12 October 2023
EMA/CHMP/487533/2023
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Albrioza

International non-proprietary name: sodium phenylbutyrate/ ursodoxicoltaurine

Procedure No. EMEA/H/C/005901/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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List of abbreviations

AA	Group of subjects randomized to AMX0035 in the main phase and who stayed on AMX0035 upon enrollment in the open-label extension phase
AE	Adverse event
AMX0035	Coformulation of 3 g phenylbutyrate (PB) and 1 g ursodoxicoltaurine (TURSO)
ALT	Alanine aminotransferase
ALS	Amyotrophic Lateral Sclerosis
ALSFRS-R	Amyotrophic Lateral Sclerosis Functional Rating Scale - Revised
ATLIS	Accurate Test of Limb Isometric Strength
ASMF	Active Substance Master File
AST	Aspartate aminotransferase
AUC	Area under the curve
BCRP	Breast cancer resistance protein
BID	Twice Daily
BMI	Body Mass Index
(S)(D)BP	(Systolic) (Diastolic) Blood Pressure
CI	Confidence Interval
CL/F	Apparent Clearance
CMAP	Compound Muscle Action Potential
C_{max}	Maximal plasma Concentration
CNS	Central Nervous System
COSY	Correlated Spectroscopy NMR
CQA	Critical quality attribute
CSF	Cerebrospinal Fluid
CSR	Clinical Study Report
CYP	Cytochrome P450
DEA	Diethanolamine
DEPT	Distortionless Enhancement by Polarization Transfer
DDI	Drug-drug interaction
DSC	Differential Scanning Calorimetry
ECG	Electrocardiogram
eGFR	Estimated glomerular filtration rate
EMG	electromyography

ENCALS	European Network to Cure ALS
ER	Endoplasmic reticulum
FTIR	Fourrier Transform Infrared Spectroscopy
FUS	Fused-in-sarcoma
GC	Gas Chromatography
GMP	Good Manufacturing Practice
GUDCA	Glycoursodeoxycholic acid
HDAC	Histone deacetylase
HR	Hazard ratio
hERG	human ether-a-go-go related gene
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear multiple quantum coherence
HPLC	High performance liquid chromatography
hrCYP	Human recombinant CYP enzymes
IC_{50}	50% inhibitory concentration
IPC	In-process control
IR	Infrared
ITT	Intent-to-Treat
IV	Intravenous
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LLDPE	Linear low density polyethylene
LLOQ	Lower limit of quantification
LMN	Lower Motor Neuron
LS	Least-squares
MAR	Missing At Random
MATE	Multidrug and toxin extrusion proteins
mITT	Modified Intent-to-Treat
MO	Major objection
NMR	Nuclear Magnetic Resonance
MNAR	Missing Not At Random
MS	Mass spectrometry
NOESY	Nuclear Overhauser Effect Spectroscopy
OAT	Organic anion transporter

OATP	Organic anion transporting polypeptide
OCT	Organic cation transporter
OD	Once daily
OLE	Open-Label Extension
PA	Group of subjects randomized to placebo in the main phase and who switched to AMX0035 upon enrollment in the open-label extension phase
PAA	Phenylacetic acid
PAGN	Phenylacetylglutamine
PAV	Permanent Assisted Ventilation
PB	Phenylbutyrate
PD	Pharmacodynamics
PET	Positron Emission Tomography
P-gp	P-glycoprotein
Ph. Eur.	European Pharmacopoeia
PK	Pharmacokinetics
pNF-H	phosphorylated axonal neurofilament H subunit
PPN	Percent Predicted Normal
PSM	Propensity score matching
QTcF	QT interval correct for heart rate using the Fridericia formula
QTTP	Quality target product profile
RA	Group of subjects randomized to AMX0035 and stayed on AMX0035 in the open-label extension phase, plus the AMX0035 subjects who did not enter the open-label extension
RP	Group of subjects randomized to placebo in the main phase and switched from placebo to AMX0035 in the open-label extension phase, plus the placebo subjects who did not enter the open-label extension
RPSFT	Rank-preserving structural failure time
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SD	Standard deviation
SE	Standard error
SOD1	Superoxide Dismutase 1
SOC	System Organ Class
SVC	Slow Vital Capacity
TAR	Trans-Activation Response element

TEA	Triethanolamine
TEAE	Treatment-Emergent Adverse Event
TDP43	DNA binding protein 43
TGA	Thermo-Gravimetric Analysis
TLC	Thin layer chromatography
T_{max}	Time of maximal plasma concentration
$T_{1/2}$	Half-live
TSPO	18 kDa translocator protein
TUDCA	Tauroursodeoxycholic acid
TURSO	Ursodoxicoltaurine
UDCA	Ursodeoxycholic acid
UMN	Upper Motor Neuron
USP	United States Pharmacopeia
UV	Ultraviolet
Vz/F	Apparent volume of distribution

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Amylyx Pharmaceuticals EMEA B.V. submitted on 2 January 2022 an application for marketing authorisation to the European Medicines Agency (EMA) for Albrioza, through the centralised procedure falling within the Article 3(1) and point 4 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 25 March 2021.

Albrioza was designated as an orphan medicinal product EU/3/20/2284 on 4 June 2020 in the following condition: treatment of amyotrophic lateral sclerosis (ALS).

The applicant applied for the following indication:

"*for the treatment of ALS*."

1.2. Legal basis, dossier content

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on Applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

1.3. Information on Paediatric requirements

Pursuant to Article 8 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/0495/2020 on the granting of a (product-specific) waiver.

1.4. Information relating to orphan market exclusivity

1.4.1. Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

1.5. Applicant's request(s) for consideration

1.5.1. Conditional marketing authorisation

The applicant requested consideration of its application for a Conditional marketing authorisation in accordance with Article 14-a of Regulation (EC) 726/2004.

1.5.2. New active Substance status

The applicant initially requested that the combination of the active substances sodium phenylbutyrate and taurursodiol contained in the above medicinal product to be considered as a new active substance. On 23 January 2023, the applicant withdrew their claim for new active substance status.

1.6. Protocol assistance

The applicant received the following Protocol assistance on the development relevant for the indication subject to the present application:

Date	Reference	SAWP co-ordinators
23 July 2020	EMEA/H/SA/4527/1/2020/PA/SME/III	*Marion Haberkamp* *Mario Miguel Coelho da Silva Rosa*
29 January 2021	EMA/SA/0000046306	*Marion Haberkamp* *Mario Miguel Coelho da Silva Rosa*

The Protocol assistance pertained to the following quality, non-clinical, and clinical aspects:

- Quality: The specifications and test procedures for the drug substances, proposed drug product specifications, approach to provide stability data.
- Non-clinical: Adequacy of the proposed toxicological studies to support a MAA. Deferral of carcinogenicity and fertility (segment 1 and 3) studies until post-MA.
- Clinical: Adequacy of the primary outcome of ALSFRS-R rate of progression in combination with a long-term assessment of survival to assess the effectiveness of AMX0035 for the treatment of ALS. Whether compelling evidence of clinical benefit from CENTAUR and CENTAUR OLE could support CMA, including acceptability of the study population and duration. Acceptability of study (A35-004, PHOENIX) to support full approval.

1.7. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Johann Lodewijk Hillege Co-Rapporteur: Martina Weise

The application was received by the EMA on	2 January 2022
The procedure started on	24 February 2022
The CHMP Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	16 May 2022
The CHMP Co-Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	31 May 2022
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC	30 May 2022

and CHMP members on	
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	23 June 2022
The applicant submitted the responses to the CHMP consolidated List of Questions on	8 September 2022
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Questions to all CHMP and PRAC members on	21 October 2022
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	27 October 2022
The CHMP agreed on a list of outstanding issues in writing and/or in an oral explanation to be sent to the applicant on	10 November 2022
Working Party experts were convened to address questions raised by the CHMP on The CHMP considered the views of the Working Party (as appropriate) as presented in the minutes of this meeting.	21 November 2022
The applicant submitted the responses to the CHMP List of Outstanding Issues on	23 January 2023
SAG-N was convened to address questions raised by the CHMP on The CHMP considered the views of the SAG-N as presented in the minutes of this meeting.	15 February 2023
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	16 February 2023
The CHMP agreed on a list of outstanding issues in writing and/or in an oral explanation to be sent to the applicant on	23 February 2023
The applicant submitted the responses to the CHMP List of Outstanding Issues on	24 April 2023
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	17 May 2023
The outstanding issues were addressed by the applicant during an oral explanation before the CHMP during the meeting on	24 May 2023
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a negative opinion for granting a marketing authorisation to Albrioza on	22 June 2023

1.8. Steps taken for the re-examination procedure

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Kristina Dunder Co-Rapporteur: John Joseph Borg

The applicant submitted written notice to the EMA, to request a re-examination of Albrioza CHMP opinion of 22 June 2023, on	5 July 2023
The CHMP appointed Kristina Dunder as Rapporteur and John Joseph Borg as Co-Rapporteur on	20 July 2023
The applicant submitted the detailed grounds for the re-examination (Appendix 1 of Final Opinion) on	15 August 2023
The PRAC appointed Rhea Fitzgerald as Rapporteur on	N/A
The re-examination procedure started on	16 August 2023
The CHMP Rapporteur's re-examination assessment report was circulated to all CHMP members on	14 September 2023
The CHMP Co-Rapporteur's assessment report was circulated to all CHMP and PRAC members on	14 September 2023
The PRAC rapporteur's re-examination assessment report was circulated to all PRAC members on	N/A
SAG Neurology were convened to address questions raised by the CHMP on The CHMP considered the views of the SAG Neurology as presented in the minutes of this meeting	2 October 2023
The CHMP Rapporteurs circulated the CHMP Rapporteurs Joint Assessment Report on the detailed grounds for re-examination to all CHMP members on	06 October 2023
The PRAC agreed on the PRAC Assessment Overview and Advice on	N/A
The detailed grounds for re-examination were presented by the applicant during an oral explanation before the CHMP on	10 October 2023
The CHMP, in the light of the scientific data available and the scientific discussion within the Committee, re-examined its initial opinion and in its final opinion concluded that the application did not satisfy the criteria for authorisation and did not recommend the granting of the conditional marketing authorisation on	12 October 2023

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that affects motor neurons in the brain and spinal cord. Rapid progression of symptoms directly results from degeneration in motor neurons, causing motor function loss. Most people will eventually need assistance with activities of daily living (unless they pass away prior to this), with subsequent progression leading to respiratory

compromise and eventually respiratory failure, which is the leading cause of death in ALS. There is both a familial and a sporadic form.

