo (p=0.0340) according to the applicant analysis.
- The AMX0035 treatment group had an estimated LS mean ALSFRS-R Total score (29.06) that was 2.32 points higher at Week 24 compared to the placebo group (estimated LS mean=26.73).

Upon completion of the DB phase of AMX0035, a total of 90 participants (66% of all randomized patients) continued into the open-label phase, 34 of whom had been originally randomized to placebo and 56 to AMX0035. During the open label phase, the placebo treatment group was crossed over to receive AMX0035 with the same dose as in the DB phase. The applicant reported survival analyses results from the initial randomization into the CENTAUR study through the data cutoff date of March 1st, 2021 with survival status for 136 out of 137 participants.

During the late review cycle, the applicant submitted new analyses of previously submitted efficacy and survival data as confirmatory evidence. These analyses included responder analysis based on the ALSFRS-R of the proportion of patients in the original trial who had an unusually strong response, new post-hoc survival analysis with rank preserving structural failure time model (RPSFTM), and new post-hoc survival analysis with comparisons to external data. On September 7, 2022, an additional PCNS AC Meeting was held to continue discussion of the application in the context of the additional analyses and data submitted by the applicant. Assessment of these data are discussed by the clinical and statistical teams (Refer to the reviews from Dr. Tandon and Dr. Massie for details).

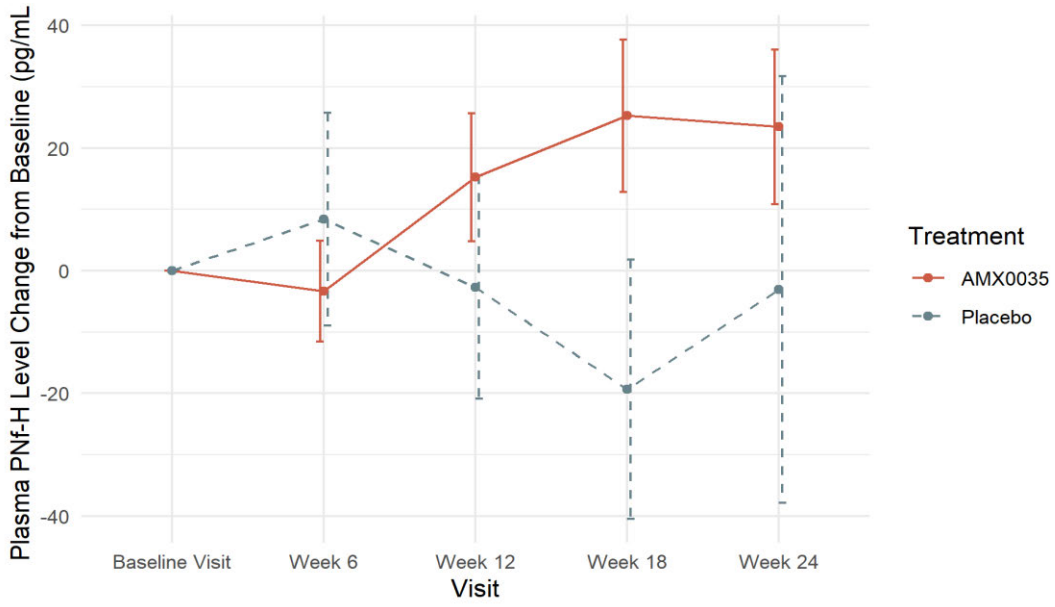
In addition to the analyses listed above, the applicant submitted biomarker data obtained from the CENTAUR study in ALS patients and from a recent study with AMX0035 in Alzheimer disease patients (PEGASUS) to support the mechanistic activity of AMX0035 in the central nervous system (CNS). These data are discussed below.

The bioanalytical method validation has not been established for any of these biomarkers, thus all biomarker analyses discussed below were considered as exploratory.

Biomarker Data from ALS Patients

Literature studies have reported that neurofilament proteins (NFs), the byproducts of neuroaxonal breakdown increase following axonal injury in neuronal degenerative diseases [1]. It was reported that NFs, including neurofilament heavy chain (pNF-H), neurofilament light chain (NF-L), increased significantly in patients with ALS compared to other neurodegenerative diseases. It has been reported that the NF-L levels correlated with disease severity and thus may offer supporting value in ALS drug development as a prognostic biomarker [2]. It was also hypothesized that a reduction in NF-L level with treatment may signal a potential treatment benefit in ALS. The biomarker data of pNF-H and NF-L in plasma from CENTAUR study were evaluated to supplement the evaluation of the treatment effect of AMX0035. The plasma biomarker data of pNF-H and NF-L collected in the CENTAUR study did not show statistically significant difference in change from baseline (CFB) at week 24 between patient groups receiving AMX0035 and placebo (Figure 1 and 2).

Figure 1 Plasma pNF-H Change from Baseline from CENTAUR Study

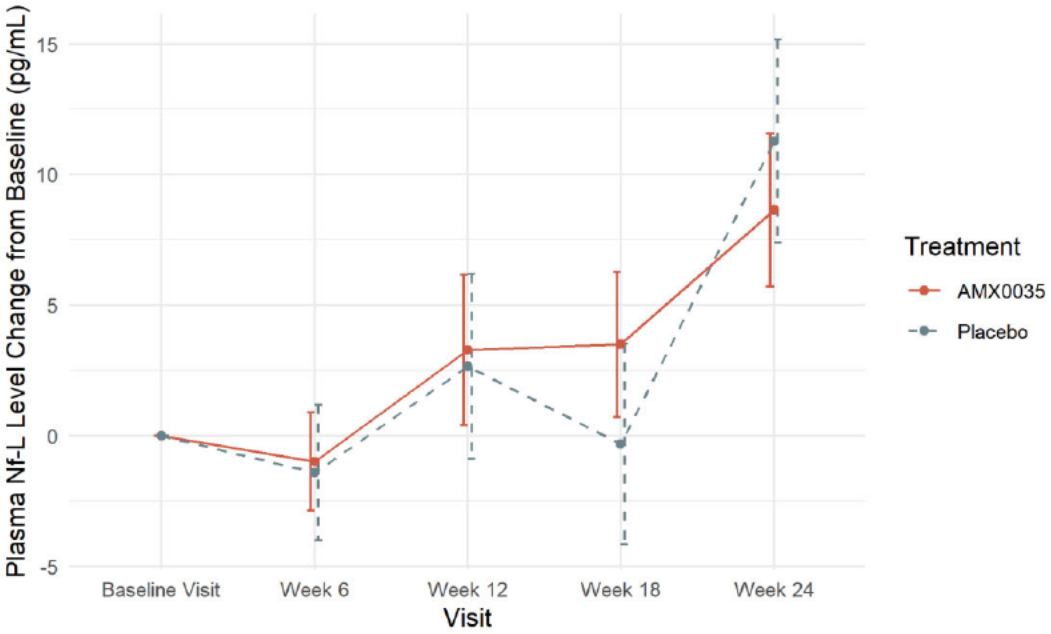


Reviewer's Analysis; Line plot: Mean± SE

[1] Verber NS, Shepheard SR, Sassani M, et al. Biomarkers in Motor Neuron Disease: A State of the Art Review. Front Neurol. 2019;10:291. Published 2019 Apr 3. doi:10.3389/fneur.2019.00291

[2] Lu, Ching-Hua et al. "Neurofilament light chain: A prognostic biomarker in amyotrophic lateral sclerosis." *Neurology* vol. 84,22 (2015): 2247-57. doi:10.1212/WNL.0000000000001642

Figure 2 Plasma NF-L Change from Baseline from CENTAUR Study



Reviewer's Analysis; Line plot: Mean± SE

In August 2022, the applicant submitted additional plasma biomarkers summary level data related to two neuroinflammatory biomarkers Chitinase-3-like protein 1 (CHI3L1 or YKL-40) and Chitinase1(CHIT1) from CENTAUR Study. The applicant's analysis showed that significant differences in CFB was observed at week 24 between AMX0035 (+1.05 ng/mL) and placebo (+7.64 ng/mL) groups on plasma YKL-40, but not for CHIT1. The applicant claimed that AMX0035 treatment demonstrated a significant decrease in plasma YKL-40, a reported marker of neuroinflammation. The applicant indicated that several literatures supported that the cerebrospinal fluid (CSF) concentration of YKL-40 correlated with ALS severity, disease progression rate, and survival. In the applicant presentation during the AC meeting, the applicant also asserted that the plasma YKL-40 correlated with ALSFRS-R score ($r = -0.21$, $p = 0.0001$) and ALSFRS-R progression rate ($r = -0.19$, $p = 0.004$) in CENTAUR study, thus further supporting the efficacy finding of CENTAUR study. However, the reliability and translatability of these results are not established for the following reasons:

1. Details of bioanalysis and validation are not available for any of the biomarkers.
2. Only descriptive summary statistics for these biomarkers in the plasma and CFB values were provided. Individual level data was not submitted to the NDA to establish the correlation between change in the biomarker levels and clinical benefit (ALSFRS-R);
3. There was imbalance in baseline in plasma YKL-40 levels between AMX0035 and placebo group;

4. Several literatures suggested prognostic value for YKL-40 in the CSF for ALS patients[3][4] to predict disease severity, however, only plasma data were collected from CENTAUR study and no CSF data was available in this study. The association between YKL-40 in CSF and plasma is not clear. We also note that YKL-40 is not a specific biomarker to the neuronal damage and changes in plasma concentration may not be selective to pharmacological effect in motor neurons for ALS patients. Increased levels of YKL-40 in plasma have been described in many diseases running with inflammation.
5. Correlation between YKL-40 reduction and clinical benefit is not established for either CSF or plasma in ALS patients.

In conclusion, the biomarker results of plasma YKL-40 and CHIT1 from CENTAUR study do not provide clear evidence supporting AMX0035's clinical benefit in treating ALS.

Biomarker Data from Alzheimer's Disease (AD) Patients

In additional to the biomarker data from CENTAUR study, the applicant submitted biomarker information from a different study to support this NDA. The applicant asserted that the improvement in selected cerebrospinal fluid (CSF) biomarkers in AD patients supported the mechanistic activity of AMX0035 in the central nervous system (CNS). The biomarker information included CSF biomarker data from Study PEGASUS. In study PEGASUS, AMX0035 was investigated in 95 patients with Alzheimer's Disease (AD) or mild cognitive impairment (MCI) with a randomized, placebo-controlled study design. Patients received AMX0035 or placebo twice a day for 24-week. As an objective of exploratory analysis, a total of eighteen biomarkers in CSF that may be relevant to AD, including biomarkers of AD pathology, neurodegeneration, synaptic activity, and inflammation, were evaluated for the treatment effect of AMX0035. Comparing to the placebo group, several CSF biomarkers including total Tau, pTau 181, FABP3, neurogranin, YKL-40, IL-15 had significant reduction after the treatment of AMX0035. There was no significant change in other biomarkers including NF-L, interleukin-6 (IL-6), interleukin-8 (IL-8), glial fibrillary acidic protein (GFAP), monocyte chemoattractant protein-1 (MCP-1), 24-S-hydroxycholesterol (24-OHC), leptin, soluble insulin receptor (sIR), matrix metalloproteinase-10 (MMP-10) in AMX0035 group comparing to placebo. The applicant claimed that several CSF biomarkers, including total tau, pTau 181, YKL-40, Aβ 42/40 ratio may be associated with ALS clinical function or survival based on several literature publications. However, it was challenging to interpretate or draw conclusion on biomarker results in the context of ALS treatment. Not all neurodegeneration or inflammation markers in study PEGASUS showed consistent trend following AMX0035 treatment. For example, while the AMX0035 treatment appeared to lower some neurodegeneration biomarkers (e.g. total Tau, pTau 181, and FABP3), such trend was not observed on many other neurodegeneration biomarkers including NF-L. In addition, due to different disease characteristics, underlying pathophysiology, and biomarker baseline

[3] Vu L, An J, Kovalik T, Gendron T, Petrucelli L, Bowser R. Cross-sectional and longitudinal measures of chitinase proteins in amyotrophic lateral sclerosis and expression of CHI3L1 in activated astrocytes. J Neurol Neurosurg Psychiatry. 2020;91(4):350-358. doi:10.1136/jnnp-2019-321916

[4] Dreger M, Steinbach R, Otto M, Turner MR, Grosskreutz J. Cerebrospinal fluid biomarkers of disease activity and progression in amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry. 2022;93(4):422-435. doi:10.1136/jnnp-2021-327503

levels of AD and ALS, it is unclear if the biomarker findings observed in AD patients can be generalized or extrapolated to ALS patients. Lastly, the bioanalytical method validation has not been established for any of these CSF biomarkers. In conclusion, the submitted biomarker data are not clear evidence of a CNS effect or a potential for clinical benefit in patients with ALS.

There are limited other clinical pharmacology information including exposure-response analysis to provide supportive evidence of effectiveness for AMX0035. A PPK model for TUDCA and its metabolites has not been established and thus the PPK characterization was conducted for only one of the components (only for PB and its metabolite phenylacetic acid (PAA)).

3.3.2 Is the proposed dosing regimen appropriate for the general patient population for which the indication is being sought?

Yes, the proposed dosing regimen, 1 packet once daily for the first three weeks of dosing and increased dose to 1 packet twice daily (BID) as tolerated, is same as the dosing regimen tested in CENTAUR study. In the Phase 2 CENTAUR study, majority of participants (89%) were up titrated to 1 packet BID dosing after the initial 1 packet QD dosing of 3 weeks.

The rationale for dose selection was mainly based on the previous clinical experience that supported the safety of the individual components along with adequate nonclinical safety studies. PB and TUDCA were both studied (as monotherapy) in patients with ALS in two clinical studies reported in published literature.

In one study, PB was evaluated in a 20-week open-label, dose-escalation study at doses of 9-21 g/day in 26 completers, in which the biomarker of interest, histone acetylation, was the primary endpoint[5]. In another study, TUDCA was studied in a randomized study of 34 patients with ALS in Italy[6]. Patients were treated with 1 g TUDCA twice daily for 54 weeks and was well tolerated. Both clinical studies were not designed to show contribution of individual components in the treatment of ALS on clinical endpoints. The applicant used these prior clinical experiences of PB and TUDCA to support the safety of the selected dose in the registration study CENTAUR. The contribution of the individual components of AMX0035 (i.e., PB and TUDCA) on clinical endpoints is not clear.