2.1.2. Epidemiology

The incidence (average around 1/50,000 per year) and prevalence (average around 1/20,000) are relatively uniform in Western countries, although foci of higher frequency have been reported in the Western Pacific. The mean age of onset for sporadic ALS is about 60 years. Overall, there is a slight male preponderance (male to female ratio of around 1.5:1).

2.1.3. Aetiology and pathogenesis

Although the precise aetiology of ALS is unknown, ALS and other neurodegenerative diseases dare characterized by nerve cell death and inflammation. Together, these pathologic processes are considered key drivers of the progressive loss of motor neurons.

Recent research has highlighted endoplasmic reticulum (ER) stress and mitochondrial stress as key mediators of neuronal death and neuroinflammatory processes (Manfredi 2016).

ER stress and mitochondrial bioenergetic deficits can independently activate necrotic and apoptotic cell death pathways. These pathways are also closely linked through mitochondrial-associated ER membranes (Lau 2018). The brain is extremely sensitive to both ER and mitochondrial stress, and these pathways have been strongly implicated in causing neurodegenerative diseases such as ALS (Area-Gomez 2018; Berg 2001).

2.1.4. Clinical presentation, diagnosis

ALS patients typically first present with dysarthria, fasciculations, weakness in a limb, or some combinations thereof. Diagnosis of ALS requires excluding other conditions, such as stroke or multiple sclerosis, which cause similar symptoms. Within a year of diagnosis, most patients suffer from significant and increasing morbidities and impaired ability to function independently, including losing the ability to swallow, speak, or ambulate independently (Manera 2019, Galvin 2017; Labra 2016; Jordan 2015; Paganoni 2014; Ruoppolo 2013; Traxinger 2013; Fujimura-Kiyono 2011; Turner 2010a).

Limb-onset ALS presents a combination of upper and lower motor neuron (UMN and LMN) signs in the limbs, and bulbar onset ALS is presented with speech and swallowing difficulties and with limb features developing later in the course of the disease. Bulbar-onset ALS occurs less frequently and has been associated with a worse prognosis than limb onset.

Median survival is approximately 27-41 months from symptom onset (Labra 2016; Turner 2010a).

Diagnosis is mainly clinical and based on the revised El Escorial criteria. The El Escorial revised Airlie House diagnostic criteria grade the certainty of the diagnosis based upon 4 clinical grades, clinically definite, probable, probable - laboratory supported and possible ALS.

2.1.5. Management

There is currently only one approved ALS product, riluzole (Rilutek®, 50 mg tablets, Sanofi Aventis Pharma S.A., 1996), in Europe.

Riluzole is a drug that blocks glutamatergic neurotransmission in the CNS. Riluzole is taken orally and can cause side effects such as dizziness, gastrointestinal conditions and liver function changes.

Edaravone is a free radical scavenger intended to counter the oxidative damage that occurs in the nervous system of ALS patients. A marketing authorisation application was submitted into EMA but subsequently withdrawn by the applicant on 24 May 2019.

2.2. About the product

Albrioza (AMX0035) is a fixed-dose combination of sodium phenylbutyrate and ursodoxicoltaurine.

The mechanism by which Albrioza exerts its therapeutic effects in patients with ALS is unknown. According to the applicant, sodium phenylbutyrate and ursodoxicoltaurine, in combination, demonstrate synergistic activity in reducing neuronal death, hypothesised to occur through simultaneous inhibition of ER and mitochondrial stress-related cell death. Sodium phenylbutyrate is a Histone deacetylase (HDAC) inhibitor that decreases ER stress through the upregulation of chaperone proteins and acts as a small molecular chaperone. Ursodoxicoltaurine decreases mitochondrial stress by reducing mitochondrial permeability and increasing the apoptotic threshold of the cell through inhibition of the BAX protein.

The claimed indication is: "*for the treatment of amyotrophic lateral sclerosis (ALS).*"

AMX0035 is recommended to be administered prior to a meal according to the following regimen:

- Run-in Period: The recommended starting dose of AMX0035 is 1 sachet once daily (OD) for 1-21 day
- Maintenance Dose: The recommended maintenance dose of AMX0035 is 1 sachet twice daily, morning and evening (BID).

To improve overall acceptance and reduce bitter after taste AMX0035 may be followed by a glass of milk, meal or snack.

2.3. Type of Application and aspects on development

The applicant requested consideration of its application for a Conditional Marketing Authorisation (CMA) in accordance with Article 14-a Regulation (EC) 726/2004, based on the following criteria:

- The benefit-risk balance is positive.

The applicant claimed that clinical results from the randomised phase 2 trial (CENTAUR; NCT03127514) indicate that treatment with Albrioza slow down the rate of functional decline and improve survival in patients with ALS together with a favourable safety profile.

- It is likely that the applicant will be able to provide comprehensive data.

Study A35-004 (PHOENIX) is a randomized, double-blind, parallel-groups, placebo-controlled 48-week study aiming to recruit ALS patients from approximately 65 sites in some European Countries, the UK and the US. Patients were randomized to receive study medicinal product (Albrioza) or placebo in addition to investigator-selected standard of care. This study intends to provide larger safety and efficacy data of Albrioza in ALS patients with a diagnosis of ALS definite or clinically probable of the revised El Escorial Criteria, and with a time since onset of the first symptoms of ALS <24 months. Its recruitment began in the fourth quarter of 2021, with the First Patient Dosed, and concluded in February 2023 as the applicant informed EMA during the procedure. A total of 664 participants were enrolled including 552 participants from EU. A final clinical study report (CSR) would have been submitted to the CHMP approximately by Q2 2024.

The design of Study A35-004 is similar to the Phase 2 study AMX3500 (CENTAUR; NCT03127514) in order to confirm the effects of Albrioza in ALS patients. It has also been taken into consideration

measures to preserve the blinding of the study such as the flavour to mask the different taste from AMX0035 and placebo, and the prevalence of SAEs between groups in the Phase 2 clinical study.

Albrioza was approved in the US in September 2022. This approval could result in up to 200 subjects in the Study prematurely discontinuing study participation and, therefore, administratively censored before the week 48 timepoint. To minimise the impact of US approval on the completion of the Study, the applicant proposed to cap subject enrolment to 200 in the US and ensure that 400 or more European subjects will remain in the study until completion of the 48-week planned follow-up. The applicant has assessed the statistical impact of the US scenario as described above and confirmed that sufficient power is retained and a robust clinical interpretation will be achieved. Statistical power was modelled with a functional treatment effect at 20% (more conservative than what was observed in CENTAUR) and no survival differences, a worst-case scenario compared to CENTAUR Study that demonstrated a functional treatment effect at 25% and a survival advantage. In this circumstance, adequate power (>80%) will still be achieved with 400 subjects. The applicant has additionally modelled different circumstances of patient dropout after approvals, and in all circumstances, adequate power is retained in the proposed study to detect even conservative treatment differences. Therefore, Amylyx Pharmaceuticals EMEA B.V. is taking active steps in the implementation of Study A35-004 to ensure that approximately 400 patients at week 48 will be completed in Europe (UK and EU). During the assessment, it was confirmed that as per the data cut-off 16 January 2023, of the 112 US subjects enrolled, 79 discontinued the study prematurely, as the applicant actively transitioned subjects to commercial product. It is also acknowledged that there could be an impact on the retention of EU subjects in Study A35-004 should a CMA be granted. To ensure that EU subjects completed the 48-week study, the applicant proposes to delay marketing of the drug until the last patient out (date Dec 2023/Jan 2024) in the whole EU.

- Unmet medical needs

The applicant claimed that the unmet medical need has been addressed by means that currently, only one medicinal product is approved in the EU for the treatment of ALS, riluzole (Rilutek® EMEA/H/C/000109 1996). Riluzole survival benefit was shown in a randomised controlled trial. This benefit was mainly found in patients with bulbar onset ALS treated over 12 months, but it did not show a statistical benefit for patients with limb onset, which comprise in 70% of the population (Bensimon et al., 1994). The functional and survival advantages demonstrated by Albrioza in the clinical trial compare favourably with riluzole, the current reference therapy for ALS in the EU. In a recent review, riluzole studies showed a 9% gain in the probability of surviving one year (49% in the placebo and 58% in the riluzole group) but a small beneficial effect on bulbar and limb function but not muscle strength. No data from the Amyotrophic Lateral Sclerosis Functional Rating Scale - Revised (ALSFRS-R) score is available from riluzole because the scale was not yet created by the time riluzole was studied (Miller RG et al, 2012). Due to the modest survival results, lack of data on function, and no effect on muscle strength, riluzole cannot be considered a fully satisfactory treatment method. Apart from riluzole, ALS treatment is mainly symptomatic and palliative (Miller 2009; EFNS guideline 2012).

The applicant claimed that results of sensitivity analyses accounting for concomitant riluzole retained the statistically significant benefit of Albrioza over placebo in the analysis of functional outcome and overall survival. The applicant´s position is that these results suggest that the benefit of Albrioza is independent of baseline riluzole use and confirm that the medicinal product has a benefit also as an add-on therapy.

- The benefits to public health of the immediate availability outweigh the risks inherent in the fact that additional data are still required.

While it is acknowledged that there is a value in further confirmatory data generation for Albrioza, the burden of mortality and morbidity of ALS remains larger despite the availability of riluzole.

Results from CENTAUR have demonstrated statistically significant and clinically meaningful retention of function and long-term survival together with a favourable safety profile. Due to the average 2-5 years of life expectancy from the first symptoms (Chio et al., 2017) and the considerable morbidity of ALS, the immediate availability of Albrioza would benefit ALS patients with a high unmet medical need. Furthermore, the availability of Albrioza could prolong survival, slow disease progression, and maintain function for ALS patients alive today; therefore, immediate availability of Albrioza would benefit these patients with a current high unmet need.

Therefore, the applicant believes that a CMA could provide an option that allows patients with this fatal disease more rapid access while allowing for additional generation of evidence in parallel.

2.4. Quality aspects

2.4.1. Introduction

The finished product is presented as a powder for oral suspension in sachets containing 3 g of sodium phenylbutyrate and 1 g of ursodoxicoltaurine as active substances.

Other ingredients are: hydrated dextrates, sorbitol (E420), sodium phosphate dibasic anhydrous (E339), silicon dioxide (E551), sucralose (E955), sodium stearyl fumarate, maltodextrin (E1400), flavour masking and mixed berry flavouring.

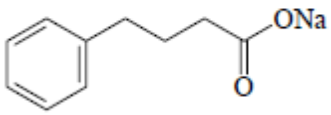
The product is available in a laminated pouch sachet with a linear low density polyethylene (LLDPE) inner lining.

2.4.2. Active substance - Sodium phenylbutyrate

General information

The chemical name of sodium phenylbutyrate is sodium 4-phenylbutanoate corresponding to the molecular formula $C_{10}H_{11}NaO_2$. It has a molecular mass of 186.2 g/mol and the following structure, as per Figure 1.

Figure 1: Sodium phenylbutyrate structure



The chemical structure of sodium phenylbutyrate was elucidated by a combination of FTIR spectroscopy, ^{1}H-NMR spectroscopy and ^{13}C-NMR spectroscopy.

Sodium phenylbutyrate is a white or yellowish-white powder, hydroscopic and freely soluble in water.

Sodium phenylbutyrate does not exhibit stereoisomerism due to the absence of chiral centres.

Polymorphism has not been observed for sodium phenylbutyrate.

Although there is a monograph of sodium phenylbutyrate in the European Pharmacopoeia, the active substance is supported by an ASMF.

Manufacture, characterisation and process controls

Detailed information on the manufacturing of the active substance has been provided in the restricted part of the ASMF and it was considered satisfactory. One manufacturer has been proposed for sodium phenylbutyrate.

Sodium phenylbutyrate is synthesized in three main synthetic steps using commercially available well defined starting materials with acceptable specification.

Following a major objection (MO) raised by the CHMP during the procedure, the starting material was re-defined and additional steps were included in the synthetic process.

Adequate in-process controls are applied during the synthesis. There are no critical steps in the process, and the control strategy has been shown to be adequate to yield a high-purity substance of acceptable quality. The specifications and control methods for intermediate products, starting materials and reagents have been presented.

The characterisation of the active substance and its impurities are in accordance with the EU guideline on the chemistry of active substances.

Potential and actual impurities were well discussed with regards to their origin and characterised.

Apart from the redefinition of the starting materials and increase number of synthetic steps performed under GMP, the manufacturing process for the active substance has remained unchanged during the development of the finished product; active substance manufactured via the commercial process has been used through the various phases of the development.

The active substance is packaged in aluminium-polyolefin laminated foil bags which comply with the Commission Regulation (EU) 10/2011 as amended.

Specification

Sodium Phenylbutyrate specification includes tests for appearance (visual), identity (IR, sodium test, HPLC), assay (titration and HPLC), impurities (HPLC), pH (USP), water content (Ph. Eur.), heavy metals (Ph. Eur.), residual solvents (GC-headspace), bulk density (in-house method).

Sodium Phenylbutyrate is controlled in line with the Ph. Eur monograph with additional controls for residual solvents methanol and acetone have been defined. Although reference to Ph. Eur. should be made for pharmacopeial methods, reference to USP is accepted as the methods are harmonised across the two regions.

All additional methods have been adequately validated and described according to ICH Q2. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented.

Batch analysis data from three commercial scale batches of the active substance are provided. The results are within the specifications and consistent from batch to batch.

Stability

Stability data on 3 commercial scale batches of the active substance from the proposed manufacturer stored in the intended commercial package under long term conditions (25 ºC / 60% RH) for up to 24 months and under accelerated conditions (40 ºC / 75% RH) according to the ICH guidelines were provided.

The following parameters were tested: appearance, assay, impurities, pH and water content. All tested parameters were within the specifications.

Results under stressed conditions (acidic, basic and oxidizing conditions) were also provided on samples of the active substance: degradation has been observed under stressed conditions.

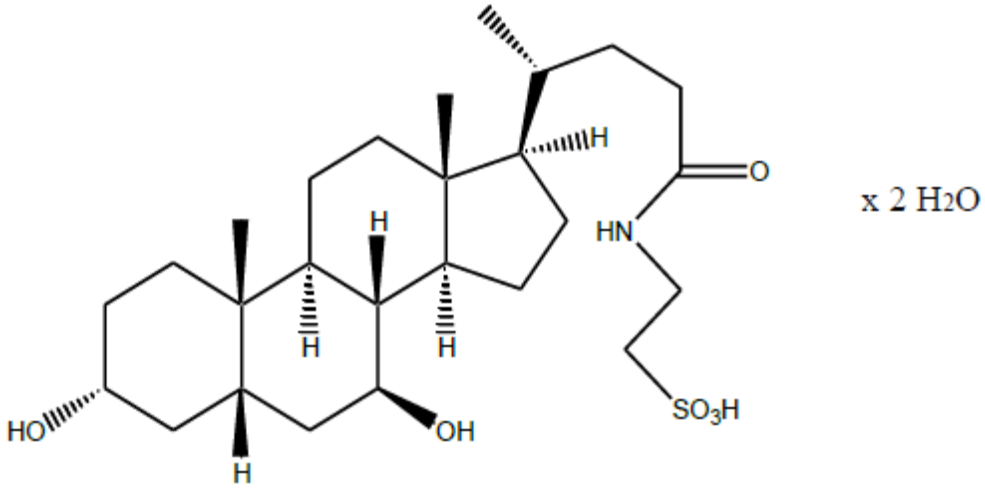
The stability results indicate that the active substance manufactured by the proposed supplier is stable. The stability results justify the proposed retest period of 2 years with no storage conditions in the proposed container.

2.4.3. Active substance - Ursodoxicoltaurine

General information

The chemical name of ursodoxicoltaurine is 2-[(3α, 7β-dihydroxy-24-oxo-5β-cholan-24-yl) amino] ethane sulfonic acid, dihydrate corresponding to the molecular formula $C_{26}H_{45}NO_6S \cdot 2H_2O$. It has a relative molecular mass of 535.74 g/mol and the following structure, as per Figure 2.

Figure 2: Ursodoxicoltaurine structure



Ursodoxicoltaurine is referred to tauroursodeoxycholic acid thought out the report interchangeably. The chemical structure of tauroursodeoxycholic acid was elucidated by a combination of NMR, IR, MS, and elemental analysis. The solid-state properties of tauroursodeoxycholic acid were measured by DSC and TGA.

Tauroursodeoxycholic acid is a white microcrystalline powder, non-hygroscopic and sparingly soluble in water. Literature reports two solid forms of tauroursodeoxycholic acid, form I and an amorphous form. Tauroursodeoxycholic acid form I can be converted to amorphous material by milling, while the amorphous form converts spontaneously to form I, the most stable form. Tauroursodeoxycholic acid manufactured by the proposed manufacturer has been demonstrated to be form I.

Despite the presence of several chiral centres, tauroursodeoxycholic acid presents only one diasteroemeric impurity with a different orientation of the hydroxyl group in position 7: taurochenodeoxycholic acid. Taurochenodeoxycholic acid may form during the manufacturing process, when chenodeoxycholic acid, a related impurity in the intermediate ursodeoxycholic acid, is conjugated with taurine. Taurochenodeoxycholic acid is controlled as related impurity in the active substance specification.

Manufacture, characterisation and process controls

One manufacturer is proposed for the manufacture of tauroursodeoxycholic acid. Detailed information on the manufacturing of the active substance has been provided in the restricted part of the ASMF and it was considered satisfactory.

Following a request from the CHMP (MO) during the procedure, the synthetic process has been updated and the starting materials redefined in the applicant's Part of the ASMF, in line with the Restricted Part of the ASMF. Taurochenodeoxycholic acid is synthesized by coupling two known substances, taurine (an amino acid) and ursodeoxycholic acid (Ph. Eur), both isolated from bovine bile. The staring materials, cholic acid and taurine, are well defined starting materials with acceptable specifications. The manufacturer performs all steps of the process and supplies both taurine and ursodeoxycholic acid.

Adequate in-process controls are applied during the synthesis. The specifications and control methods for intermediate products, starting materials and reagents have been presented.

The manufacturing process for the active substance has remained unchanged during the development of the finished product; active substance manufactured with the commercial process has been used through the various phases of the development.

The characterisation of the active substance and its impurities are in accordance with the EU guideline on the chemistry of active substances. Potential and actual impurities were well discussed with regards to their origin and characterised. To address a MO, suitable data showing absence of NDEA and its related vulnerable amines, TEA and DEA, have been provided. To address a MO, absence of routine control for benzene, TEA and DEA in acetone and recovered acetone has been adequately justified and accepted.

The active substance is packaged in polyethylene bags which complies with the Commission Regulation (EU) 10/2011 as amended.