However, the sponsor submitted in vitro studies to support the selected doses of each component of AMX0035. In vitro pharmacology studies submitted by the applicant concluded that PB and TUDCA at 500 µM and 250 µM yielded optimal pharmacological effects in several in vitro models, including a hydrogen peroxide-induced oxidative cell death model and a spinal cord motor neurons model with injury induced by glutamate exposure. However, in the clinical study A35-002 in healthy subjects, the mean C_{max} of unbound TUDCA was about 0.026 µM, which was significantly lower than the TUDCA concentrations tested in the in vitro models. The mean C_{max} of unbound PB in Study A35-002 ranged

[5] Cudkowicz, Merit E et al. "Phase 2 study of sodium phenylbutyrate in ALS." Amyotrophic lateral sclerosis : official publication of the World Federation of Neurology Research Group on Motor Neuron Diseases vol. 10,2 (2009): 99-106. doi:10.1080/17482960802320487

[6] Elia, A E et al. "Tauroursodeoxycholic acid in the treatment of patients with amyotrophic lateral sclerosis." European journal of neurology vol. 23,1 (2016): 45-52. doi:10.1111/ene.12664

from 66 to 218 µM. While these studies showed pharmacological effects for PB and TUDCA in the in vitro setting, there are some differences in the tested concentrations and clinical exposures, as mentioned above. We also note that the translatability of these in vitro models to clinical benefits in treating ALS is not clear.

3.3.3 Is an alternative dosing regimen and/or management strategy required for subpopulations based on intrinsic factors?

No dose adjustments are needed based on mild renal impairment (CLcr 60-89 mL/min), or mild hepatic impairment (NCIc). However, use in patients with moderate and severe renal/hepatic impairment should be avoided because the PK or safety data are not available in these populations. Further, there are no dose adjustment recommendations based on (body weight, age, sex and race). Please see details below.

Renal Impairment:

A clinical pharmacology study to evaluate the impact of renal impairment on the pharmacokinetics of AMX0035 was not conducted. A population PK approach was used to assess the impact of renal impairment on the exposure of PB and PAA. Approximate 10 ALS patients and 10 healthy subjects with mild renal impairment (60 <eGFR < 90 mL/min/1.73m^2) were included in this analysis. Renal function, as measured by creatinine clearance estimated using the Cockcroft-Gault (C-G) equation did not have a statistically significant effect on the clearance (CL/F) of PB or PAA for patients with mild renal impairment compared to subjects with normal renal function. The effect of mild renal impairment on the PK exposure of TUDCA and its metabolites cannot be evaluated because a PPK model for TUDCA and its metabolites has not been established due to complicated enterohepatic recirculation and interconversion of TUDCA with UDCA (please see section **4.4.1** TUDCA, UDCA, and GUDCA for details). Safety profile in ALS patients with mild renal impairment was acceptable. For patients treated with AMX0035, there are no differences in AEs and no increase in gastrointestinal events in patients with mild renal impairment, comparing to the patients with normal renal function. Based on safety data, no dose adjustment is proposed for patients with mild renal impairment. Avoid use in moderate and severe renal impairment patients because the PK or safety data are not available in these populations.

Hepatic Impairment:

A clinical pharmacology study to evaluate the impact of hepatic impairment on the pharmacokinetics of AMX0035 was not conducted. A PPK approach was used to assess the impact of elevated liver enzyme ALT on the exposure of PB and its metabolite PAA. Only 7 ALS patients, who had elevated ALT and non-BLQ concentration data, were included in this covariate analysis. Although the PPK analysis concluded that elevated ALT (50 -116 U/L) did not have a statistically significant effect on the clearance (CL/F) of PB or PAA for patients, such analysis is considered exploratory due to the limited sample size. In addition, although elevated ALT can be classified as mild hepatic impairment based on National Cancer Institute Classification system (NCIc), NCIc is different from the Child-Pugh classification recommended by the FDA for evaluating PK in patients with impaired hepatic function[7]. The effect of mild function impairment on

[7] Guidance for Industry: Pharmacokinetics in patients with impaired hepatic function: Study design, data analysis, and impact on dosing and labeling (2003).

the PK exposure of TUDCA and its metabolites cannot be evaluated because a PPK model for TUDCA and its metabolites has not been established due to complicated enterohepatic recirculation and interconversion of TUDCA with UDCA (please see section **4.4.1** TUDCA, UDCA, and GUDCA for details). Safety profile in ALS patients with mild hepatic impairment was acceptable. For patients treated with AMX0035, there are no differences in AEs and no increase in gastrointestinal events in patients with elevated ALT, comparing to the patients with normal hepatic function. Based on the safety data, no dose adjustment is proposed for patients with mild hepatic impairment. Avoid use in moderate and severe hepatic impairment patients because the PK or safety data are not available in these populations.

Body Weight:

The PPK analysis revealed that body weight had a significant effect on the exposure of PAA, but not PB exposure. Comparing to a subject with mean body weight of 82 kg for ALS patients in study AMX0035, a subject with body weight of 50 kg is expected to have 45% and 100% higher PAA C_{max} and $AUC_{0\text{-}inf}$, respectively (see ***Table 6*** in section **4.4.4** PK Simulations for PB, PAA for details). A subject with body weight of 115 kg is expected to have 32% and 42% lower PAA C_{max} and $AUC_{0\text{-}inf}$, respectively (see ***Table 6***). The impact from body weight on TUDCA and its metabolites is unknown because a PPK model was not established for TUDCA and its metabolites due to complicated enterohepatic recirculation and interconversion between TUDCA and UDCA (please see section **4.4.1** TUDCA, UDCA, and GUDCA for details). Because the clinical significance of the changes in PAA exposure due to different body weight is not clear, no dose adjustment recommendation can be provided.

Age, sex, race:

The PPK analysis showed that age, sex, or race do not have a significant effect on the exposure of PB and PAA exposure. The effect of age, sex or race on the PK of TUDCA and its metabolites is unknown, because a PPK model for TUDCA and its metabolites has not been established due to complicated enterohepatic recirculation and interconversion between TUDCA and UDCA (please see section **4.4.1** TUDCA, UDCA, and GUDCA for details).

3.3.4 Are there clinically relevant food-drug or drug-drug interactions and what is the appropriate management strategy?

Food Effect:

Food effect was evaluated in a food effect study in healthy volunteers in Study A35-002. Co-administration of AMX0035 with a standard high-fat meal resulted in reducing the C_{max} by 76% and AUC by 55% for PB and increasing the $AUC_{0\text{-}24h}$ by 38% for TUDCA. The high-fat meal did not change the C_{max} of TUDCA or the time to maximum plasma concentrations (T_{max}) of PB and TUDCA.

In Phase 2 CENTAUR study, AMX0035 was administered before a meal or snack. However, patients had the option to take a snack or meal after the drug administration to lessen the bitter aftertaste of AMX0035. Considering the key efficacy study was conducted in this manner with respect to food intake, the review team recommends the same dosing instruction on administering AMX0035 as used in Phase 2 study.

Feeding Tube Administration:

In Centaur study, AMX0035 was dissolved before oral administration or via feeding tube. Because the active ingredients dissolves in 8 oz. of water, AMX0035 can be administer via feeding tube (Refer to OPQ integrated Review for details).

Drug-Drug Interactions (DDI):

DDI Based on In Vitro Results:

Based on results from in vitro DDI evaluation on AMX0035, AMX0035 has the DDI potential with CYPs and transporter-mediated pathways. AMX0035's DDI potential are summarized below:

Transporter (in vitro)	The following information was concluded from in vitro studies: • AMX0035 is a substrate of OATP1B3, MATE2-K, OAT3, and BSEP. • AMX0035 inhibited OAT1, P-gP, BRCP at clinically relevant concentration.
CYPs Inhibitor/Inducer (in vitro)	• AMX0035 has the inhibition potential on CYP2B6 and CYP2C8 at clinically relevant concentration. • AMX0035 has the induction potential on CYP1A2, CYP2B6 and CYP3A4.

However, clinical DDI studies have not been conducted to confirm AMX0035's potential as a victim or perpetrator drug. Based on the in vitro DDI results, it is recommended to avoid concomitant use of substrates of CYP1A2, CYP2C8, CYP2B6, CYP3A4, OAT1, P-gP, BCRP where a small change in substrate plasma concentration may lead to serious toxicities and loss of efficacy, and OATP1B3 inhibitors with AMX0035. Several clinical DDI studies are required at post-marketing setting to inform the DDI labeling recommendation for AMX0035:

- In vivo drug interaction study to evaluate the effect of AMX0035 on inhibiting and/or inducing concomitant drugs that substrates of CYP2C8, CYP1A2, CYP2B6, and CYP3A4.

  Rationale: AMX0035 showed inhibition of CYP2C8 and CYP2B6 and induction potential on CYP1A2, 2B6, and 3A4 in vitro. The clinical DDI should be evaluated to provide dose adjustment recommendations for substrates of these enzymes concomitantly administered with AMX0035.

- In vivo drug interaction study to evaluate the effect of a transporter inhibitor of OATP1B3 on the exposure of AMX0035

  Rationale: AMX0035 is a substrate of OATP1B3 in vitro. The clinical DDI should be evaluated to provide dose adjustment recommendations with OATP1B3 inhibitors.

- In vivo drug interaction study to evaluate the effect of AMX0035 on inhibiting concomitant drugs that are substrates of OAT1, BCRP, and P-gP.

Rationale: AMX0035 has the inhibition potential on OAT1, and BCRP, P-gP at clinically relevant concentration in vitro. The clinical DDI should be evaluated to provide dose adjustment recommendations.

Other DDIs:

DDIs for other drugs' effects on AMX0035 are proposed by the applicant based on their known effect on bile acids metabolism or potential pharmacodynamic interaction with PB as following:

- Bile Acid Sequestering Agents: Bile acid sequestering agents may interfere with the absorption of bile acids such as TUDCA. Because disposition of TUDCA may involve enterohepatic recirculation, the recommendation for avoiding concomitant use of bile acid sequencing agent with AMX0035 is acceptable.
- Inhibitors of Bile Acid Transporters: Inhibitor of bile acid transporters such as the BSEP may affect the PK of TUDCA and its metabolites. The applicant proposes to exercise caution and monitor serum transaminases and bilirubin for concomitant use of a strong inhibitor of BSEP. However, due to the unknown PK and clinical impact, we recommend avoiding concomitant use of strong inhibitors of bile acid transporters with AMX0035.
- Aluminum-based Antacids: Aluminum-based antacids have been shown to adsorb bile acids based on literature. Because disposition of TUDCA may involve enterohepatic recirculation, there is inadequate evidence suggesting that the 2-hour staggered dosing will be adequate to mitigate the DDI potential. We recommend avoiding concomitant use with aluminum-based antacids with AMX0035.
- Probenecid: Probenecid is known to inhibit the renal excretion of many compounds and may affect renal excretion of sodium phenylbutyrate metabolites. AMX0035 is also found as a substrate of renal transporter OAT3 in vitro. The recommendation for avoiding concomitant use of probenecid with AMX0035 is acceptable.
- HDAC Inhibitors: PB is reported as a pan-histone deacetylase (HDAC) inhibitor. It is acceptable to avoid use of AMX0035 with other HDAC inhibitors.

4. APPENDICES

4.1 Summary of Bioanalytical Method Validation and Performance

PK analysis conducted and included in this submission used two validated liquid chromatography with tandem mass spectrometric detection (LC-MS/MS) assays for the quantification of PB, PAA, TUDCA (Method ATM-2492) and UDCA and GUDCA (Method ATM-2484). The method performance characteristics, including precision, accuracy, stability, are provided in below table.

Table1: Summary of Bioanalytical Method Validation Information

Parameter	Analyte				
	PB	PAA	TUDCA	UDCA	GUDCA
Contract Research Organization	(b) (4)				
Matrix	Plasma				

Method	HPLC-MS/MS (ATM-2492)			HPLC-MS/MS (ATM-2484)	
Validation Report #	4008198			1004536	
Calibration range	0.800 to 400 µg/mL	1.60 to 800 µg/mL	20.0 to 10,000 ng/mL	20.0 to 10,000 ng/mL	20.0 to 10,000 ng/mL
Intrarun precision (%CV)	1.7-6.3	0.8-4.5	2.1-8.4	0.6-8.6	1.8-8.3
Intrarun accuracy (%)	-8.1-11.3	-9.2-1.3	-2.5-2.7	-3.1-2.8	-2.0-5.0
Interday precision (%CV)	2.4-4.5	2.0-6.0	2.5-5.5	17-5.2	2.2-4.9
Interrun accuracy (%)	-6.9-8.1	-5.0-0.3	-1.5-0.8	-2.5-1.7	-1.3-2.1
Freeze/thaw stability, -20°C	5 Cycles			5 Cycles	
Long-term freezer stability, -20°C	440 days			1184 days	
Benchtop stability, room temperature	24 hours			24 hours	
Studies Supported	A35-002, CENTAUR study				

%CV = coefficient of variation; GUDCA = glycoursodeoxycholic acid; HPLC-MS/MS = high-performance liquid chromatography–tandem mass spectrometry;
PAA = phenylacetate ; PB = phenylbutyrate ; TUDCA = tauroursodeoxycholic acid; UDCA = ursodeoxycholic acid;

Reviewer's Comments:

1. *For the bioanalytical sample analysis of CENTAUR study, study samples were stored up to 967 days at -20 °C, exceeding the validated long-term stability (LTS) duration of 440 days at -20 °C for PB, PAA, and TUDCA in human plasma. Therefore, the concentration data for PB, PAA, and TUDCA from study samples stored beyond 440 days are considered as not reliable. The population PK analysis was subsequently updated with a restricted data, containing data from samples analyzed within established stability duration (Refer to section 4.4, Population PK analysis).*
2. *The validated LTS duration for UDCA and GUDCA in human plasma was 1184 days at -20 °C, which is adequate to cover the CENTAUR study samples for the quantitation of UDCA and GUDCA.*
3. *For the bioanalysis of Study A35-002, all study samples were covered by the validated LTS for all analytes (PB, PAA, TUDCA, UDCA, and GUDCA) in human plasma.*
4. *In bioanalytical methods ATM-2492 (PB, PAA, and TUDCA) and ATM-2484 (UDCA and GUDCA), all the calibrators were prepared in surrogate matrix (2X charcoal-scrubbed human plasma). In addition, several QC levels, including LLOQ and LQC levels for PA, PAA, and TUDCA, and LLOQ, LQC, and MQC levels for UDCA and GUDCA, used for validation and sample analysis were prepared in the surrogate matrix. An information request was sent to the applicant for justification and impact on the bioanalytical results for using surrogate matrix. The applicant stated that the equivalence of using the surrogate and unaltered matrix was supported through selectivity testing in unaltered matrix, and reproducible results in QC samples prepared in unaltered matrix. In addition, the reviewer noted that matrix effect, evaluated in six different lots of unaltered plasma, was minimal for all three analytes. Therefore, the use of surrogate matrix during validation and sample analysis was unlikely to have significant impact on the bioanalytical results.*

4.2 In Vitro Studies

Studies 19AMLXP1_CYP Phenotyping, 19AMLXP2R1, 19AMLXP1_CYP Inhibition, and 19AMLXP1_CYP Induction

In vitro testing for the DDI potential of AMX0035 was conducted by incubating PB and TUDCA together under various experimental conditions in studies 19AMLXP1_CYP Phenotyping, 19AMLXP2R1, 19AMLXP1_CYP Inhibition, and 19AMLXP1_CYP Induction. Therefore, the substrate, inhibition, or induction potential was determined as a combinational effect from PB and TUDCA at the tested concentrations. The in vitro studies concluded the following findings:

- PB/TUDCA combination was not found as substrates of CYP Enzymes or transporters of P-gP or BCRP
- PB/TUDCA combination at 7400/1600 µM showed more than 50% inhibition for CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4, with the highest inhibition on CYP2B6 (IC_{50} < 2467/553 µM). The R_1 values were above the cut-off of 1.02 for all evaluated CYPs
- In the in vitro induction tests, the induction potential by PB and TUDCA was tested up to 826/108 µM because cytotoxicity of PB/TUDCA was observed for concentration levels at 826/108 µM and beyond. The results showed that PB/TUDCA did not induce CYP1A2 or CYP3A4 mRNA at up to 826/108 µM but induce CYP2B6 mRNA at 826/108 µM using hepatocyte from all three donors.