Specification

The tauroursodeoxycholic acid specification includes tests for: description (visual), identification (IR), water content (USP), pH (USP), specific rotation (in-house), residue on ignition (in house), related substances (HPLC), assay (HPLC), residual solvents (GC), particle size (laser diffraction).

Although reference to Ph. Eur. should be made for pharmacopeial methods, reference to USP is accepted as the methods are harmonised across the two regions.

To address a MO, the limit of unspecified impurities has been tightened.

Impurities present at higher than the qualification threshold according to ICH Q3A were qualified by toxicological and clinical studies and appropriate specifications have been set. To address a MO, taurochenodeoxycholic acid in tauroursodeoxycholic acid were successfully qualified during the procedure, their proposed control is considered acceptable.

The analytical methods used have been adequately described and non-compendial methods appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented.

Batch analysis data on three commercial scale batches of the active substance are provided. The results are within the specifications and consistent from batch to batch.

Stability

Stability data from three commercial scale batches of tauroursodeoxycholic acid from the proposed manufacturers stored in the intended commercial package for up to 60 months under long term conditions (25 ºC / 60% RH) and for up to 6 months under accelerated conditions (40 ºC / 75% RH) according to the ICH guidelines were provided. For the historic, batches appearance, pH, water

content, assay (titration), related substances (TLC) were investigated; in view of the use of the TLC method, these are considered are supportive only. Stability data from three pilot scale batches recently manufactured, placed at long term stability for 12 months were also provided; test parameters tested were: appearance, pH, water content, assay (HPLC), related substances (HPLC) and taurine (by TLC). Since during the procedure the specification limits have been tightened, the initially proposed 60 months re-test time was no longer acceptable. Additionally, the updated stability data showed out of specification results for ursodeoxycholic acid at 40°C after 6 months, a very detailed study was undertaken to understand the root cause for this increase, indicating that the OOS result was linked to sample preparation.

Samples of the active substance were placed under stress testing in the following conditions: acidic (pH 1), basic (pH 13.5), oxidative and heat (50°C and 80°C): no degradation was observed in all the conditions.

The stability results indicate that the active substance manufactured by the proposed supplier is sufficiently stable. The stability results justify the proposed retest period of 36 months when stored in the proposed container.

2.4.4. Finished medicinal product

Description of the product and pharmaceutical development

Albrioza, the finished product, is presented as a powder for oral suspension containing 3 g of sodium phenyl butyrate and 1 g of tauroursodeoxycholic acid as active substances.

The pharmaceutical development of the finished product contains QbD elements. The quality target product profile (QTPP) has been provided.

Critical quality attributes were identified and appropriately justified and found to be acceptable.

The choice of the pharmaceutical form was dictated by the amounts needed for each active substance as the large quantity did not allow for the development of a tablet or capsule.

The formulation and manufacturing development was simple: excipients typically used for this pharmaceutical form were selected. The manufacturing process consists of blending two active substances with buffer, glidant, diluents, and taste maskers.

All of the excipients are known pharmacopoeial excipients, with the exception of "dextrates, hydrated" which is described in the NF. The flavour components are proprietary blends, comprising mostly of Ph. Eur. excipients of maltodextrin and acacia gum (both close to 85% of total weight). Upon request of the CHMP the applicant committed to conduct a 12-week excipient compatibility study. The 4-weeks data did not show any significant incompatibilities at the 25°C ± 2°C/ 60% RH ± 5% storage condition with the exception of the combination of maltodextrin and sodium 4-phenylbutanoate; nevertheless, as no new impurities were found, the study was considered adequate to demonstrate compatibility of the active substance and excipients.

During the development and the clinical phase, only 2 formulations were used. The initial study indicated the severe bitterness and unpleasantness of swallowing the suspension, which was addressed with the addition of flavour masking and increased sucralose sweetener. The rapid dissolution of both active substances justifies the absence of a bioequivalence study between the two formulations.

An in-use study confirmed the stability of the finished product once reconstituted. The compatibility of the finished product in its administration form with feeding tubes has been sufficiently demonstrated.

The primary packaging is laminated pouch sachet with a linear low density polyethylene (LLDPE) inner lining. The bulk packaging material in contact with the finished product is a double low density polyethylene (LDPE). The materials of contact comply with Ph. Eur. and EC requirements. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product.

Manufacture of the product and process controls

The selected manufacturing process is a standard manufacturing process: granulation (by compaction) of the powder blend. It consists of five main steps: 1. Blending and screening of the active substances and excipients, 2. compaction, 3. blending, 4. bulk packaging, 5. packaging in sachets.

The batch analysis data confirm that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner. No process validation has been performed, and the commitment to perform the validation based on the submitted validation protocol before marketing is sufficient for this standard process.

Product specification

The finished product release and end of shelf-life specifications include appropriate tests for this kind of dosage form: appearance (visual examination), appearance of reconstituted solution (visual examination), identification of sodium phenylbutyrate (HPLC and UV), identification of tauroursodeoxycholic acid (HPLC and UV), reconstitution time, uniformity of dosage units (USP), sodium phenylbutyrate assay (HPLC), tauroursodeoxycholic acid assay (HPLC), degradation products ((HPLC), pH (USP), moisture (Karl Fischer, USP), microbial limits (USP).

Although reference to Ph. Eur. should be made for pharmacopeial methods, reference to USP is accepted as the methods are harmonised across the 2 regions.

To address a multidisciplinary MO (quality/safety), the applicant was asked to elucidate the structures of the unidentified specified impurities. Despite the efforts, these were not elucidated during the procedure. Further analytical work and structural characterisation to confirm the non-genotoxicity of the impurities, if appropriate, are necessary. It is agreed that the applicant could have finalised the structural characterisation of the impurities and if necessary, could have evaluated their genotoxic potential according to ICH M7 during the post-authorisation phase. Aspects related to this MO have also been discussed in the non-clinical section.

During the procedure, the applicant has improved the way the assay is calculated using a weight correction, to address the high variability issue of the assay; as a consequence the assay range at release and at shelf life was tightened to 95.0-105.0%.

Although there are several references to 'reconstituted solution' the applicant has clarified, whilst addressing a MO on the appearance of the reconstituted solution, that the reconstituted powder is in fact a suspension, as reflected in the PI, due to the presence of insoluble excipients. It was further clarified that after reconstitution into oral suspension, the two active substances are in fact in solubilised form into the suspension.

The potential presence of elemental impurities in the finished product has been assessed following a risk-based approach in line with the ICH Q3D Guideline for Elemental Impurities. Based on the risk assessment it can be concluded that it is not necessary to include any elemental impurity controls. The information on the control of elemental impurities is satisfactory.

A risk assessment concerning the potential presence of nitrosamine impurities in the finished product was updated during the procedure to address a MO. The risk assessment now considers all suspected

and actual root causes in line with the "Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products" (EMA/409815/2020) and the "Assessment report-Procedure under Article 5(3) of Regulation EC (No) 726/2004- Nitrosamine impurities in human medicinal products" (EMA/369136/2020). Based on the information provided, it is accepted that there is no risk of nitrosamine impurities in the active substance or the related finished product. Therefore, no specific control measures are deemed necessary.

The analytical methods used have been adequately described and appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented.

Batch analysis results are provided for three commercial scale batches confirming the consistency of the manufacturing process and its ability to manufacture to the intended product specification.

The finished product is released on the market based on the above release specifications, through traditional final product release testing.

Stability of the product

Stability data from 2 pilot scale and 1 full commercial scale batches of finished product stored for up to 36 months under long term conditions (25 ºC / 60% RH), for up to 12 months under intermediate conditions (30 ºC / 65% RH) and for up to 6 months under accelerated conditions (40 ºC / 75% RH) according to the ICH guidelines were provided. The batches of medicinal product are identical to those proposed for marketing and were packed in the primary packaging proposed for marketing.

Samples were tested in line with the shelf-life specification. The analytical procedures used are stability indicating. No significant changes have been observed during long term stability testing. However, OOS results have been observed under intermediate and accelerated conditions for some specified and unspecified impurities.

In addition, samples of the finished product were subjected to forced degradation studies which show that the finished product is susceptible to oxidation as well as temperature and humidity, but stable under acidic or basic conditions.

Based on available stability data, the proposed shelf-life of 12 months and storage conditions "Do not store above 25 °C. Store in the original package." as stated in the SmPC (section 6.3) are acceptable.

Adventitious agents

No excipients derived from animal or human origin have been used. TSE risk for the active substances have been adequately covered in the restrictive part of the respective ASMF.

2.4.4.1. Discussion on chemical, and pharmaceutical aspects

Both active substances are known active substances which are supported by ASMFs.

The selection of the starting materials for sodium phenylbutyrate and tauroursodeoxycholic acid has been adequately addressed and the starting materials were re-defined upstream for sodium phenylbutyrate, whilst for tauroursodeoxycholic acid the starting materials and manufacturing process described in the applicant part of the ASMF were brought in line with those of the restrictive part of the ASMF.

The absence of routine control of benzene for tauroursodeoxycholic acid and the qualification of taurochenodeoxycholic acid have been justified when addressing two MOs.

The finished product is a powder for oral suspension in sachets. During the procedure two quality MOs, one on nitrosamine and one regarding the appearance of the reconstituted oral suspension have been adequately addressed. To address a MO related to the unknown degradation impurities, data have been provided indicating a potential source of formation of these impurities, hence they are suitably controlled by the proposed specification limits. However, a recommendation was raised for the attention of the applicant to provide the structural characterisation of the unknown degradation impurities and confirming their non-genotoxicity post-approval.

Information on development, manufacture and control of the active substances and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

As described above, at the time of the CHMP opinion, there was one minor unresolved quality issues, having no impact on the Benefit/Risk ratio of the product, pertaining the structural characterisation of the unknown degradation impurities. Further analytical work and structural characterisation to confirm the non-genotoxicity of the impurities, if appropriate, are necessary. It is agreed that the applicant could have finalised the structural characterisation of the impurities and if necessary, could have evaluated their genotoxic potential according to ICH M7 during the post-authorisation phase.