- In vitro study results showed that PB/TUDCA combination had potential to inhibit OAT1 (IC_{50} > 6.8/0.07 µM), P-gP (1790/222 µM < IC_{50} < 5371/267 µM), and BCRP (1790/222 µM < IC_{50} < 5371/267 µM) at clinically relevant concentration. PB/TUDCA combination did not inhibit OAT3, OCT1, OCT2, OATP1B3, MATE1, or MATE2-K at clinically relevant concentration. The inhibition effect on OATP1B1 was not conclusive, with $I_{max,\,u}/IC_{50}$ at a range of 0.05-0.18.

Studies 8478622, 8478623, and 8478624:

During the NDA review, the applicant submitted in vitro DDI reports (studies 8478622, 8478623, and 8478624) for additional in vitro experiments. These experiments included the evaluation on CYPs inhibition (CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4), CYPs induction (CYP1A2, CYP2B6, and CYP3A4), transporter substrate and inhibition (OAT1, OAT3, BCRP, P-gP, OATP1B1, OATP1B3, OCT1, OCT2, MATE1, MATE2-K, and BSEP) for the DDI potential of each individual parent drugs (PB and TUDCA) and metabolites (PAA, UDCA, and GUDCA). A summary of key findings from above studies are listed below:

- Potential as CYPs inhibitor
  - PB was found to have IC_{50} for CYP2C8 as above 250 µM and R_1 as 3.1
  - PB's inhibition potential on other CYPs (CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4) was not found for up to 250 µM; However, the highest PB concentration (250 µM) used in the testing condition was less than 50 fold of $I_{max,u}$ (144 µM) as recommended in the in vitro drug-drug interaction guidance[8].
  - The potential of TUDCA, UDCA, GUDCA as CYPs inhibitors at clinically relevant concentrations is low.
- Potential as CYPs inducer
  - PB was found to have induction potential for CYP1A2, CYP2B6, and CYP3A4 at 250 µM
  - TUDCA was found to have induction potential for CYP1A2, CYP2B6, and CYP3A4 at 0.5 µM
- Potential as transporter substrate:
  - PB was found as a substrate of MATE2-K
  - TUDCA was found as a substrate of OATP1B3, P-gP, BCRP, OAT3, and BSEP
- Potential as transporter inhibitor:
  - PB was found to be an inhibitor of OAT1 (IC_{50} less than 25 µM), OAT3 (IC_{50} less than 25 µM), and MATE1 (IC_{50} less than 250 µM).
  - PB was not found to be an inhibitor of BCPR or P-gP at up to 250 µM
  - The potential of TUDCA, UDCA, GUDCA as transporter inhibitors at clinically relevant concentrations is low.

Reviewer's comments:

- *AMX0035 showed most inhibition on CYP2C8 and CYP2B6, comparing to other evaluated CYPs in vitro. AMX0035 showed induction potential on CYP1A2, 2B6, and 3A4 in vitro. For CYP1A2, 2C9, 2C19, 2D6, and 3A4, AMX0035 showed inhibition effect in vitro, with R1 marginally above the cut-off of 1.02. Therefore, the risk of AMX0035's DDI potential with CYP2C9, 2C19, and 2D6 is considered lower than other CYPs.*

[8] In Vitro Drug Interaction Studies – Cytochrome P450 Enzyme- and Transporter-mediated Drug Interactions Guidance for Industry, January 2020: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/vitro-drug-interaction-studies-cytochrome-p450-enzyme-and-transporter-mediated-drug-interactions

- *Although TUDCA was found as a substrate of P-gP and BCRP, the tested concentration used in the in vitro substrate experiment (1 µM and 10 µM) was not relevant to the intestinal concentration (I_2 at 8000 µM).*
- *Different in vitro study results were concluded for PB/TUDCA's inhibition effects on OAT3 and MATE1. PB showed inhibition effect on OAT3 (IC_{50} less than 25 µM) and MATE1 (IC_{50} less than 250 µM) in study 8478624. However, IC_{50} of PB/TUCDA combination was observed to be higher than 4928/54 µM for both OAT3 and MATE1 in study 19AMLXP2R1. Therefore, PB/TUDCA's inhibition effects on OAT3 and MATE1 cannot be ruled out.*
- *Although PB was not found to be an inhibitor of BCRP or P-gP at up to 250 µM in study 8478624, the testing concentration was significantly lower than the intestinal concentration of PB. In addition, PB/TUDCA combination was found to have inhibition effect on BCRP and P-gP at clinically relevant concentrations in Study 19AMLXP2R1. Therefore, the clinical DDI potential of PB as an inhibitor of BCRP or P-gP cannot be ruled out.*

4.3 Clinical PK Assessments

Food Effect: (Study A35-002)

Study Design:

Study A35-002 was an open-label, 2-period, 2-sequence, single dose, crossover study conducted in healthy subjects. Subjects received a single oral dose of AMX0035 on two occasions: under fasted conditions and after a high-fat standardized meal. The washout between periods was a minimum of 4 days.

Table 1 Dosing Regimens

Sequence	Period	Investigational Medicinal Product	Route of Administration
1	1	AMX0035	Oral, Fasted
	2		Oral, Fed (High-Fat)
2	1	AMX0035	Oral, Fed (High-Fat)
	2		Oral, Fasted

Prior to dosing of Period 1, baseline samples with a 24 h matched sampling were collected for TUDCA and metabolites to determine endogenous levels. All subjects were fasted for a minimum of 10 h prior to dosing on Day 1. Under fed conditions, subjects were provided a high-fat breakfast and dosing occurred 30 min after the start of breakfast. Under 24-h baseline and fasted conditions, subjects remained fasted up to 4 hr post-dose and received standardized meal between 4-5 h post-dose.

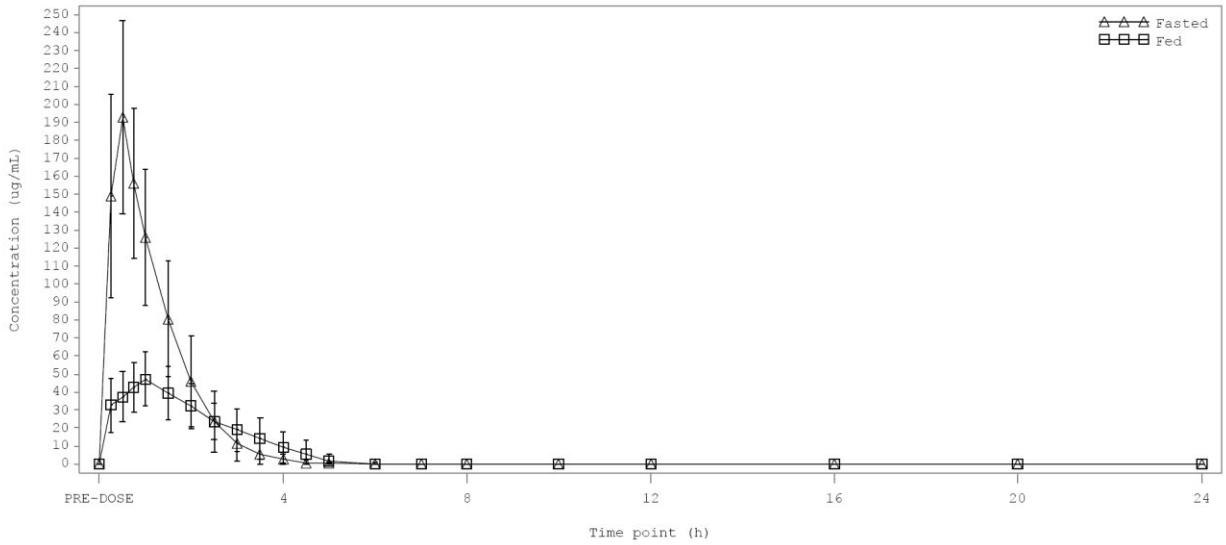
On Day -1, blood samples for baseline of TUDCA and metabolites were collected at 0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 16, 20, and 24 hr. On Day 1 of period 1 and 2, blood samples were collected at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 10, 12, 16, 20, and 24 hr. A total of 14 subjects enrolled in the study and were dosed. One subject (Subject (b) (6)) discontinued due to an AE event before dosing of Period 2. All 14 subjects were included in the PK population.

Results:

For PB and PAA, the PK analysis was based on the actual plasma concentration. For TUDCA, UDCA, GUDCA, the PK analysis was based 24 h baseline corrected (BC) concentration and concentration data from P1 and P2 were corrected using the baseline data collected on Day -1 0-24 h. For TUDCA, the baseline samples were BLQ for all subjects at all Day -1 0-24 h.

PB:

Figure 1 PK concentration time profile for PB



Source: CSR A35-002, Figure 14.2.1.1.1.1

Table 1 PK Parameters for PB

	Fasted (N=13)		Fed (N=14)	
	Mean	CV%	Mean	CV%
T_{max} (h)[1]	0.5	0.25-0.5	1	0.25-1.5
C_{max} (µg/mL)	198	28.0	50.1	31.2
$AUC_{0\text{-}last}$ (µg.h/mL)	253	34.0	117	35.7
$AUC_{0\text{-}inf}$ (µg.h/mL)	254	33.8	126	27.8
$T_{1/2}$ (h)	0.466	17.4	0.645	41.7

[1] T_{max} is expressed as median and range (min, max)

Source: CSR A35-002, Table 14.2.2.1.1.1

Table 2 Statistical Analysis Results for the Assessment of Food Effect for PB

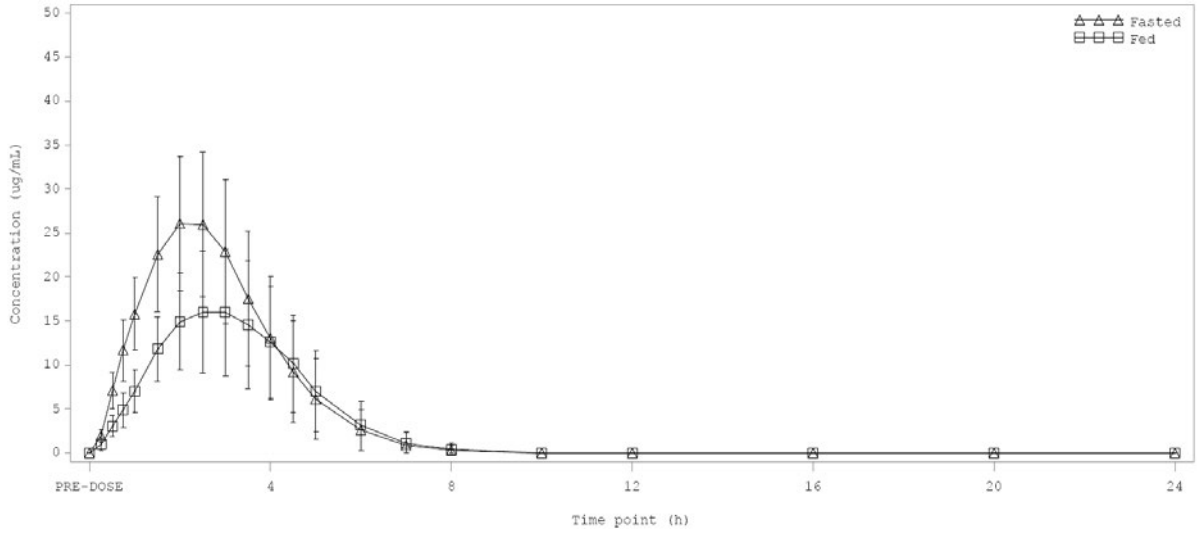
Parameter	Comparison	Fed		Fasted		Ratio (%)(2)	90% CI (3)	CVw (%) (4)
		N	Adj Geo Mean (1)	N	Adj Geo Mean (1)			
C_{max} (µg/mL)	Fed/Fasted	13	46.5	13	191	24.4	(22.3, 26.6)	12.8
$AUC_{0\text{-}last}$ (µg.h/mL)	Fed/Fasted	13	109	13	241	45.4	(41.1, 49.9)	13.8

$AUC_{0\text{-}inf}$ (µg.h/mL)	Fed/Fasted	12	120	12	261	46.0	(41.7, 50.7)	13.3

(1) Adj geo mean = adjusted geometric mean from model, (2) Ratio of adj geo means for Fed/Fasted, (3) CI = confidence interval for ratio of adj geo means, (4) CVw = Intra-subject variability
Source: CSR A35-002, Table 11

PAA:

Figure 2 PK concentration time profile for PAA



Source: CSR A35-002, Figure 14.2.1.1.1.2

Table 3 PK Parameters for PAA

	Fasted (N=13)		Fed (N=14)	
	Mean	CV%	Mean	CV%
T_{max} (h)	2.5	1.5-3.5	2.5	2.0-4.5
C_{max} (µg/mL)	27.4	27.6	17	42.4
$AUC_{0\text{-}last}$ (µg.h/mL)	86.8	34.8	61.5	44.2
$AUC_{0\text{-}inf}$ (µg.h/mL)	88.4	34.4	63	43.3
$T_{1/2}$ (h)	0.818	11.5	0.790	16.2

[1] T_{max} is expressed as median and range (min, max)
Source: CSR A35-002, Table 14.2.2.1.1.2

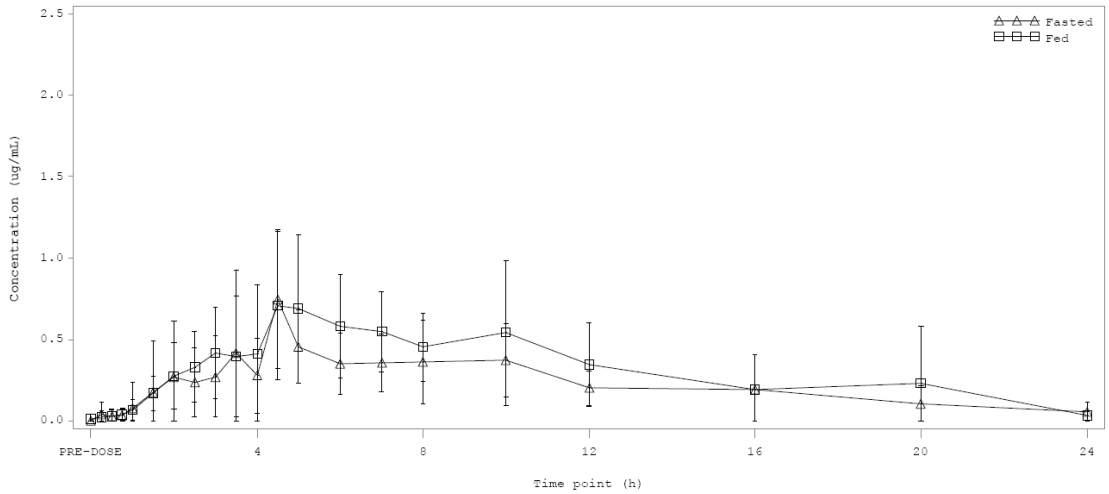
Table 4 Statistical Analysis Results for the Assessment of Food Effect for PAA

Parameter	Comparison	Fed		Fasted		Ratio (%)[2]	90% CI [3]	CVw (%) [4]
		N	Adj Geo Mean [1]	N	Adj Geo Mean [1]			
C_{max} (µg/mL)	Fed/Fasted	13	16.1	13	26.6	60.3	(54.3, 67.1)	15.0
$AUC_{0\text{-}last}$ (µg.h/mL)	Fed/Fasted	13	58.2	13	83.2	70.0	(64.6, 75.8)	11.3
$AUC_{0\text{-}inf}$ (µg.h/mL)	Fed/Fasted	13	59.8	12	84.8	70.5	(65.3, 76.2)	11.0

(1) Adj geo mean = adjusted geometric mean from model, (2) Ratio of adj geo means for Fed/Fasted, (3) CI = confidence interval for ratio of adj geo means, (4) CVw = Intra-subject variability
Source: CSR A35-002, Table 12

TUDCA:

Figure 3 PK concentration time profile for TUDCA (with time matched baseline correction)



Source: CSR A35-002, Figure 14.2.1.3.1.1

Table 5 PK Parameters for TUDCA

	Fasted			Fed		
	N	Mean	CV%	N	Mean	CV%
T_{max} (h) [1]	13	4.5	1.5-10	14	5.0	4.5-10
C_{max} (ug/mL)	13	0.871	51.7	14	0.836	52.3
$AUC_{0\text{-last}}$ (µg.h/mL)	13	5.30	59.6	14	7.27	70.8
$AUC_{0\text{-inf}}$ (µg.h/mL)	5	4.35	46.2	6	4.94	20.6
$T_{1/2}$ (h)	5	4.76	50.5	6	3.59	35.8

[1] T_{max} is expressed as median and range (min, max)
Source: CSR A35-002, Table 14.2.2.2.1.1

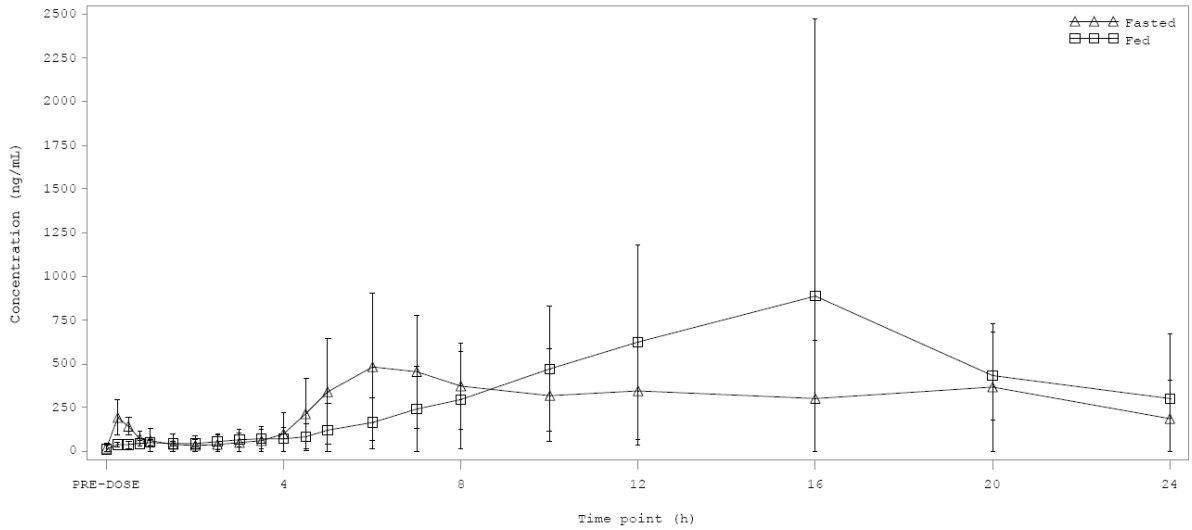
Table 6 Statistical Analysis Results for the Assessment of Food Effect for TUDCA

Parameter	Comparison	Fed		Fasted		Ratio (%) (2)	90% CI (3)	CVw (%) (4)
		N	Adj Geo Mean (1)	N	Adj Geo Mean (1)			
C_{max} (µg/mL)	Fed/Fasted	13	0.767	13	0.755	102	(78.5, 131)	37.7
$AUC_{0\text{-last}}$ (µg.h/mL)	Fed/Fasted	13	6.19	13	4.46	139	(105, 183)	40.5

(1) Adj geo mean = adjusted geometric mean from model, (2) Ratio of adj geo means for Fed/Fasted, (3) CI = confidence interval for ratio of adj geo means, (4) CVw = Intra-subject variability
Source: CSR A35-002, Table 13

UDCA:

Figure 4 PK concentration time profile for UDCA (with time matched baseline correction)



Source: CSR A35-002, Figure 14.2.1.1.1.4

Table 7 PK Parameters for UDCA

	Fasted			Fed		
	N	Mean	CV%	N	Mean	CV%
T_{max} (h) [1]	13	6.0	0.25-20	14	16.0	6-24
C_{max} (µg/mL)	13	0.766	57.6	14	1.13	139
$AUC_{0\text{-}last}$ (µg.h/mL)	13	6.67	61.9	14	9.50	88.5
$AUC_{0\text{-}inf}$ (µg.h/mL)	1	11.3	NC	1	8.58	NC
$T_{1/2}$ (h)	2	5.29	61.1	1	5.31	NC

[1] T_{max} is expressed as median and range (min, max)

Source: CSR A35-002, Table 14.2.2.2.1.2

Table 8 Statistical Analysis Results for the Assessment of Food Effect for UDCA

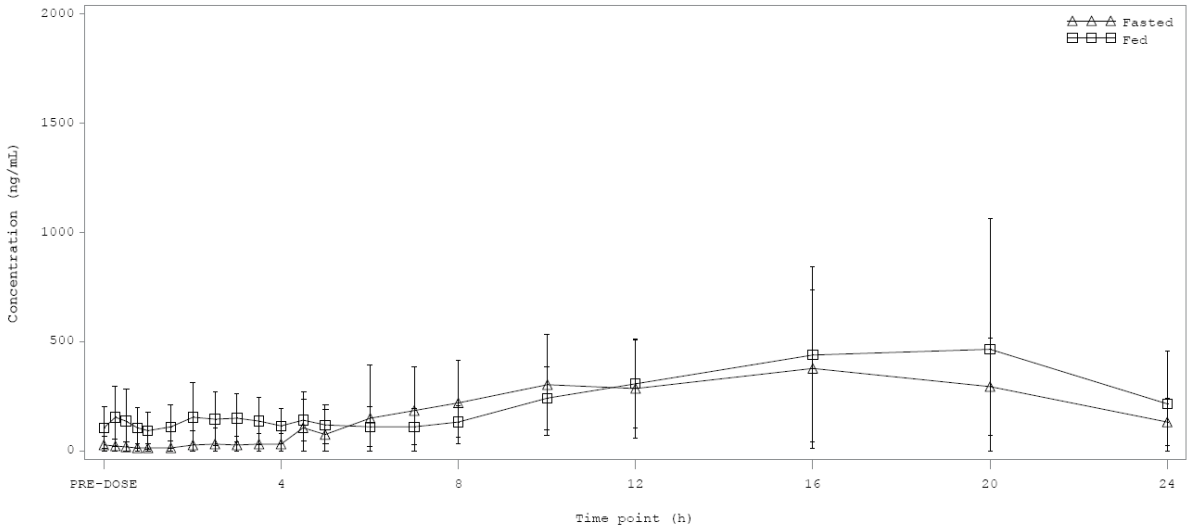
Parameter	Comparison	Fed		Fasted		Ratio (%) (2)	90% CI (3)	CVw (%) (4)
		N	Adj Geo Mean (1)	N	Adj Geo Mean (1)			
C_{max} (µg/mL)	Fed/Fasted	13	0.712	13	0.635	112	(65.8, 191)	87.7
$AUC_{0\text{-}last}$ (µg.h/mL)	Fed/Fasted	13	7.54	13	5.53	136	(88.5, 210)	67.3

(1) Adj geo mean = adjusted geometric mean from model, (2) Ratio of adj geo means for Fed/Fasted, (3) CI = confidence interval for ratio of adj geo means, (4) CVw = Intra-subject variability

Source: CSR A35-002, Table 14

GUDCA:

Figure 5 PK concentration time profile for GUDCA (with time matched baseline correction)



Source: CSR A35-002, Figure 14.2.1.1.1.5

Table 9 PK Parameters for GUDCA

	Fasted			Fed		
	N	Mean	CV%	N	Mean	CV%
T_{max} (h) [1]	13	16.0	6.0-20	14	16	0.5-24
C_{max} (µg/mL)	13	0.478	77.3	14	0.613	92.3
$AUC_{0\text{-}last}$ (µg.h/mL)	13	5.06	71.8	14	6.36	68.4
$AUC_{0\text{-}inf}$ (µg.h/mL)	0	NC	NC	0	NC	NC
$T_{1/2}$ (h)	1	12.7	NC	2	17.9	54.1

[1] T_{max} is expressed as median and range (min, max)

Source: CSR A35-002, Table 14.2.2.2.1.3

Table 8 Statistical Analysis Results for the Assessment of Food Effect for GUDCA

Parameter	Comparison	Fed		Fasted		Ratio (%) (2)	90% CI (3)	CVw (%) (4)
		N	Adj Geo Mean (1)	N	Adj Geo Mean (1)			
C_{max} (µg/mL)	Fed/Fasted	13	0.514	13	0.382	134	(96.0, 188)	50.6
$AUC_{0\text{-}last}$ (µg.h/mL)	Fed/Fasted	13	5.76	13	4.20	137	(106, 178)	38.4

(1) Adj geo mean = adjusted geometric mean from model, (2) Ratio of adj geo means for Fed/Fasted, (3) CI = confidence interval for ratio of adj geo means, (4) CVw = Intra-subject variability

Source: CSR A35-002, Table 15

Conclusion:

Following single oral administrations of AMX0035, plasma exposure to PB, based on C_{max}, $AUC_{0\text{-}last}$ and $AUC_{0\text{-}inf}$, showed decreases of approximately 76%, 55% and 54%, respectively, in the fed state relative to the fasted state.

The geometric mean C_{max}, $AUC_{0\text{-}last}$ and $AUC_{0\text{-}inf}$ of PAA showed decreases of approximately 40%, 30% and 29%, respectively, under fed state relative to the fasted state.

The geometric mean $AUC_{0\text{-}last}$ of TUDCA showed increase of approximately 39% under fed state relative to the fasted state. The C_{max} was unchanged.

Following administration in the fed state, the geometric mean C_{max} and $AUC_{0\text{-}last}$ for metabolite UDCA showed increases of approximately 12% and 36%, respectively, compared to the fasted state. However, due to the high variability these changes did not achieve statistical significance.

Following administration in the fed state, the geometric mean C_{max} and $AUC_{0\text{-}last}$ for metabolite GUDCA showed increases of approximately 34% and 37%, respectively, in the fed state; however, only the difference in AUC(0-last) achieved statistical significance.

The evaluation of $AUC_{0\text{-}inf}$ is not available for TUDCA, UDCA, and GUDCA due to inadequate characterization of terminal slopes. Therefore, $AUC_{0\text{-}inf}$ was not included in statistical analysis of the food effect for TUDCA, UDCA, or GUDCA.

Gender effect was not found for the C_{max} and AUC of PB, PAA, TUDCA, UDCA, and GUDCA in healthy volunteers.

Reviewer's Comments:

- *Due to limited sample size, the estimation of $AUC_{0\text{-}inf}$ and elimination phase PK parameters (e.g. $T_{1/2}$) may not be reliably determined for TUDCA, UDCA, and GUDCA.*
- *GUDCA pre-dose level are different for most subjects comparing to P1 to P2. The PK data of GUDCA should be interpretated with caution.*
- *The reviewer was able to verify and confirm the applicant's analyses and results.*

4.4. Population PK Analyses

The population PK report (pop-pk-report.pdf), submitted to sequence 0001, module 5335, is titled "*Population Pharmacokinetics of Phenylbutyrate (PB) and Phenylacetate (PAA) following the Administration of AMX0035 for the Treatment of Amyotrophic Lateral Sclerosis (ALS)*". The objectives of these analyses are to develop a population PK model to describe the plasma concentrations of PB and its metabolite, PAA, to identify covariates that explain PK variability, and to perform simulations to examine the influence of different covariates on PB and PAA exposure. The Applicant addresses why a PPK model has not been established for TUDCA, UDCA, and GUDCA.