2.4.4.2. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. Data have been presented to give reassurance on viral/TSE safety.

2.4.5. Recommendations for future quality development

Not applicable.

2.5. Non-clinical aspects

2.5.1. Pharmacology

2.5.1.1. Primary pharmacodynamic studies

AMX0035 is a fixed-dose combination of sodium phenylbutyrate (PB) and ursodoxicoltaurine (TURSO). The exact pathological mechanisms leading to the progressive degeneration of motor neurons within cortex, brainstem and spinal cord in human ALS remain to be unravelled. Nevertheless, mutations in the genes of superoxide dismutase 1 (SOD1), Trans-Activation Response element (TAR) DNA binding protein 43 (TDP43), C9ORF72 and Fused-in-sarcoma (FUS) have been linked to familial and sporadic forms of ALS, respectively. Based on previously and newly submitted scientific publications, the applicant delineated that these gene mutations are associated with insoluble cytoplasmic protein aggregates and impaired nucleocytoplasmic transport due to an unfolded protein response and stress condition in the ER as well as mitochondrial dysfunction that ultimately lead to neuronal apoptosis via oxidative stress.

As oxidative stress is implicated in ALS pathology, the applicant evaluated if PB, TURSO or the combination could protect from cell death induced in primary rat mixed cortical neurons by the oxidant

hydrogen peroxide. Pre-treatment of the cultures with the PB/TURSO combination significantly improved cellular viability compared to incubation with either compound alone. Across 20 different PB/TURSO combinations, optimal cell viability was observed when 500 µM PB and 150 – 200 µM TURSO were combined.

Mitochondrial dysfunction may trigger excitotoxicity, which is thought to be inhibited by riluzole, the only approved clinical therapy for ALS in the EU. The applicant had therefore analysed the presumptive protective effect of PB and TURSO after induction of excitotoxicity by glutamate in primary rat spinal motor neurons. Overall, TURSO effectively protected neurite networks, improved neuronal survival and reduced abnormal TDP 43 cytoplasmic translocation, which decreased upon prolonged pre-incubation intervals of the neuronal cultures ⩾8 h before induction of excitotoxicity, when TURSO was added with a delay of ⩾1 h after glutamate, or when excitotoxicity was induced with 10 µM glutamate. However, subsequent investigations indicated the effective inhibition of mitochondrial dysfunction, cellular apoptosis and ER stress. During the assessment, the applicant evaluated statistically whether there was a benefit of AMX0035 in these studies. When comparing PB, TURSO, and AMX0035, there is an overall trend that AMX0035 outperforms the individual components. Of note, the tested PB/TURSO combinations did not consistently unveil superiority to a single TURSO treatment but mostly mirrored the effects seen with TURSO alone. In contrast, PB alone was less efficacious than TURSO or the PB/TURSO combination. Therefore, it is considered that the combination might have an additive effect, but a synergistic effect was not consistently demonstrated.

The applicant supplemented the *in vitro* data with a recently published study on comparative genomic and metabolomic analysis on the mechanism of action of AMX0035 (Fels JA et al. Ann Clin Transl Neurol. Available online since 2022 Sep 9). Each 12 fibroblast cell lines derived from individuals with sporadic ALS and healthy subjects were treated for 5 days with either 100 µM PB, 10 µM TURSO, or both. Motor neurons, the primary cell type affected in ALS, were not investigated. More gene expression and metabolomic changes for the PB/TURSO combination were found involving nucleocytoplasmic transport, unfolded protein response (incl. the wolframin 1 gene), mitochondrial function, RNA metabolism, and innate immunity.

In vivo, twice daily oral administrations of the 100 mg/kg PB/ 200 mg/kg TURSO combination in transgenic SOD1 G93A mice did not improve survival or locomotor performance. Another study in TDP43 mutant Drosophila revealed enhanced locomotor function by individual PB at ⩾1 µM or 30 µM TURSO but did not evaluate the PB/TURSO combination. Other mouse models of multiple sclerosis or Alzheimer's disease did not convincingly demonstrate beneficial effects and are of minor relevance for the present CMA in ALS due to differences in doses of both agents and the specific characteristics of the disease models.

Furthermore, the applicant presented a new investigation in response to the Multi PD MO based on transgenic mice expressing the mutant human profilin 1 C71G (PFN1), an animal model of familial ALS characterized by insoluble protein aggregates in progressively degenerating motor neurons. Neuromuscular function was measured using terminal electromyography (EMG) to determine peak compound muscle action potential (CMAP), an indicator of muscle innervation and activation. Loss of CMAP amplitude is a non-specific observation relating to motor axon loss or muscle dysfunction. The combination of PB (400 mg/kg/day) and TURSO (400 mg/kg/day) resulted in a significant improvement in CMAP that was not observed in animals treated with PB or TURSO alone, suggesting benefits of treatment with the PB/TURSO combination. It is noted that only one PB/TURSO combination dose was tested; thus a dose-response effect could not be established. In addition, the evaluation of vehicle-treated controls revealed about 10 % variability of CMAP measurements and that neuromuscular function was more severely compromised in older mice. Accordingly, the PB/TURSO combination was less efficacious when administrations started at 12 weeks of age. It should also be noted that mutations in the Pfn1 gene are associated with familial ALS. The applicant did not elaborate on the relevance of this

mouse model for the whole ALS population, but the prevalence of PFN1 mutations is low, around 1% of inherited forms of the disease. The rationale for a fixed dose combination of PB and TURSO can be followed. Published data indicate that PB and TURSO mitigate ER and oxidative stress, the unfolded protein response, mitochondrial dysfunction and neuronal apoptosis. All these processes are known to play a role in ALS pathology, but the exact mechanistic contribution of PB or TURSO to ameliorate the disease could not be unravelled. Despite the trend in favour of PB/TURSO combination, synergistic efficacy is not convincingly demonstrated.

2.5.1.2. Secondary pharmacodynamic studies

An *in vitro* receptor binding panel for PB and TURSO, as well as their major metabolites including phenylacetate (PAA), phenylacetyl-l-glutamine (PAGN), ursodeoxycholic acid (UDCA), and glycoursodeoxycholic acid (GUDCA) was performed. No inhibition or stimulation (>50%) was seen for UDCA, PB, TURSO, or GUDCA. PAGN had >50% activity at 2500 µM for NMDA (antagonist), 5-HT_{2B}*(h)* (agonist) and GABA transporter (antagonist). *In vitro* activity of PAGN was further evaluated in a binding assay, where 50% inhibitory concentration (IC_{50}) values for these receptors were determined to be 680 µM and 1300 µM for the GABA transporter and NMDA receptor, respectively. No IC_{50} could be determined for 5-HT_{2B}*(h)* with 39.5% inhibition seen at 2.5 mM, the highest dose tested. Following single and multiple AMX0035 dosing in healthy volunteers, maximum plasma concentration (C_{max}) values of 158.2 µM and 143.4 µM were determined. Hence, IC_{50} values for GABA transporter and NMDA are approximately 4.5- and 8.6-fold higher than those reached clinically. Thus, there is a marginal exposure multiple concerning the potential of PAGN for pharmacologic interaction with the GABA transporter. Because PAGN is also a metabolite in rats, where no CNS effects were seen as part of the functional observational battery, a potential risk regarding AMX0035 effects on the GABA transporter has been sufficiently addressed.

2.5.1.3. Safety pharmacology programme

The safety pharmacology package comprised GLP-compliant central nervous system (CNS) (rats), respiratory (rats) and cardiovascular (minipigs) safety studies. No stand-alone studies concerning renal function/urinary parameters were performed, but these were evaluated in the general toxicity studies.

In a GLP-compliant 28-day repeated-dose toxicity study in rats, AMX0035 (250/83 mg/kg PB/TURSO, 750/250 mg/kg PB/TURSO, 1000/333 mg/kg PB/TURSO) was evaluated for CNS observations using the Functional Observation Battery. In line with ICH S7A, motor activity, behavioural changes, coordination, sensory/motor reflex responses and body temperature were evaluated. There were no notable changes attributed to AMX0035 up to 1000/333.28 mg/kg/day. Similarly, in GLP-compliant respiratory safety study, a single administration of PB/TURSO in rats had no effects on tidal volume, minute volume and respiratory rate at doses up to 1000/333.28 mg/kg.

In a GLP-compliant cardiovascular safety study in telemetered minipigs, there were no effects on clinical signs, body weights, body temperature, qualitative ECG, QRS, QT, and QTca observed up to doses of 1000/333.3 mg/kg PB/TURSO. However, this dose was associated with sustained increases in heart rate (and decreased PR interval) and decreases in pulse height up to 20 hours post-dose. The applicant attributes this observation to the large quantities of sodium present in PB. In one animal, acute, transient decreases in blood pressure were observed, which were treatment-related and occurred at all dose levels (125/41.7 mg/kg - 1000/333.3 mg/kg). However, no effects on the cardiovascular system were noted in the repeated dose toxicology studies with the same doses; thus, the clinical relevance of these effects seems limited.

The human ether-a-go-go related gene (hERG) studies are generally recommended as a standard addition to the assessment of safety pharmacology. It is acknowledged there were no acute effects on heart rhythm in the cardiovascular safety study. The provided secondary pharmacology studies do not indicate cardiac risks of PB, TURSO or major metabolites. For the authorized product Ravicti (glycerol phenylbutyrate), no hERG inhibition was seen at clinically relevant concentrations (Ravicti EPAR EMA/676925/2015). Overall, the cardiovascular safety of AMX0035 has been sufficiently evaluated and the absence of hERG studies can be accepted.