4.4.1 TUDCA, UDCA, and GUDCA

The Applicant was unable to generate a population PK model for TUDCA or its metabolites UDCA and GUDCA. The Applicant provides the following statement:

"TUDCA is well-absorbed and generally has a concentration profile with multiple peaks usually occurring approximately 4 and 6 hours after oral dosing. This is characteristic of a biliary salt and is consistent with enterohepatic recirculation. Population PK modeling of TUDCA was investigated and it was determined not to be feasible to characterize the complex PK profile of TUDCA and its major metabolites, UDCA, and glycoursodeoxycholic acid (GUDCA), based on the limited plasma concentration data available. This potential problem was anticipated in the population PK analysis plan. Instead, plasma concentrations of TUDCA and its metabolites for ALS patients in the Phase 2 study are described in separate report using a non-model-based approach as specified in the population PK analysis plan."

Source: Sequence 0001, module 5335, pop-pk-report.pdf, page 16 to 17 of 191

[*Reviewer comment: A population PK model has not been established for TUDCA, UDCA, or GUDCA. OCP agrees that the presence of multiple peaks in the concentration profile after oral dosing is consistent with enterohepatic circulation and is a factor that likely contributes to this outcome. Another factor that likely contributes to this outcome is that TUDCA and UDCA interconvert between one another. For example, the Applicant's statement above from page 17 of pop-pk-report.pdf indicates that UDCA is a major metabolite of TUDCA. However, the Applicant also provides this statement:*

"When these secondary bile acids are recirculated back to the liver, conjugation with glycine or taurine can further differentiate them, such as the addition of taurine to UDCA forms TUDCA" *(summary-clin-pharm.pdf, page 11 of 44).*]

4.4.2 PB, PAA

The PPK analyses for PB and its metabolite PAA included 860 PK samples collected from a subset of n=85 subjects (n=14 healthy subjects from Phase 1 study A35-002 and n=71 subjects with ALS in Phase 2 study CENTAUR study). Information about these two studies from which the data were collected are summarized in the table below:

Table 1: Clinical Studies from which data were collected for inclusion in the analysis in the main PPK Report (pop-pk-report.pdf)

Type of Study	Study Number	Location of Study Report in CTD	Study Objectives	Study Design and Type of Control	Test Products; Dosage Regimen; Route of Administration	Number of Subjects	Healthy Subjects or Diagnosis of Patients	Duration of Treatment	PK Sample Timing
Phase 1, Food Effect	A35-002	5.3.3.1	Determine the plasma levels and PK parameters of sodium phenylbutyrate, taurursodiol, and major metabolites following single oral dose administration of AMX0035; Evaluate the effect of a high-fat standardized breakfast on the extent and rate of absorption of sodium phenylbutyrate, taurursodiol and active metabolites	Open label, 2 sequence, 2-period, cross-over	AMX0035; 1 sachet (1 g taurursodiol and 3 g sodium phenylbutyrate); Oral	14	Healthy	Single dose	pre-dose, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 16, 20, and 24 hours post-dose, during both study periods

Phase 2, Efficacy	AMX3500	5.3.5.1	Confirm the safety and tolerability of a fixed-dose combination of phenylbutyrate and taurursodiol; Measure the impact of a fixed-dose combination of PB and taurursodiol using the slope of progression with the (ALSFRS R)	Randomized, Placebo-controlled	AMX0035; 1 sachet BID (1 sachet = 1 g taurursodiol and 3 g sodium phenylbutyrate); Oral	137	ALS	24 weeks	baseline: pre-dose. Wk 12 24: either 1- or 4-hrs post-dose.

Source: Sequence 0001, module 52, tabular-listing-all-clinical-studies.pdf, page 1 to 2 of 2

The final model includes one compartment, first-order absorption, first-order metabolism of PB into PAA assuming 100% conversion, a transit compartment to represent the delay in appearance of PAA into the plasma, and non-linear elimination of PAA. Structural PK parameters include PB apparent clearance (CL_{PB}/F), PB apparent volume of distribution (V_{PB}/F), Volume of distribution of PAA (V_{PAA}), absorption rate constant (Ka_t), mean transit time for appearance of PAA into plasma after formation (MTT), a non-linear elimination process for PAA [maximum rate of PAA elimination (V_{maxPAA}), and plasma concentration at which PAA elimination rate is half-maximum (Km)].

Allometric Scaling: Applied to $V_{max\text{-}PAA}$ using a power model with weight normalized to 70 kg. The scaling exponent was estimated.
Interindividual Variability: exponential. Variance terms for CL_{PB} /F, V_{PB}/F, V_{PAA}, and V_{maxPAA}. Covariance term for CL_{PB}/F with V_{PB}/F.
Residual Variability: proportional for PB, proportional for PAA.
Covariates: Meal state (fasted versus fed) is a covariate on Ka (Ka_{Fast} versus Ka_{Fed}) and relative bioavailability versus a fasted state (F_{Food}). ALS diagnosis is a covariate on $V_{max\text{-}PAA}$ and V_{PAA}. Parameter estimations for the final run (Run PB_PAA_model728l3015016 or Run 5016) are shown in the table below.

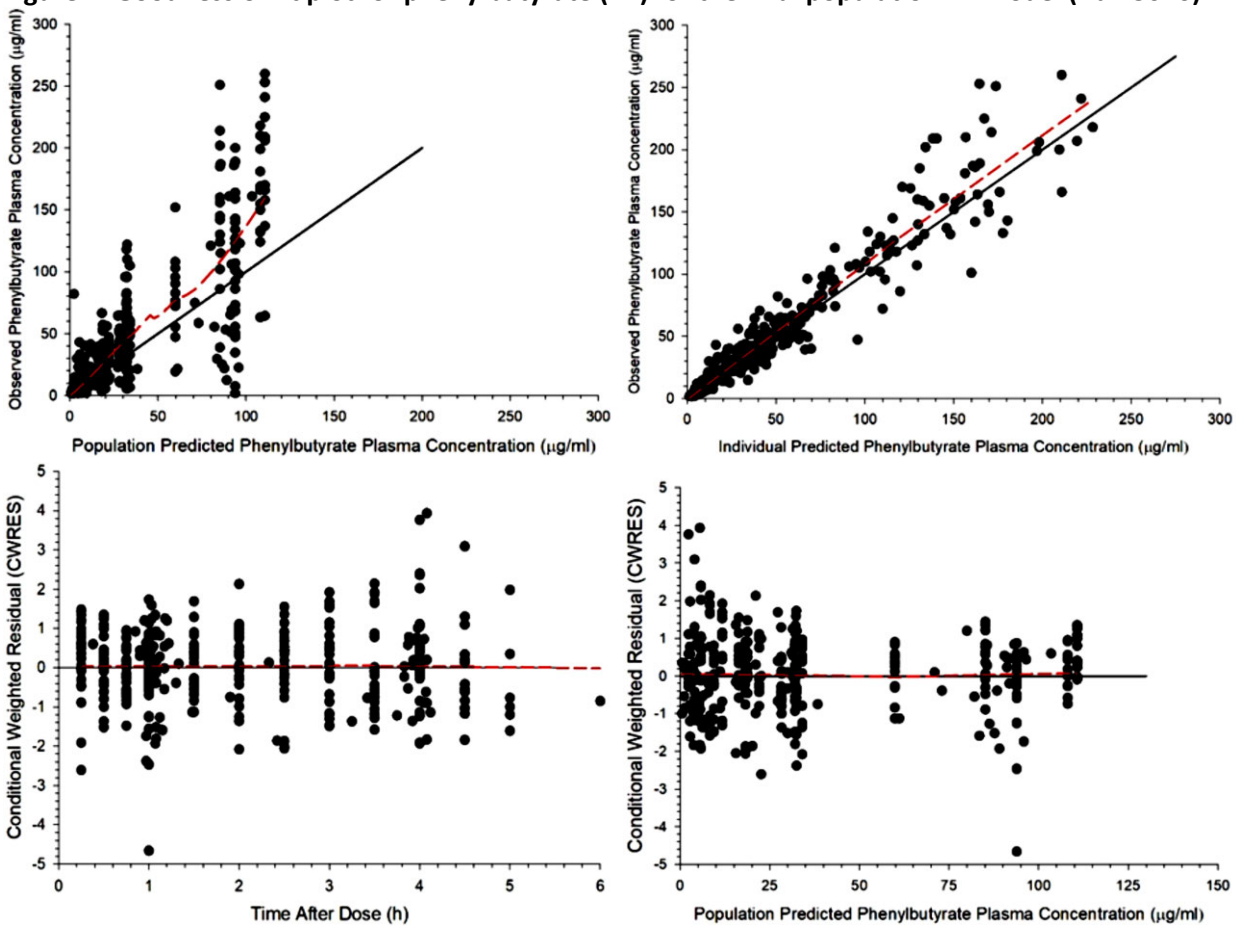
Table 2: Parameter Estimates for the Final PPK Model (Run 5016)

	Base Model	Final Model	Bootstrap	
Fixed Effect Parameters*			**Median**	**2.5th - 97.5th Percentiles**
CL_{PB}/F (L/h)	14.7 (9.9)	14.8 (9.9)	14.7	11.8 – 18.6
V_{PB}/F (L)	9.1 (17.4)	9.6 (16.7)	9.7	6.3 – 14.5
V_{PAA} (L)	44.2 (6.7)	56.3 (6.7)	56.1	48.3 – 65.9
Ka_{Fast} (h^{-1})	1.98 (9.9)	1.92 (8.7)	1.96	1.58 – 3.00
$V_{max\text{-}PAA}$	811 (6.5) mg/h	585 (6.5) mg/h/70 kg	585 mg/h/70 kg	506 – 676 mg/h/70kg
Km (µg/mL)	8.4 (10.6)	7.8 (10.7)	7.7	5.2 – 10.9
Mean Transit Time (h)	0.124 (16.1)	0.123 (16.6)	0.128	0.059 – 0.249
Ka_{Food} (h^{-1})	0.775 (5.6)	0.767 (5.2)	0.779	0.667 – 0.917
F_{Food}	0.480 (4.2)	0.476 (4.2)	0.475	0.423 – 0.544
$V_{max\text{-}PAA}$ Covariates				
Exponent for weight effect	---	0.92 (13.2)	0.930	0.649 – 1.19
ALS diagnosis	---	0.275 (25.5)	0.271	0.154 – 0.405
V_{PAA} Covariates				
ALS diagnosis	---	-0.360 (17.7)	-0.350	-0.491 - -0.189
Inter-individual Variability**				
CL_{PB}/F	0.802 (9.3) [9.1]	0.807 (9.3) [9.2]	0.791	0.600 – 0.968
V_{PB}/F	1.118 (10.4) [12.8]	1.131 (9.6) [11.8]	1.12	0.805 – 1.37
V_{PAA}	0.336 (16.6) [30.6]	0.233 (19.6) [42.4]	0.223	0.126 – 0.360
$V_{max\text{-}PAA}$	0.314 (10.2) [12.1]	0.180 (11.7) [15.3]	0.174	0.136 – 0.224
Corr (CL_{PB}/F,V_{PB}/F)	0.925 (2.7)	0.930 (2.4)	0.905	0.656 – 1.13
Residual Variability (theta derived epsilon)*				
Log additive error- PB	0.320 (4.7)	0.324 (4.6)	0.317	0.274 - 0.365
Log additive error- PAA	0.241 (4.0)	0.243 (4.0)	0.238	0.211 – 0.263
Shrinkage (%)	13.7	12.8	---	---

Source: Sequence 0001, module 5335, pop-pk-report.pdf, page 59 to 60 of 191

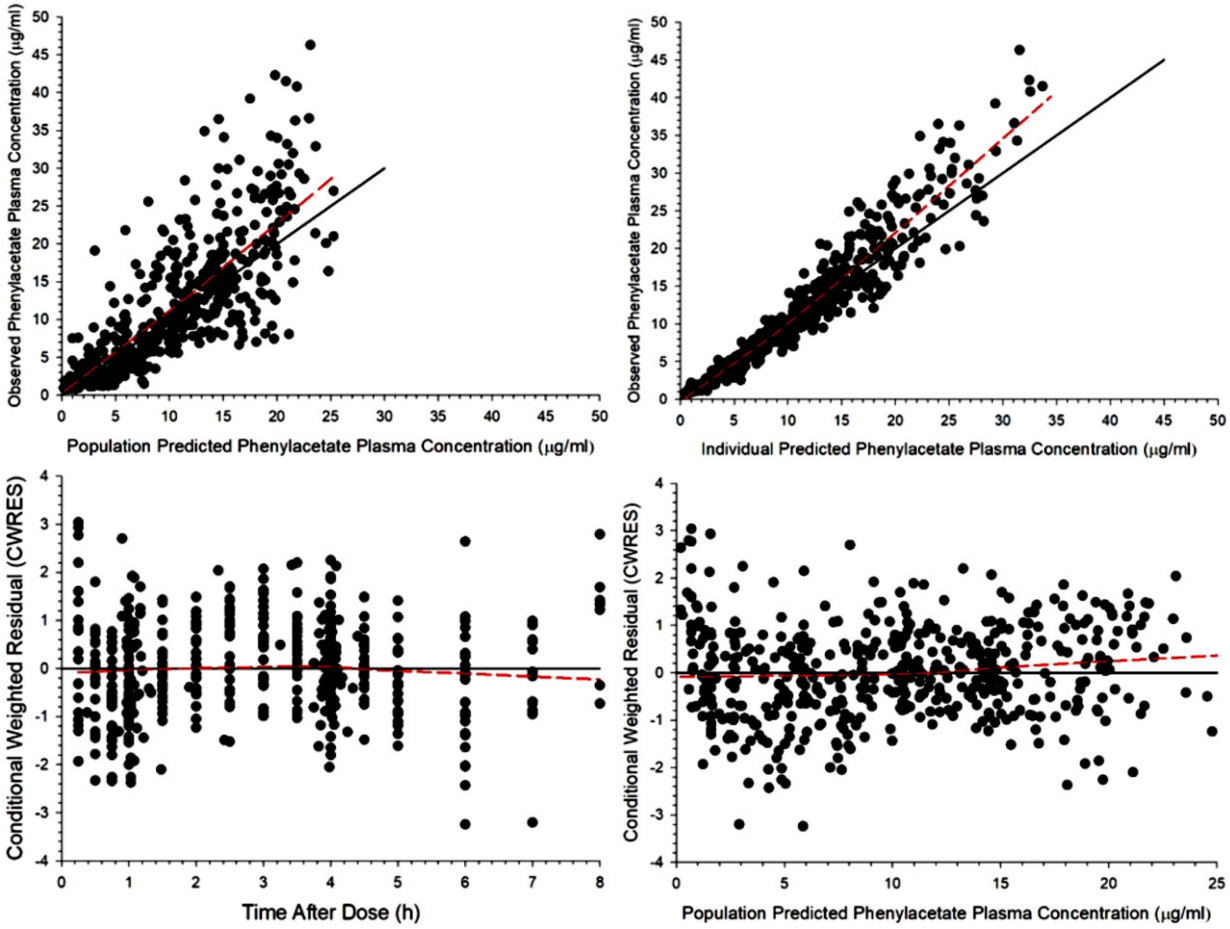
Epsilon shrinkage is 12.8%. Eta shrinkage is < 20 % for all parameters except the eta for VPAA (which is 42.4%). Key model diagnostics plots are presented in the figures below.