Thus, apart from possible increases in heart rate and decreases in pulse height, *in vivo* studies did not reveal reasons for concern. However, it should be noted that the exposures of PB and TURSO were around or only slightly above clinical exposures, respectively.

2.5.1.4. Pharmacodynamic drug interactions

Neuroprotective effects of AMX0035 in primary spinal cord motor neurons after glutamate exposure were not reduced or enhanced when combined with riluzole and edaravone, suggesting that these compounds are not interacting on a pharmacodynamic (PD) level.

2.5.2. Pharmacokinetics

Methods of analysis

Concentrations of PB and TURSO were measured in the plasma of rats and minipigs with validated Liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods. The validation was adequate regarding calibration, accuracy, precision, dilution integrity, matrix effect and short-term stability. Lower limit of quantification (LOQ) was 50 ng/mL for PB and 10 ng/mL for TURSO. The methods used in the pharmacokinetic (PK) studies were fit for purpose.

Absorption

Following oral administration of PB to rats, area under the curve (AUC) and C_{max} increased dose-proportionally or approximately 1.5x more than dose-proportionally. Time of maximal plasma concentration (T_{max}) was 1 – 4 h. Half-live ($T_{1/2}$) was 0.3 – 0.7 h after single-dose and 0.7 – 3.6 h after multiple-dose. In general, there were no consistent gender differences. In general, exposure after multiple dosing was comparable to exposure on day 1 (after 7 and 28 days) or only slightly higher (≤ 2-fold) (after 182 days).

Following multiple oral dosing of PB to minipigs, exposure increased approximately dose-proportionally or slightly more than dose-proportionally (< 2x). There were no consistent or marked gender differences. Exposure after multiple dosing was comparable to exposure on day 1, after 7 or 28 days, or slightly higher (maximally 2-fold) after 273 days. $T_{1/2}$ was 0.9 – 7.7 h. T_{max} was 1 – 2 h.

Following oral administration of TURSO to rats, exposure increased with dose, but in a variable way, without a clear pattern in dose-proportionality. T_{max} was 1 – 12 h. Exposure was either comparable in males and females or higher in females (up to 12x). Exposure was slightly higher on day 28 than on day 1 and clearly higher on day 182 than on day 1 (AUC up to 18x). $T_{1/2}$ was 6.2 – 96.4 h in the 7-day and 28-day studies and not reportable in the 6-month study due to limited data following C_{max} or due to a value not meeting acceptance criteria. According to the applicant, $T_{1/2}$ of TURSO could not be determined very well due to the lack of time points in the terminal elimination phase.

Following multiple oral dosing of TURSO to minipigs, dose-proportionality varied between the studies from no increase with dose to more than dose-proportional increases. Exposure was higher in females than in males in the 7-day- and 28-day studies (up to 4 – 6-fold). In the 9-month study, exposure was

very variable, also between the sexes, but there were no consistent gender differences. Exposures on day 7 or day 28 were comparable to or slightly higher than exposure on day 1. After 273 days, accumulation was observed in females (up to 9-fold), but not in males. The exposure was, however, still below the expected human exposure. $T_{1/2}$ was 4.3 – 11.6 h. T_{max} was 4 – 16 h.

Absolute bioavailability was not determined, but based on a mass balance study in rats, absorption in rats was at least 90% for PB and TURSO.

Distribution

In vitro protein binding in human plasma was 81.5-82.7% for PB and 98.5-98.6% for TURSO.

In tissue distribution studies in rats, PB-derived radioactivity was widely distributed. The highest concentrations were found in the kidneys, liver, urinary bladder, Harderian gland, lung, small intestine and stomach (gastric mucosa). Low concentrations were found in the brain. Measurable amounts of PB were found in the cerebrospinal fluid (CSF) of rats, with relatively high concentrations at the highest dose of 300 mg/kg. Concentrations in the skin and eye (uvea) were higher in LE rats than in SD rats, indicating possible binding to melanin. At 168 h after dosing, concentrations were below Lower limit of quantification (LLOQ) in most tissues except for low levels in brown and white adipose tissue and in the Harderian gland.

TURSO-derived radioactivity was widely distributed. The highest concentrations were found in the small intestine, stomach (gastric mucosa) and liver. Levels in the brain were below LLOQ. TURSO-derived radioactivity was still quantifiable at 120 h post-dose in the kidney, liver, Harderian gland, brown adipose tissue, small intestine and stomach (gastric mucosa). At 673 h, concentrations in all tissues were below LLOQ. In SD rats, concentrations at 24 h were higher in blood and lymphatic tissues than in LE rats. Concentrations in skin and eye were low in both LE rats and SD rats. This indicates that TURSO does not bind to melanin. TURSO was not detectable in the CSF of rats. However, this may be due to a lack of sensitivity of the non-validated method used.

Metabolism

Following oral administration of [^{14}C]PB to rats, the parent compound in plasma comprised 19% of total AUC. However, the parent compound was not detected in urine and bile and only found in trace amounts in faeces. Major metabolites in plasma were M135 (phenyl acetate), M161 (phenyl crotonate) and M192 (phenylacetyl glycine), comprising 16%, 18% and 46% of total AUC, respectively. Also, a small amount (approximately 1%) of M179 (hydroxyl phenylbutyrate) was found in plasma. In urine, the major metabolite was M192 (88% of the dose). Also, M179 was found in urine at 1% of the dose. In bile, a low amount of M192 was found (1.4%). M135 and M192 were detected in faeces, but no specific amounts were determined because of the low total amount of radioactivity recovered from faeces. Overall, PB is primarily converted into phenylacetyl glycine and then excreted into urine (see also section 3.6). In humans, phenylacetic acid (= M135) is the most important metabolite of PB (i.e. 42-60% of AUC).

Following oral administration of [^{14}C]TURSO to rats, the parent compound in plasma comprised 9% of the total AUC. The major metabolite in plasma was M516_2 (metabolite formed by oxidation), comprising 91% of total AUC. In urine, only trace amounts were found of the parent compound, M391_1, M516_1 and M516_2. In bile, the parent compound was the main component, comprising 83% of the dose. M516_1 (metabolite formed by oxidation) was also found in bile at 8% of the dose. In faeces (of intact rats), major metabolites were M391_1, M391_4 and M391_5 (all formed by hydrolysis + dehydrogenation), comprising 46%, 13% and 15% of the dose, respectively. A number of other metabolites were found in faeces at ≤ 2% of the dose. The parent compound was found only at trace amounts in faeces. The study indicates that TURSO is primarily oxidated to M516 or hydrolysed to M391 (ursodeoxycholic acid), and TURSO is excreted primarily into faeces (see section 3.6). The fact that M391 was found in faeces and not in bile indicates that it is formed in the intestines. This is confirmed by the

fact that M4 (which is the same as M391) was only found in low amounts in hepatocytes *in vitro*. In humans, ursodeoxycholic acid (corresponding to M4 *in vitro* and M391 in the rat) and its glycine conjugate glycoursodeoxycholic acid are the most important metabolites of TURSO (AUC after a single dose was approximately similar to TURSO).

In conclusion, there are no relevant human-specific metabolites of PB. Regarding TURSO, the metabolite glycoursodeoxycholic acid was not found in this particular rat study; however, TURSO and its human metabolites mentioned are all endogenous compounds in humans.

Excretion

In rats, PB was primarily excreted in urine (89 – 91% of dose). PB was excreted in small amounts in faeces (2.3% in intact rats) and bile (1.4% in bile-duct cannulated rats). Total recovery was 94% after 96 h and 96% after 168 h. The study indicates that systemic absorption of PB was approximately 91% in rats.

TURSO was primarily excreted into faeces (87% of the dose in intact rats) via the bile (92% in bile-duct cannulated rats). Only a very minor part was excreted in urine (0.1%). Total recovery was 93% after 96 h and 99% (including 12% in carcass) after 168 h. The study indicates that systemic absorption of TURSO was approximately 92% in rats.

Pharmacokinetic drug interactions

For *in vitro* studies, see Clinical section

Two *in vivo* drug-drug interaction (DDI) studies were performed in rats.

AMX0035 caused a moderate increase (2.2-fold) in AUC_{last} of tenofovir (a substrate for OAT1) in rats following combined treatment of TDF with AMX0035 compared to administration of TDF alone.

In rats, exposure to both PB and TURSO was lower in combination than when administered alone. For PB it was slightly lower, and for TURSO up to two-fold lower. This is not expected to have influenced overall conclusions, because in all PK and toxicokinetic studies, PB and TURSO were administered together. Also if this is the case in humans, it would not influence overall conclusions because all PK data are based on PB and TURSO being administered together.

2.5.3. Toxicology

The applicant did not consider the available non-clinical data from earlier licenses of PB ("Ammonaps", EMEA/H/219) and non-conjugated ursodeoxycholic acid (UDCA; see SmPC of "Ursofalk 500 mg film-coated tablets") in concert with European recommendations for an abbreviated toxicology program of the fixed combination containing one or more approved active substances (EMEA/CHMP/SWP/258498/2005). Instead, the toxicity of AMX0035 was evaluated after single dosing at a 3:1 mass ration of PB and TURSO in non-GLP dose escalation studies in rats, dogs and minipigs as well as in GLP-compliant repeat-dose studies for up to 6 months in rats and 9 months in minipigs, respectively.

2.5.3.1. Single dose toxicity

A single dose of AMX0035 at 1500/500 mg/kg in rats and 750/250 mg/kg in minipigs was well tolerated with no mortality or other adverse effects. Dogs were very sensitive to the effects of AMX0035. The emesis in dogs might be related to the oral gavage administration of the acidic drug combination and could hence also correspond to the stomach, and intestinal findings noted in the 28 days repeat-dose toxicity studies in rats and minipigs. Dogs were not further pursued in the non-clinical studies.