Figure 1: Goodness of fit plot for phenylbutyrate (PB) for the final population PK model (Run 5016)



Source: Sequence 0001, module 5335, pop-pk-report.pdf, page 117 to 118 of 191

Figure 2: Goodness of fit plot for phenylacetate (PAA) for the final population PK model (Run 5016)



Source: Sequence 0001, module 5335, pop-pk-report.pdf, page 119 to 120 of 191

[*Reviewer comment: The plots of observed versus population predicted concentrations appear to suggest modest over prediction at higher concentrations for PB and PAA. However, the conditional weighted residual plots for PB and PAA do not suggest the presence of systemic bias with respect to time after dose nor with respect to magnitude of predicted concentrations.*

The visual predictive check plots (figures 11 and 12 on pages 61 and 62 of pop-pk-report.pdf; figures not shown in review) demonstrate that the model represents the central tendency over time well for both PB and PAA in a fasted state and fed state.

Overall, the population PK model for PB and PAA is acceptable. *The data support the inclusion of the covariates of weight and ALS diagnosis for predicting PAA PK. Please refer to section* ***4.4.4*** PK Simulations for PB, PAA *for discussion of the expected effect size of these and other covariates based on PK simulations.*]

OCP determined that a subset of the PK samples of PB and PAA present in the original analytical dataset for the population pharmacokinetic analysis were analyzed after being stored beyond the established duration of long-term stability (LTS; please see section **4.1** Summary of Bioanalytical Method Validation and Performance for details). This storage of samples in excess of the LTS duration raises the concern about the reliability of these samples and how it may have affected the final population PK model. On 4 March 2022, OCP sent an information request (IR) to the Applicant addressing the concerns regarding the stability issue and how it may impact on the PK analyses:

> "*For the bioanalytical sample analysis of Study AMX3500, some study samples were stored up to 967 days at -20 °C, exceeding the validated long-term stability (LTS) duration of 440 days at -20 °C for phenylbutyrate (PB), phenylacetate (PAA), and tauroursodeoxycholic acid (TUDCA) in human plasma. Therefore, this subset of PK samples for PB, PAA, and TUDCA, from Phase 2 study AMX350 (Centaur) that were stored beyond 440 days cannot be considered reliable. You should assess the effects of excluding these pharmacokinetic (PK) samples on the results of the population PK (PPK) analyses. Your assessment should include a comparison of PPK parameter estimates from the original analyses versus analyses where the PK samples exceeding LTS are excluded. One approach you may consider is to include a "flag" variable in the input dataset identifying the PK samples exceeding the LTS and adjusting your analysis code to ignore the flagged samples. You should submit an addendum to the pop-pk-report.pdf showing a comparison of the PPK parameter estimates for the original dataset versus the PPK parameter estimates with exclusion of the flagged PK samples. The addendum report should comment on the relevance of any differences in the PPK parameter estimates upon exclusion of the flagged PK samples.*
>
> *We acknowledge your determination that you were unable to characterize the complex PK profile of TUDCA and its major metabolites using a population PK modeling approach. We also acknowledge the non-model-based approach for analyzing TUDCA and its metabolites. You should characterize the summary statistics of observed TUDCA PK samples by age group (< 65 years versus ≥ 65 years), by sex, by race/ethnicity, and renal function (normal versus mild renal impairment) for the data subset remaining after exclusion of the flagged TUDCA PK samples. While the bioanalytical issue does not impact UDCA and GUDCA, you should conduct a similar analysis for them without the exclusion process.*
>
> *You should submit an addendum report showing the summary statistics of observed TUDCA, UDCA, and GUDCA PK samples for each group for the treatment arm and again for each group in the placebo arm. The addendum report should comment on the relevance of any differences in observed PK across the groups. Along with the report addendum, you should submit an updated TUDCA PK dataset which identifies the flagged TUDCA PK samples. This can be submitted as part of the updated dataset for the PPK analyses or as a separate dataset. If these analyses require construction of a new dataset for UDCA or GUDCA, please submit that dataset as well. If these analyses will use a previously-submitted dataset for UDCA and GUDCA, you should specify the file name and location of the dataset within the NDA submission.*"

The Sponsor provided responses to the IR in sequence 0033 and 0034. In module 5335 of sequence 0033, received on 11 March 2022, the Applicant submitted report pop-pk-report-addendum.pdf titled "*ADDENDUM 1: Population Pharmacokinetics of Phenylbutyrate (PB) and Phenylacetate (PAA) Following the Administration of AMX0035 for the Treatment of Amyotrophic Lateral Sclerosis (ALS)*" which includes the results of the PPK re-analysis following exclusion of the samples which exceeded the LTS duration. There were 196 PK samples which exceeded the LTS duration, all of which came from Protocol AMX3500 conducted in ALS patients. The samples in excess of LTS fit into one of six categories:

i. 44 samples from participants receiving active drug and providing PB and PAA concentrations above the lower limit of quantitation (LLOQ),
ii. 17 samples from participants receiving active drug and providing only one analyte concentration above the LLOQ,
iii. 1 sample from a participant receiving active drug with no concentrations above the LLOQ,
iv. 10 samples from participants receiving active drug and previously excluded from the dataset (refer to Amylyx PopPK Master Plasma Conc List.xlsx for causes),
v. 59 baseline samples from participants receiving active drug, and
vi. 65 samples from participants receiving placebo.

Source: Sequence 0033, module 5335, pop-pk-report-addendum.pdf, page 5 of 8

The Applicant determined that the first three categories (i, ii, and iii) were applicable the PK dataset. The elimination of the samples in categories i, ii, and iii lead to the elimination of 105 observation records of plasma concentrations for either PB or PAA. The reduction represents 50% of the 210 observations records provided from ALS patients in the original PPK dataset.

The following table which includes a comparison of the PK parameter estimates for the final model with the original (full) dataset versus the model estimates with the restricted dataset (where the samples that exceed the stability time were excluded).

Table 3: Comparison of PPK Parameters for PB and PAA for the Final Model (Model 5016) using the Original Dataset Versus the Restricted Dataset (where Samples Analyzed Beyond the Established Long-term Stability were Censored)

	Final Model with Original Dataset	Final Model with Restricted Dataset	Bias between Datasets (%)*
Objective Function Value	-781.039	-848.036	
Number of Subjects	85	85	
Observation Records**	860	755	
Condition Number	528	1428	
Fixed Effect Parameters†			
CL_{PB}/F (L/h)	14.8 (9.9)	15.5 (11.5)	-4.7
V_{PB}/F (L)	9.6 (16.7)	9.99 (17.5)	-4.1
V_{PAA} (L)	56.3 (6.7)	56.5 (7.4)	-0.4
Ka_{Fast} (h^{-1})	1.92 (8.7)	2.08 (8.5)	-8.3
$V_{max\text{-}PAA}$ (mg/h/70 kg)	585 (6.5)	577 (6.2)	1.4
Km (µg/ml)	7.8 (10.7)	7.85 (10.7)	-0.6
Mean Transit Time (h)	0.123 (16.6)	0.120 (16.4)	2.4
Ka_{Food} (h^{-1})	0.767 (5.2)	0.785 (4.7)	-2.3
F_{Food}	0.476 (4.2)	0.468 (4.1)	1.7
$V_{max\text{-}PAA}$ Covariates			
Exponent for weight effect	0.92 (13.2)	1.07 (15.7)	-16.3
ALS diagnosis	0.275 (25.5)	0.290 (23.9)	-5.5
V_{PAA} Covariates			
ALS diagnosis	-0.360 (17.7)	-0.342 (22.6)	5.0
Inter-individual Variability‡			
CL_{PB}/F	0.807 (9.3) [9.2]	0.736 (11.6) [27.1]	8.8
V_{PB}/F	1.131 (9.6) [11.8]	1.000 (12.1) [28.4]	11.6
V_{PAA}	0.233 (19.6) [42.4]	0.260 (21.2) [44.5]	-11.6
$V_{max\text{-}PAA}$	0.180 (11.7) [15.3]	0.157 (14.2) [32.8]	-12.8
Corr (CL_{PB}/F,V_{PB}/F)	0.930 (2.4)	0.963 (1.7)	-3.5
Residual Variability (theta derived epsilon)*			
Log additive error - PB	0.324 (4.6)	0.31	4.3
Log additive error - PAA	0.243 (4.0)	0.243	0.0
Shrinkage (%)	12.8	10.9	

$$* \; Bias\ (\%) = \frac{parameter_{original\ dataset} - parameter_{restricted\ dataset}}{parameter_{original\ dataset}} \times 100$$

Source: Sequence 0033, module 5335, pop-pk-report-addendum.pdf, page 6 to 7 of 8

The Applicant reports that the relative standard error is <22% for fixed and random effect parameters and 15.7 to 23.9% for covariates. Epsilon shrinkage is 10.9%. Applicant reports that eta shrinkage was at or slightly above the acceptable limit (20%-30%) for etas associated with CL_{PB}/F, V_{PB}/F, and $V_{max\text{-}PAA}$. However, when estimating eta shrinkage based on the average empirical Bayes variances (EBVs), the shrinkage fell within acceptable limits for all 4 etas (ranging from 6.3% to 19.4%). Fifteen of the parameters changed by < 10%. Four parameters changed by more than 10% (-16.3% for Exponent for Weight Effect, 11.6% for V_{PB}/F, -11.6% for V_{PAA}, and -12.8% for $V_{max\text{-}PAA}$). The condition number increased from 528 to 1428, a change which can suggest ill conditioning of the model and may reflect an inability of the data to support the current number of parameters. However, the Applicant states that the parameter precision magnitude and close agreement of parameters in the censored dataset compared to the original dataset support the ability of the current model to suitably describe the population PK of the restricted dataset. The applicant concludes that results confirm the robustness of the population model for describing the PK of PB and PAA.

[*Reviewer comment: The finding that the condition number increases is expected based on the reduction of the number of observations. Overall, the change in the PK parameter estimates is no greater than 16.3%. Furthermore, only 4 parameters underwent a change of magnitude greater than 10% (that is, greater than a +10% change or less than a -10% change) when comparing the results of the restricted dataset versus the original dataset.*

Overall, the model estimation results with restricted dataset are reasonably close to the model estimates with the original dataset. As such, the re-analysis supports the acceptability of the original model 5016.

Covariates evaluated in the final PPK model include age, sex, race, body weight, BMI, BSA, CrCL, diagnosis of ALS, (mildly) abnormal serum ALT level, concurrent administration of CYP inducing antiepileptic drugs, and food. The analyses support inclusion of food and ALS diagnosis as covariates. Please refer to section ***4.4.3*** Observed PK by Group *for details regarding covariates based on observed PK data for PB, PAA, TUDCA, UDCA, and GUDCA. Please refer to section* ***4.4.4*** PK Simulations for PB, PAA *for discussion of the expected effect size of covariates based on PK simulations.*]

The Applicant's subsequent response to the 4 March 2022 IR (sequence 0034) is discussed in section **4.4.3** Observed PK by Group.

4.4.3 Observed PK by Group

The Applicant's original population PK analysis report includes boxplots the provide a graphical presentation how observed PK for PB and PAA vary according to various intrinsic factors such as patient versus healthy volunteer status, fed versus fasted status, age (< 65 years versus ≥ 65 years), male versus female, weight, renal function, and hepatic function (see Figure 3 for PB, and Figure 4 for PAA, on pages 37 and 39, respectively, of pop-pk-report.pdf; figures not shown in review). However, due to the concern about a subset of TUDCA PK samples being analyzed after exceeding the LTS period, the OCP sent an IR on 4 March 2022 IR requesting re-analysis of the PK data after excluding the samples of concern (see section **4.4.2** PB, PAA for details on the information request). Part of the IR is a request for the Applicant to provide descriptive statistics by group (i.e. the analyses presented in Figure 3 and Figure 4 of the population PK report) after excluding the TUDCA PK samples that were stored in excess of the LTS duration. Though there is no known issue with the assay for GUDCA or UDCA, tables of these descriptive statistics are requested for these analytes as well (as the box plots in Figure 3 and Figure 4 of the population PK report provide only a graphical comparison between these groups).

The Applicant submitted the report tudca-pk-report-addendum.pdf titled "*ADDENDUM 1: Summary Statistics of TUDCA, UDCA and GUDCA Following the Administration of AMX0035 for the Treatment of Amyotrophic Lateral Sclerosis (ALS)*" to module 5335 of sequence 0034 on 14 March 2022. Key results from report tudca-pk-report-addendum.pdf are summarized below. Mild renal insufficient or mild renal impairment (RI) is defined as eGFR > 60 mL/min and < 90 mL/min and normal renal function is defined as eGFR ≥ 90 mL/min.

Figure 3: TUDCA PK By Age (<65 Years Versus ≥65 Years) in Study AMX3500 at Baseline, and 1- and 4-hours Post-Dose Pooled Across Weeks 12 and 24 for Original Data and Re-Analysis

Pooled Visit for AMX0035 Treatment Group	Statistic	TUDCA[1] (Reanalysis)		TUDCA[2] (Original)	
		<65 yrs	≥ 65 yrs	<65 yrs	≥ 65 yrs
Pre-dose (Baseline visit)	N	15	10	56	21
	Mean (SD); ng/mL	21.7 (6.61)	20.0 (0.00)	47.0 (96.44)	20.0 (0.00)
	Median (IQR); ng/mL	20.0 (0.0)	20.0 (0.0)	20.0 (0.0)	20.0 (0.0)
	(Q1, Q3); ng/mL	(20.0, 20.0)	(20.0, 20.0)	(20.0, 20.0)	(20.0, 20.0)
	Min, max; ng/mL	20.0, 45.6	20.0, 20.0	20.0, 577.0	20.0, 20.0
	Mean to Std Dev ratio (%)	328%	--	48.8%	--
1 hr post dose (Week 12 and Week 24 pooled)	N	21	14	48	19
	Mean (SD); ng/mL	361.2 (450.78)	188.3 (153.93)	468.6 (564.76)	394.1 (679.82)
	Median (IQR); ng/mL	189.0 (284.9)	142.5 (151.9)	263.0 (555.6)	145.0 (235.2)
	(Q1, Q3); ng/mL	(72.1, 357.0)	(91.1, 243.0)	(67.9, 623.5)	(89.8, 325.0)
	Min, max; ng/mL	20.0, 1830.0	34.9, 602.0	20.0, 2440.0	20.0, 2570.0
	Mean to Std Dev ratio (%)	80.1%	122%	83.0%	58.0%
4 hr post-dose (Week 12 and Week 24 pooled)	N	22	13	49	21
	Mean (SD); ng/mL	491.2 (438.10)	427.7 (251.98)	560.6 (489.01)	579.0 (707.82)
	Median (IQR); ng/mL	387.0 (629.0)	325.0 (328.0)	446.0 (704.0)	340.0 (385.0)
	(Q1, Q3); ng/mL	(125.0, 754.0)	(287.0, 615.0)	(147.0, 851.0)	(230.0, 615.0)
	Min, max; ng/mL	20.0, 1800.0	42.2, 828.0	20.0, 1840.0	20.0, 3250.0
	Mean to Std Dev ratio (%)	112%	170%	115%	81.8%

Source: Sequence 0034, module 5335, tudca-pk-report-addendum.pdf, page 8 of 12

Figure 4: UDCA and GUDCA PK By Age (<65 Years Versus ≥65 Years) in Study AMX3500 at Baseline, and 1- and 4-hours Post-Dose Pooled Across Weeks 12 and 24 for the Original Dataset

Pooled Visit for AMX0035 Treatment Group	Statistic	UDCA[1]		GUDCA[1]	
		<65 yrs	≥ 65 yrs	<65 yrs	≥ 65 yrs
Pre-dose (Baseline visit)	N	56	21	56	21
	Mean (SD); ng/mL	177.3 (798.54)	32.8 (28.64)	257.8 (626.10)	78.3 (97.13)
	Median (IQR); ng/mL	20.0 (39.0)	20.0 (3.5)	82.4 (219.4)	37.3 (58.6)
	(Q1, Q3); ng/mL	(20.0, 59.0)	(20.0, 23.5)	(31.1, 250.5)	(22.5, 81.1)
	Min, max; ng/mL	20.0, 5970.0	20.0, 116.0	20.0, 4540.0	20.0, 403.0
	Mean to Std Dev ratio (%)	22.2%	115%	41.2%	80.7%
1 hr post dose (Week 12 and Week 24 pooled)	N	48	19	48	19
	Mean (SD); ng/mL	903.5 (1011.33)	926.2 (1160.05)	1162.7 (1112.87)	850.9 (634.57)
	Median (IQR); ng/mL	637.0 (780.0)	516.0 (1051.0)	812.5 (1328.5)	815.0 (803.0)
	(Q1, Q3); ng/mL	(335.0, 1115.0)	(219.0, 1270.0)	(346.5, 1675.0)	(277.0, 1080.0)
	Min, max; ng/mL	20.0, 6020.0	33.3, 4890.0	20.0, 4600.0	194.0, 2260.0
	Mean to Std Dev ratio (%)	89.3%	79.8%	105%	134%
4 hr post-dose (Week 12 and week 24 pooled)	N	49	21	49	21
	Mean (SD); ng/mL	989.2 (1372.03)	1588.0 (1323.65)	1330.8 (1116.01)	1292.1 (621.92)
	Median (IQR); ng/mL	607.0 (710.0)	1070.0 (1478.0)	1300.0 (1571.0)	1180.0 (778.0)
	(Q1, Q3); ng/mL	(245.0, 955.0)	(702.0, 2180.0)	(379.0, 1950.0)	(872.0, 1650.0)
	Min, max; ng/mL	20.0, 7340.0	46.3, 5140.0	20.0, 5290.0	42.5, 2840.0
	Mean to Std Dev ratio (%)	72.1%	120%	119%	208%

Source: Sequence 0034, module 5335, tudca-pk-report-addendum.pdf, page 8 of 12

[*Reviewer comment: Upon the re-analysis, observed TUDCA plasma PK are numerically lower in subjects ≥ 65 years compared to subjects < 65 years. The difference is greatest at the 1 hour time ((361.2-188.2)/361.2*100 = 48% lower) and smaller at 4 hours post-dose ((491.2-427.7)/491.2*100 = 13% lower). In the context of the PK variability, the TUDCA PK difference between age groups at 4 hours appears modest. The effect of age on UDCA mean PK or GUDCA mean PK is modest compared to the variability in UDCA PK and variability of GUDCA PK.*]

Figure 5: TUDCA PK By Sex (Male versus Female) in Study AMX3500 at Baseline, and 1- and 4-hours Post-Dose Pooled Across Weeks 12 and 24 for Original Data and Re-Analysis

Pooled Visit for AMX0035 Treatment Group	Statistic	TUDCA[1] (Reanalysis)		TUDCA[2] (Original)	
		Female	Male	Female	Male
Pre-dose (Baseline visit)	N	7	18	23	54
	Mean (SD); ng/mL	20.0 (0.00)	21.4 (6.03)	23.4 (9.55)	46.6 (98.29)
	Median (IQR); ng/mL	20.0 (0.0)	20.0 (0.0)	20.0 (0.0)	20.0 (0.0)
	(Q1, Q3); ng/mL	(20.0, 20.0)	(20.0, 20.0)	(20.0, 20.0)	(20.0, 20.0)
	Min, max; ng/mL	20.0, 20.0	20.0, 45.6	20.0, 62.2	20.0, 577.0
	Mean to Std Dev ratio (%)	--	355%	245%	47%
1 hr post dose (Week 12 and Week 24 pooled)	N	9	26	20	47
	Mean (SD); ng/mL	275.7 (326.59)	297.7 (388.16)	505.6 (676.34)	422.7 (563.33)
	Median (IQR); ng/mL	145.0 (264.0)	152.5 (235.2)	251.5 (546.1)	195.0 (355.3)
	(Q1, Q3); ng/mL	(46.0, 310.0)	(89.8, 325.0)	(77.4, 623.5)	(89.7, 445.0)
	Min, max; ng/mL	20.0, 968.0	20.0, 1830.0	20.0, 2570.0	20.0, 2440.0)
	Mean to Std Dev ratio (%)	84.4%	76.7%	75%	75%
4 hr post-dose (Week 12 and Week 24 pooled)	N	9	26	21	49
	Mean (SD); ng/mL	422.7 (338.09)	483.2 (394.29)	733.9 (718.1)	494.2 (464.24)
	Median (IQR); ng/mL	348.0 (271.0)	422.5 (493.0)	702.0 (652.0)	399.0 (503.0)
	(Q1, Q3); ng/mL	(287.0, 558.0)	(158.0, 651.0)	(287.0, 939.0)	(148.0, 651.0)
	Min, max; ng/mL	22.1, 1080.0	20.0, 1800.0	20.0, 3250.0	20.0, 1840.0
	Mean to Std Dev ratio (%)	125%	123%	102%	107%

Source: Sequence 0034, module 5335, tudca-pk-report-addendum.pdf, page 9 of 12

Figure 6: UDCA and GUDCA PK By Sex (Male versus Female) in Study AMX3500 at Baseline, and 1- and 4-hours Post-Dose Pooled Across Weeks 12 and 24 for the Original Dataset

Pooled Visit for AMX0035 Treatment Group	Statistic	UDCA[1]		GUDCA[1]	
		Female	Male	Female	Male
Pre-dose (Baseline visit)	N	23	54	23	54
	Mean (SD); ng/mL	46.4 (50.17)	176.9 (813.52)	134.9 (169.58)	240.4 (635.85)
	Median (IQR); ng/mL	20.0 (29.1)	20.0 (37.3)	55.2 (184.7)	75.1 (155.5)
	(Q1, Q3); ng/mL	(20.0, 49.1)	(20.0, 57.3)	(30.3, 215.0)	(22.5, 178.0)
	Min, max; ng/mL	20.0, 188.0	20.0, 5970.0	20.0, 680.0	20.0, 4540.0
	Mean to Std Dev ratio (%)	92%	22%	80%	38%
1 hr post dose (Week 12 and Week 24 pooled)	N	20	47	20	47
	Mean (SD); ng/mL	634.1 (458.81)	1027.3 (1199.01)	1033.9 (905.4)	1091.5 (1054)
	Median (IQR); ng/mL	523.0 (706.5)	658.0 (972.0)	699.0 (1333.5)	847.0 (917.0)
	(Q1, Q3); ng/mL	(260.5, 967.0)	(288.0, 1260.0)	(341.5, 1675.0)	(343.0, 1260)
	Min, max; ng/mL	20.0, 1690.0	25.6, 6020.0	25.5, 3540.0	20.0, 4600.0
	Mean to Std Dev ratio (%)	138%	86%	114%	104%
4 hr post-dose (Week 12 and Week 24 pooled)	N	21	49	21	49
	Mean (SD); ng/mL	1130.2 (983.79)	1185.5 (1522.52)	1252.1 (823.89)	1348.0 (1058.99)
	Median (IQR); ng/mL	950.0 (1149.0)	647.0 (861.0)	1060.0 (1163.0)	1360.0 (1531.0)
	(Q1, Q3); ng/mL	(311.0, 1460.0)	(339.0, 1200.0)	(677.0, 1840.0)	(379.0, 1910.0)
	Min, max; ng/mL	20.0, 3830.0	20.0, 7340.0	20.0, 3350.0	20.0, 5290.0
	Mean to Std Dev ratio (%)	115%	78%	152%	127%

Source: Sequence 0034, module 5335, tudca-pk-report-addendum.pdf, page 9 of 12

[*Reviewer comment: Upon the re-analysis, observed TUDCA plasma PK are 8-18% lower in females compared to males. This difference appears modest in the context of variability. There is no consistent effect of sex on UDCA PK. The reduction in mean GUDCA PK in females is modest compared to GUDCA PK variability.*]

Figure 7: TUDCA PK By Renal Function (eGFR between 60 to 90 mL/min versus eGFR ≥ 90 mL/min) in Study AMX3500 at Baseline, and 1- and 4-hours Post-Dose Pooled Across Weeks 12 and 24 for Original Data and Re-Analysis

Pooled Visit for AMX0035 Treatment Group	Statistic	TUDCA[1] (Reanalysis)		TUDCA[2] (Original)	
		eGFR >60, <90 mL/min	eGFR ≥ 90 mL/min	eGFR >60, <90 mL/min	eGFR ≥ 90 mL/min
Pre-dose (Baseline visit)	N	6	19	29	48
	Mean (SD); ng/mL	20.0 (0.00)	21.3 (5.87)	59.5 (124.37)	27.7 (38.96)
	Median (IQR); ng/mL	20.0 (0.0)	20.0 (0.0)	20.0 (0.0)	20.0 (0.0)
	(Q1,Q3); ng/mL	(20.0, 20.0)	(20.0, 20.0)	(20.0, 20.0)	(20.0, 20.0)
	Min, max; ng/mL	20.0, 20.0	20.0. 45.6	20.0, 577.0	20.0, 287.0
	Mean to Std Dev ratio (%)	--	364%	48%	71%
1 hr post dose (Week 12 and Week 24 pooled)	N	5	30	19	48
	Mean (SD); ng/mL	188.0 (235.19)	309.3 (386.80)	693.3 (717.41)	350.1 (516.07)
	Median (IQR); ng/mL	91.1 (47.2)	155.0 (235.3)	456.0 (1133.0)	155.0 (291.0)
	(Q1, Q3); ng/mL	(89.8, 137.0)	(89.7, 325.0)	(137.0, 1270.0)	(50.0, 341.0)
	Min, max; ng/mL	20.0, 602.0	20.0, 1830.0	20.0, 2570.0	20.0, 2440.0
	Mean to Std Dev ratio (%)	79.9%	80.0%	97%	68%
4 hr post-dose (Week 12 and Week 24 pooled)	N	8	27	19	51
	Mean (SD); ng/mL	421.8 (329.06)	481.2 (394.47)	566.1 (431.70)	566.2 (602.48)
	Median (IQR); ng/mL	322.5 (381.5)	399.0 (596.0)	403.0 (636.0)	435.0 (624.0)
	(Q1, Q3); ng/mL	(219.0, 600.5)	(158.0, 754.0)	(230.0, 866.0)	(148.0, 772.0)
	Min, max; ng/mL	20.0, 1070.0	20.0, 1800.0	20.0, 1620.0	20.0, 3250.0
	Mean to Std Dev ratio (%)	128%	122%	131%	94%

Source: Sequence 0034, module 5335, tudca-pk-report-addendum.pdf, page 11 of 12

Figure 8: UDCA and GUDCA PK By Renal Function (eGFR between 60 to 90 mL/min versus eGFR ≥ 90 mL/min) in Study AMX3500 at Baseline, and 1- and 4-hours Post-Dose Pooled Across Weeks 12 and 24 for the Original Dataset