2.5.3.2. Repeat dose toxicity

The human AUC value for PB is based on AUC0-tlast (measured over 24 h) in the food effect study in healthy subjects (A35-002). This was measured after administration of 1 sachet of Albrioza. For TURSO, to estimate $AUC_{0\text{-}24h}$ at the recommended dose (i.e. 2 sachets daily), $AUC_{0\text{-}last}$ as measured in the study, was multiplied by 2 (6130 x 2 = 12260 ng.h/mL). For PB, due to the short half live, the value of 1 sachet can be used, which is 113000 ng.h/ml.

AMX0035 was tested in rats and minipigs at up to 6 months (rats) and 9 months (minipigs).

In minipigs, AMX0035 was well tolerated up to 1000/333.33 (PB/TURSO) mg/kg/day for 28 days, and up to 254/84 (PB/TURSO) mg/kg/day for 9 months. These doses, however, are rather low. The 1-month study reaches an AUC based exposure margin of 5.0 and 5.1 for PB in males and females as well as 2.0 and 3.7 for TURSO in males and females as compared to the human exposure. For the pivotal 9 month study, lower doses were used, resulting in exposure margins of 1.2 and 1.4 in males and females for PB and below 1 for TURSO for both sexes.

In rats, AMX0035 was well tolerated up to 1000/333.33 (PB/TURSO) mg/kg/day for 28 days resulting in exposure margins of 2.0 and 2.7 for PB in males and females and below 1 for TURSO for both sexes as compared to the human exposure. In the pivotal 6-month study, some effects were seen in females that suggest hormonal imbalance. These include ovary follicular cysts from the low dose (exposure margins of 0.2 for PB and 0.2 for TURSO). From the mid-dose mucification of the vagina and cervix, and lobuloalveolar hyperplasia and dilatation of the mammary gland were seen, including a mammary gland adenocarcinoma at this dose (exposure margins of 0.4 for PB and 0.7 for TURSO). Additionally, at the high dose a disrupted oestrus cycle was observed (exposure margins of 0.5 for PB and 2.1 for TURSO). The predictive value of repeat-dose toxicity studies in animals for human safety is limited. Nonetheless, additional repeat-dose toxicity studies are not warranted, given the existing experience from human therapy with the individual components or derivatives. The remaining safety concerns should be addressed from a clinical perspective.

2.5.3.3. Genotoxicity

In vitro genotoxicity testing was performed for the individual components and the combination. These *in vitro* studies were negative. The *in vivo* study was only performed with the combination product. It is not stated what the actual doses of the active substances are in this study but is assumed that the study was performed with the formulation including excipients, as in some other studies in the dossier. This results in a high dose of 600/200 mg/kg PB/TURSO administered twice. Although this study was negative, it can be questioned whether the exposure in the rats was sufficient. No toxicokinetic analysis was performed, and from toxicokinetic data from the repeated dose studies, it appears that the exposure margin of PB would be around 2 and for TURSO below 1. However, considering the nature of the substances, a genotoxic potential is not anticipated, and these studies are considered sufficient.

It can be concluded that both PB and TURSO have no genotoxic potential.

2.5.3.4. Carcinogenicity

No carcinogenicity studies have been performed to support this CMA. This can be agreed upon based on the short-term survival of ALS patients.

2.5.3.5. Reproductive and developmental toxicity

In the reproductive toxicity studies, doses of PB and TURSO of 112.5, 225 and 450 mg/kg/day PB and 37.5, 75 and 150 mg/kg/day TURSO were used.

Effects of AMX0035 on male and female fertility and early embryonic development were evaluated in rats. No effects were seen at PB/TURSO doses up to 450/150 mg/kg/day. The exposure at these doses are, however, below the human exposure at the maximum dose, which hampers the assessment considerably.

Effects on embryo-fetal development were evaluated in mice, rats and rabbits. In rats and mice, no effects were seen at PB/TURSO doses up to 450/150 mg/kg/day. As with the fertility study, the highest dose in the rat resulted in exposures below the human exposure. For mice, no toxicokinetic data are available so it can only be assumed that the low exposures also apply to mice.

The rabbit study has not been discussed further by the applicant. This concerns a non-GLP DRF study, in which the doses of AMX0035 were above the MTD, as the 3 highest dose groups needed to be terminated due to severe maternal toxicity evidenced as severe weight loss. Also, there was some maternal body weight loss at the low dose, two litter losses, and reduced fetal weight of surviving pups. Therefore, the applicant decided that the rabbit was not suitable for reproductive toxicity testing. This results in an EFD programme including two rodent studies and no non-rodent species, which is not in line with ICH S5.

Effects on peri and postnatal development of AMX0035 were evaluated in rats. At the high dose, there appears to be an effect on pup survival, as there is an increase in stillborn pups, and increased pup death after birth. As with the other rat studies, the exposures at this dose are far below the clinical exposure, and therefore this effect could be relevant for humans.

The lack of sufficient exposure in animal studies hampers risk assessment. Considering the increased stillborn pups and pup viability in rats exposed to low doses of AMX0035, and the mention of fetal harm in product information of similar products, a risk of adverse effects during pregnancy can be considered likely. Given the presumed risk and the indication applied for, it is not considered ethical to request new studies. As ALS is a chronically debilitating and life-threatening condition, and treatment cannot be avoided or postponed, a contraindication for pregnancy is not warranted.

2.5.3.6. Toxicokinetic data

The exposure to PB was low, up to 2.7 times in rats and up to 5 times the human exposure in minipigs. Exposure in the 26-week rat study was below the human exposure.

The exposure to TURSO in rats was below the clinical exposure at almost all doses. In minipigs, the exposure was higher but still mostly below or just above the human exposure, with exposure multiples up to 3.7 times the human exposure. Exposure in the 26-week rat study and the 9-month minipig study was below the human exposure.

Exposure to PB increased dose-proportionally or more than dose-proportionally in rats and minipigs. In humans, no data were provided regarding dose-proportionality. In rats, minipigs and humans, no consistent gender differences were observed. In rats, no significant accumulation was observed. In minipigs, accumulation was maximally 2-fold after 273 days. In humans, there was no indication of accumulation. Protein binding was investigated only in human plasma (82-83%). The parent compound comprised 19% of the total AUC in plasma in rats. Major metabolites in the plasma of rats were phenyl acetate, phenyl crotonate and phenylacetyl glycine comprising 16%, 18% and 46% of total AUC, respectively. In humans, phenyl acetate is the major metabolite (42% of total AUC in fasted condition

and 60% in fed condition). The elimination $t_{1/2}$ was 0.7 – 3.6 h in rats, 0.9 – 7.7 h in minipigs and 0.5 – 0.6 h in humans. In rats, 89 – 91% of the dose was excreted in urine and 2% of the dose in faeces. In humans, 80 – 100% is excreted via the kidneys.

Exposure to TURSO increased with dose in a variable way in rats and varied from no increase with dose to more than dose-proportionally in minipigs. In humans, no data were provided regarding dose-proportionality. In rats, exposure was either comparable between the genders or higher in females. In minipigs, exposure was higher in females (up to 4-6-fold). In humans, exposure to TURSO was slightly higher in females (not more than 2-fold). In rats, accumulation was observed (AUC up to 18x on day 182). In minipigs, accumulation was observed in the 9-month study, but not in the other studies. In humans, no accumulation was observed. Protein binding was investigated only in human plasma (99%). The parent compound comprised 9% of total AUC in plasma in rats. A metabolite formed by oxidation comprised 91% of the total AUC in plasma. Ursodeoxycholic acid is formed in the intestines and was found in faeces but not in measurable amounts in plasma. In humans, the main metabolites of TURSO are ursodeoxycholic acid and glycoursodeoxycholic acid. The elimination $t_{1/2}$ was 6 – 96 h in rats, 4 – 12 h in minipigs and 3 – 4 h in humans. In rats, 87 – 92% of the dose was excreted in faeces (primarily via the bile) and 0.1% in urine. In humans, 95% is recirculated and 5% excreted via faeces. Excretion via urine was not measured but is expected to be minor.

2.5.3.7. Other toxicity studies

Certain impurities were specified at the ICH Q3B qualification limit, whereas a higher threshold was accepted for another impurity based on systemic toxicology data (NMT 0.4 %). It is agreed that the applicant could have finalised the structural characterisation of the impurities and if necessary, could have evaluated their genotoxic potential according to ICH M7 during the post-approval phase.

2.5.4. Ecotoxicity/environmental risk assessment

The active substances are natural substances, which will not alter the concentration or distribution of the substance in the environment. Therefore, tauroursodeoxycholic acid and phenylbutyrate are not expected to pose a risk to the environment.

2.5.5. Discussion on non-clinical aspects

Pharmacodynamics

In general, the applicant sufficiently elaborated on the pharmacological rationale to support the fixed dose combination of PB and TURSO. Published data indicate that PB and TURSO mitigate ER and oxidative stress, the unfolded protein response, mitochondrial dysfunction and neuronal apoptosis. The submitted data suggest a complementary protective effect from cellular stress in the ER and mitochondria. ER stress and mitochondrial dysfunction have been identified in post-mortem ALS tissue as well as in animal models of ALS, but the exact mechanistic contribution of PB or TURSO to ameliorate the disease could not be unravelled. Also disruption of the physiological interaction between these organelles may be involved in ALS pathology, pointing towards the suitability of combining PB and TURSO. Although there seems to be a connection between ER stress, mitochondrial dysfunction and ALS, it is noted that the exact mechanistic contribution of PB or TURSO to ameliorate the disease could not be unravelled. Rather, the proposed mechanism of action appears unspecific because this link is present in many neurodegenerative diseases. It is therefore not surprising that the applicant is also evaluating AMX0035 in other neurodegenerative diseases such as Alzheimer's Disease.