Pooled Visit for AMX0035 Treatment Group	Statistic	UDCA[1]		GUDCA[1]	
		eGFR >60, <90 mL/min	eGFR ≥ 90 mL/min	eGFR >60, <90 mL/min	eGFR ≥ 90 mL/min
Pre-dose (Baseline visit)	N	29	48	29	48
	Mean (SD); ng/mL	90.6 (171.75)	166.5 (856.47)	178.4 (279.44)	227.3 (652.50)
	Median (IQR); ng/mL	20.0 (43.2)	20.0 (32.2)	37.3 (59.9)	91.7 (159.8)
	(Q1,Q3); ng/mL	(20.0, 63.2)	(20.0, 52.2)	(21.2, 81.1)	(32.8, 192.5)
	Min, max; ng/mL	20.0, 673.0	20.0, 5970.0	20.0, 1060.0	20.0, 4540.0
	Mean to Std Dev ratio (%)	53%	19%	64%	35%
1 hr post dose (Week 12 and Week 24 pooled)	N	19	48	19	48
	Mean (SD); ng/mL	1153.1 (1081.12)	813.7 (1028.26)	1549.8 (1181.22)	886.1 (871.14)
	Median (IQR); ng/mL	818.0 (1247.0)	535.0 (826.5)	1110.0 (1650.0)	654.0 (912.0)
	(Q1,Q3); ng/mL	(443.0, 1690.0)	(212.0, 1038.5)	(610.0, 2260.0)	(258.0, 1170)
	Min, max; ng/mL	115.0, 4890.0	20.0, 6020.0	327.0, 4570.0	20.0, 4600.0
	Mean to Std Dev ratio (%)	107%	79%	131%	102%
4 hr post-dose (Week 12 and Week 24 pooled)	N	19	51	19	51
	Mean (SD); ng/mL	1177.7 (1407.70)	1165.6 (1378.39)	1326.8 (835.46)	1316.4 (1048.57)
	Median (IQR); ng/mL	702.0 (557.0)	841.0 (1320.0)	1300.0 (1213.0)	1060.0 (1425.0)
	(Q1,Q3); ng/mL	(437.0, 994.0)	(240.0, 1560.0)	(697.0, 1910.0)	(525.0, 1950.0)
	Min, max; ng/mL	63.9, 5140.0	20.0, 7340.0	310.0, 3650.0	20.0, 5290.0
	Mean to Std Dev ratio (%)	84%	85%	159%	126%

Source: Sequence 0034, module 5335, tudca-pk-report-addendum.pdf, page 12 of 12

[*Reviewer comment: Upon the re-analysis, there is no consistent relationship between renal function (mild RI versus normal renal function) and observed TUDCA plasma PK. The mean observed plasma values are higher for mild RI versus normal renal function both UDCA and GUDCA at 1 hour post-dose (42% higher for UDCA and 75% higher for GUDCA). However, at 4-hours post-dose, the mean observed*

plasma values for are comparable across both renal function groups for both UDCA and GUDCA. As such, there no clear relationship between renal function (mild RI versus normal renal function) and PK for either UDCA or GUDCA.]

The Applicant indicates that an effect on race could not be determined as only 2 non-white participants (1 Asian and 1 African American) had data that could be included in the analysis.

The Applicant concludes that there Is no consistent effect of age, sex, race/ethnicity, or renal function on steady-state concentrations of TUDCA, UDCA, or GUDCA.

[*Reviewer comment: These comparisons of observed PK by various intrinsic factors do not present consistent relationship for age, sex, or renal function (mild RI versus normal renal function) for TUDCA, UDCA, or GUDCA. While numerical differences are apparent at 1-hour post dose for TUDCA for age < 65 versus age ≥ 65 years, and for male versus female, these differences are not observed at the 4-hour timepoint. As such, these apparent differences at 1-hour do not likely represent a "real" difference.*

Please refer to section ***4.4.2*** PB, PAA *for additional details regarding covariates from the PPK analyses of PB and PAA. Please refer to section* ***4.4.4*** PK Simulations for PB, PAA *for discussion of the expected effect size of covariates based on PK simulations.*]

4.4.4 PK Simulations for PB, PAA

The Applicant conducted PK simulations to quantify the effect of the covariates included in the final PPK model on systemic exposure for PB and PAA (see section **4.4.2** PB, PAA for details on the model).

The Applicant utilized the demographic values from the PPK dataset to establish a reference simulation dataset for comparison with simulations conducted with a single covariate value adjusted (i.e. changing body weight from 50 to 115 kg, the presence of ALS diagnosis to absence of ALS diagnosis, and administration with food versus administration in a fasted state).

Table 4 and **Table 5** presents the summary statistics for the simulated PK values for PB for the virtual population selected to represent the effects of food versus fasting, ALS diagnosis yes versus ALS diagnosis no, and body weight (50 kg vs 115 kg).

Table 4: Impact of Covariates on Simulated PK Values for PB – Unnormalized

Parameters	Reference Dataset[1]	Dataset adjusted for PB administration with food[2]	Dataset adjusted for PB administration fasting[2]	Dataset adjusted for ALS diagnosis of no[3]	Dataset adjusted for body weight of 50 kg[4]	Dataset adjusted for body weight of 115 kg[4]
C_{max} (µg/mL)						
n[5]	71,000	71,000	71,000	71,000	71,000	71,000
Mean	152	67.5	229	151	153	153
Median	82.9	44.4	145	81.8	83.0	82.9
%CV	146	114	123	145	144	143
2.5th percentile	9.39	6.97	20.9	9.10	9.20	9.35
97.5th percentile	717	267	933	703	714	719
Geometric mean	82.2	44.0	144	81.5	82.5	82.7
Geometric %CV	159	117	125	159	159	158
$AUC_{0\text{-last}}$ (µg-h/mL)						
n[5]	71,000	71,000	71,000	71,000	71,000	71,000
Mean	189	150	326	188	189	189
Median	127	100	221	126	127	127
%CV	110	110	109	110	109	108
2.5th percentile	20.5	17.7	39.2	20.3	20.3	20.6
97.5th percentile	731	580	1246	725	730	731
Geometric mean	125	101	221	124	126	126
Geometric %CV	115	110	108	115	115	114
$AUC_{0\text{-}\infty}$ (µg-h/mL)						
n[6]	70433	70153	70950	70394	70,415	70,404
Mean	199	166	331	196	199	198
Median	135	112	225	134	135	136
%CV	229	124	108	110	127	121
2.5th percentile	24.2	21.5	41.8	24.0	24.0	24.4
97.5th percentile	745	621	1250	738	744	750
Geometric mean	134	113	226	134	135	135
Geometric %CV	108	105	106	107	107	107

Reference dataset reproduced the ALS subjects from Protocol AMX3500 in the population analytical dataset including identical demographics, clinical status and food administration characteristics.

Source: Sequence 0001, module 5335, pop-pk-report.pdf, page 137 of 191

Table 5: Impact of Covariates on Simulated PK Values for PAA – Unnormalized

Parameters	Reference Dataset[1]	Dataset adjusted for PB administration with food[2]	Dataset adjusted for PB administration fasting[2]	Dataset adjusted for ALS diagnosis of no[3]	Dataset adjusted for body weight of 50 kg[4]	Dataset adjusted for body weight of 115 kg[4]
C_{max} (µg/mL)						
n[5]	71,000	71,000	71,000	71,000	71,000	71,000
Mean	25.0	19.7	30.4	22.3	35.0	17.2
Median	22.8	18.2	28.5	20.9	33.0	15.5
%CV	46.8	43.0	40.2	38.9	36.2	47.9
2.5th percentile	8.72	7.71	12.1	9.78	16.3	6.13
97.5th percentile	53.4	40.2	59.6	42.6	65.2	37.2
Geometric mean	22.5	18.0	28.1	20.7	32.9	15.4
Geometric %CV	49.2	43.9	42.2	39.0	36.6	49.8
$AUC_{0\text{-last}}$ (µg-h/mL)						
n[5]	71,000	71,000	71,000	71,000	71,000	71,000
Mean	61.1	68.8	90.3	67.7	105	33.5
Median	53.7	55.2	73.7	62.3	100	31.0
%CV	51.2	66.3	63.8	43.0	32.8	38.4
2.5th percentile	21.8	21.5	27.7	29.3	51.9	15.6
97.5th percentile	141	197	250	136	186	64.8
Geometric mean	54.3	57.9	76.5	63	99.8	31.3
Geometric %CV	51.3	62.1	61.4	41	33.4	38.0
$AUC_{0\text{-}\infty}$ (µg-h/mL)						
n[6]	70,760	70,645	70,969	68,670	69,516	70,994
Mean	63.3	72.3	91.7	85.3	118	33.7
Median	54.2	56.1	74.1	66.2	107	31.3
%CV	98.0	103	66.7	446	150	37.9
2.5th percentile	22.2	22.0	27.8	29.9	53.8	16.0
97.5th percentile	152	213	257	200	225	64.9
Geometric mean	55.4	57.9	77.2	69.3	108	31.6
Geometric %CV	53.2	62.1	62.3	53.8	38.9	37.3

Reference dataset reproduced the ALS subjects from Protocol AMX3500 in the population analytical dataset including identical demographics, clinical status and food administration characteristics.

Source: Sequence 0001, module 5335, pop-pk-report.pdf, page 137 of 191

Table 6 provides the simulation results expressed as % change from the reference population (which contains identical demographics, clinical status, and food administration characteristics as for the ALS population from protocol AMX3500).

Table 6: Impact of Covariates on Simulated PK Values for PB and PAA - Normalized to Reference

Dataset	Administration Relative to Food	ALS Diagnosis	Body Weight (kg)	C_{max} (µg/mL)		$AUC_{0\text{-}last}$ (µg-h/mL)		$AUC_{0\text{-}\infty}$ (µg-h/mL)	
				PB	PAA	PB	PAA	PB	PAA
Reference	Mixed (53 regimens fasting, 41 regimens with food)	Yes	varies from 47 to 123.3 kg (mean: 82.1 kg)	1.0 (0.81, 1.19)	1.0 (0.96, 1.04)	1.0 (0.88,1.12)	1.0 (0.96, 1.04)	1.0 (0.76, 1.24)	1.0 (0.92, 1.08)
Dataset adjusted for PB administration with food	Fed	Yes	varies from 47 to 123.3 kg (mean: 82.1)	0.54 (0.47,0.60)	0.79 (0.77,0.82)	0.79 (0.70,0.88)	1.03 (0.97,1.09)	0.83 (0.72,0.94)	1.03 (0.99, 1.08)
Dataset adjusted for PB administration fasting	Fasting	Yes	varies from 47 to 123.3 kg (mean: 82.1)	1.75 (1.51,1.99)	1.25 (1.21,1.29)	1.74 (1.54,1.94)	1.37 (1.30,1.45)	1.66 (1.48,1.85)	1.37 (1.29,1.45)
Dataset adjusted for ALS diagnosis	Mixed (53 regimens fasting, 41 regimens with food)	No	varies from 47 to 123.3 kg (mean: 82.1)	0.99 (0.80,1.17)	0.91 (0.89, 0.94)	0.99 (0.88,1.11)	1.16 (1.12,1.20)	0.99 (0.88,1.11)	1.22 (1.16,1.28)
Dataset adjusted for body weight of 50 kg	Mixed (53 regimens fasting, 41 regimens with food)	Yes	50 kg	1.00 (0.81,1.19)	1.45 (1.41,1.49)	1.00 (0.89,1.12)	1.87 (1.82,1.92)	1.00 (0.87,1.14)	2.00 (1.91,2.03)
Dataset adjusted for body weight of 115 kg	Mixed (53 regimens fasting, 41 regimens with food)	Yes	115 kg	1.00 (0.81,1.19)	0.68 (0.65,0.70)	1.00 (0.89,1.12)	0.58 (0.56,0.60)	1.00 (0.88,1.13)	0.58 (0.56,0.59)

Reference dataset reproduced the ALS subjects from Protocol AMX3500 in the population analytical dataset including identical demographics, clinical status and food administration characteristics.

Source: Sequence 0001, module 5335, pop-pk-report.pdf, page 70 of 191

The Applicant provides the following conclusions:

1. Administration of AMX0035 with food decreases PB and, to a lesser extent, PAA exposure in plasma relative to administration in a fasted state.
2. PB and PAA PK are generally similar in ALS patients and healthy subjects
3. PB exposure is not appreciably affected by body weight while here is an inverse relationship between PAA exposure and body weight.

[*Reviewer comment: The Applicant's PK simulations assess the effects of food, ALS diagnosis, and body weight on the PK of PB and PAA. OCP disagrees with the Applicant's presentation of the effect of food as shown in* ***Table 6****. The "fed" and "fasting" groups are compared to the "reference" dataset which consists of mixed fasted and fed subjects (53 regimens fasting, and 41 regimens with food). The more appropriate assessment of food effect is to compare the simulated PK in the fed group with the simulated PK in the fasted group (using values from* ***Table 4*** *and* ***Table 5****):*

- *For PB, The C_{max}, $AUC_{0\text{-}last}$, and $AUC_{0\text{-}\infty}$ are 239%, 117%, and 99% higher in a fasted state versus a fed state, respectively { (229-67.5)/67.5*100 = 239%; (326-150)/150*100 =117%; (331-166)/166*100 = 99% }.*
- *For PAA, The C_{max}, $AUC_{0\text{-}last}$, and $AUC_{0\text{-}\infty}$ are 54%, 31%, and 27% higher in a fasted state versus a fed state, respectively { (30.4-19.7)/19.7*100 = 54% ; (90.3-68.8)/68.8*100 = 31% ; (91.7-72.3)/72.3*100 = 27%}.*

*The simulations presented in **Table 6** indicate that ALS diagnosis does not affect PB PK. The simulations in **Table 6** suggest that the ALS diagnosis is associated with 9% lower, 16% higher, and 22% C_{max}, AUC_{0-last}, and $AUC_{0-\infty}$, respectively for PAA. However, as the pivotal efficacy study was conducted in subjects with ALS, then there is no need to assess the clinical relevance of the effect of ALS on PAA PK.*

*The simulations presented in **Table 6** indicate that PB PK for a 50 kg ALS subject as well as the PB PK for a 115 kg ALS subject are both comparable to the PB PK for the reference population of ALS subjects (mean 82.1 kg; range 47 to 123.3 kg). The simulations suggest an "inverse" relationship of PAA PK with weight. A 50 kg ALS subject has 45%, 87%, and 100% higher C_{max}, AUC_{0-last}, and $AUC_{0-\infty}$, respectively, compared to the reference population of ALS subjects. A 115 kg ALS subjects has 32%, 42%, and 42% lower C_{max}, AUC_{0-last}, and $AUC_{0-\infty}$, respectively, compared to the reference population of ALS subjects.*

*Please refer to section **4.4.2*** PB, PAA *for additional details regarding covariates from the PPK analyses of PB and PAA. Please refer to section **4.4.3*** Observed PK by Group *for details regarding covariates based on observed PK data for PB, PAA, TUDCA, UDCA, and GUDCA.*]

4.5 Exposure-Response Analyses

This NDA submission does not contain exposure-response analyses for safety or efficacy.

[*Reviewer comment: OCP did not conduct independent exposure-response analyses for safety or efficacy.*]

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/s/

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