Nevertheless, the totality of non-clinical investigations suggests a trend in favour of the PB/TURSO combination over treatment with the individual compounds, which has been confirmed in recent gene expression analyses in fibroblasts from ALS patients and in a new transgenic PFN1 mouse model of familial ALS. However, the applicant did not consider the originally submitted contradictory *in vitro* results, where the benefit seemed to be primarily driven by TURSO. Synergistic efficacy is not convincingly demonstrated. In addition, transgenic SOD 1G93A mice had not shown improvement of survival and locomotor performance after twice-daily oral doses of the 100 mg/kg PB/ 200 mg/kg TURSO combination. The reason for these inconsistent data remains, therefore, unknown.

The rationale for combining PB and TURSO based on the assumed mechanisms of action can be followed and although it can be agreed that the totality of non-clinical investigations suggests a trend in favour of the PB/TURSO combination over treatment with the individual compounds, indicating a potential additive effect of the PB and TURSO combination, a synergistic benefit was not convincingly demonstrated from a non-clinical perspective. Notably, some data still suggest that the combination PB/TURSO is not more effective than TURSO alone. In view of European 3R principles and the fact that no animal model exclusively reflects human ALS symptoms, no further non-clinical data to support the fixed combination of PB/TURSO were requested and the applicant was asked to further substantiate the fixed combination based on clinical data. No additional clinical data have been provided by the applicant to support the fixed-dose combination. It can be concluded that the effect of the combination of PB and TURSO is at best additive based on non-clinical data. This is considered acceptable considering that neither of the active substances have established efficacy in the target population.

Secondary PD studies were conducted with PB, TURSO and major metabolites. Overall, there is a marginal exposure multiple (approximately 4.5-fold difference between IC_{50} and clinical C_{max}) with regard to the potential of PAGN for pharmacologic interaction with the GABA transporter. Considering that PAGN is also a metabolite in rats, where no CNS effects were seen as part of the functional observational battery, a potential risk regarding AMX0035 effects on the GABA transporter has been sufficiently addressed.

Apart from possible increases in heart rate and decreases in pulse height, *in vivo* safety pharmacology studies did not reveal concerns. However, it should be noted that the exposures of PB and TURSO were around or only slightly above clinical exposures, respectively.

Pharmacokinetics

Concentrations of PB and TURSO were measured in the plasma of rats and minipigs with validated LC-MS/MS methods. The validation was adequate regarding calibration, accuracy, precision, dilution integrity, matrix effect, short-term stability and long-term stability in rat plasma. Long-term stability of PB and TURSO in rat plasma was confirmed.

In vitro protein binding in human plasma was 81.5-82.7% for PB and 98.5-98.6% for TURSO. Protein binding was determined only in human plasma and only at one concentration. This concentration was 826 µM for PB (corresponding to 153.801 µg/mL based on a molecular weight of 186.2) and 108 µM for TURSO (corresponding to 57.860 µg/mL based on a molecular weight of 535.74). For PB, this was within the clinical concentration range (C_{max} in study A35-002 was 46.8 µg/mL in fed condition and 188 µg/mL in fasted condition). For TURSO, this was much higher than clinically relevant concentrations (C_{max} in study A35-002 was 0.762 µg/mL in fed conditions). The applicant provided, upon request, data regarding the plasma protein binding of PB in the species used in the toxicology studies, to be able to verify that plasma protein binding in animal species was not much deviating from humans and the calculated Exposure Margins are correct. The PPB study covered the clinical and preclinical plasma PB concentrations and showed that PPB was strongly PB concentration dependent yielding unbound PB values of 2.2% - 11% up to 24% - 45% at a 10-fold higher PB concentration. This concentration dependency, however, was similar for human plasma as for rat and minipig plasma. Since for all tested PB concentrations, the

PB percentages unbound/free were higher for rat and minipig as compared to human plasma, it is agreed that the exposure margins are not negatively impacted and possibly slightly higher (~2-fold for the C_{max}) in the High Doses for rat and minipig toxicology studies. Upon request, the applicant also determined the protein binding of TURSO in plasma of preclinical species and human plasma. The studied concentration range covered the clinical and preclinical plasma concentration range, was not concentration dependent and yielded mean PPB values (%) of 97.8, 96.6, 94.6, 95.5 and 98.2 for human, rat, minipig, dog and monkey, respectively. Therefore, the unbound TURSO concentration in rat or minipig plasma was found to be comparable to or slightly higher than in human plasma (1.6- and 2.5-fold in rat and minipig plasma, respectively).

Concentrations of PB and TUDCA in the brain were negligible or not measurable. Both PB and TURSO reach the brain, though concentrations are expected to be low.

AMX0035 caused a moderate increase (2.2-fold) in AUC_{last} of tenofovir (a substrate for OAT1) in rats following combined treatment of TDF with AMX0035 compared to administration of TDF alone. This cannot be directly extrapolated to humans. According to the Guideline on the Investigation of Drug Interactions (CPMP/EWP/560/95/Rev. 1 Corr. 2), PK interaction studies should generally be performed in humans. Due to species differences, direct extrapolation of results of interaction studies in animals to humans is difficult. Moreover, TURSO was not in steady-state because it was administered only once and $t_{1/2}$ of TURSO is 6.2 – 96.4 h in the rat. According to the Guideline on the Investigation of Drug Interactions, the perpetrator drug should be in a steady state to maximize inhibition. The actual increase in the AUC of tenofovir may therefore be larger. This concern was further pursued from a clinical perspective.

The overall exposure in whole blood was similar to the overall exposure in the plasma of rats. This indicates that distribution to blood cells is limited.

Toxicology

The human AUC value for PB is based on AUC0-tlast (measured over 24 h) in the food effect study in healthy subjects (A35-002). This was measured after administration of 1 sachet of Albrioza (113000 ng.h/ml), but considered acceptable given the short half-life of PB. For TURSO, to estimate AUC0-24h at the recommended dose (i.e. 2 sachets daily), AUC0-tlast as measured in the study, was multiplied by 2 (6130 x 2 =12260 ng.h/mL).). For PB, due to the short half live the value of 1 sachet can be used which is 113000 ng.h/ml.

AMX0035 was tested in rats and minipigs at up to 6 months (rats) and 9 months (minipigs). Reproduction toxicity studies were evaluated in mice and rats. The toxicology package is insufficient, and therefore, the safety assessment is hampered. The predictive value of repeat-dose toxicity studies in animals for human safety is limited. Nonetheless, additional repeat-dose toxicity studies are not warranted, given the existing experience from human therapy with the individual components or derivatives. The remaining safety concerns have been addressed from a clinical perspective.

Reproduction toxicity: The reproductive toxicity study package is not sufficient due to low exposures and lack of a non-rodents species, and therefore the safety for the fetus or offspring when exposed during pregnancy cannot be fully assessed.

Considering the increased stillborn pups and pup viability in rats exposed to low doses of AMX0035, and the mention of fetal harm in product information of similar products, a risk of adverse effects during pregnancy can be considered likely. In light of the presumed risk and the indication applied for, it is not considered ethical to request new studies. As ALS is a chronically debilitating and life-threatening condition, and treatment cannot be avoided or postponed, a contraindication for pregnancy is not warranted.

Certain impurities were specified at the ICH Q3B qualification limit, whereas a higher threshold for another impurity was accepted for based on systemic toxicology data (NMT 0.4 %). The impurities have not been structurally characterised and confirmatory studies for further structural identification are required. It is agreed that the applicant could have finalised the structural characterisation of the impurities and if necessary, could have evaluated their genotoxic potential according to ICH M7 during the post-authorisation phase.

Namely, the applicant was recommended to perform the following studies and submit the relevant information via a variation application by Q4 2023:

Complete the work on structural characterisation.

If the structures of certain impurities are successfully identified, then the applicant should follow ICH M7 and characterise (i.e. Class 1-5) the impurities by either by QSAR in silico methodologies, or by a mutagenicity assay in bacteria.

a. If found negative in QSAR – no further action is needed.
b. If found positive in QSAR, a mutagenicity assay in bacteria should be performed.
    i. If negative in Ames – no further action is needed
    ii. If positive in Ames - changes to the formulation, manufacturing process or control strategy are recommended.

If the structural characterisation work continues to be unsuccessful, the applicant is recommended to perform the following alternative: Isolate sufficient amounts of the impurities, as the root cause is known, and additional quantities of the impurities can be generated. Following the decision tree of ICH Q3B for unsuccessful identification of the structure, genotoxicity studies should be performed.

c. If found negative – no further action is needed.
d. If found positive – changes to the formulation, manufacturing process or control strategy are recommended.

The active substances are natural substances, which will not alter the concentration or distribution of the substance in the environment. Therefore, tauroursodeoxycholic acid and phenylbutyrate are not expected to pose a risk to the environment.

2.5.6. Conclusion on the non-clinical aspects

The pharmacodynamics, pharmacokinetics and toxicological properties of Albrioza were sufficiently evaluated in the non-clinical dossier. The effect of the combination of PB and TURSO is at best additive based on non-clinical data.

There is one other concern remaining regarding the structural identification of certain impurities. Further characterization is required, which could have been done during the post authorization phase.

2.6. Clinical aspects

2.6.1. Introduction

GCP aspects

The Clinical trials were performed in accordance with GCP as claimed by the applicant

The applicant has provided a statement to the effect that clinical trials conducted outside the Community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

- **Tabular overview of clinical studies**

Study number	***Study Title***	***Center(s)***	***Status***
A35-002	A Phase I Study of Single Dose Oral AMX0035 Under the Fasted and Fed State in Healthy Volunteers	Quotient Sciences in FL, USA	Completed; Final CSR
AMX3500 (CENTAUR)	Evaluation of the Safety, Tolerability, Efficacy and Activity of AMX0035, a Fixed Combination of Phenylbutyrate (PB) and Tauroursodeoxycholic Acid (TUDCA), for Treatment of Amyotrophic Lateral Sclerosis (ALS)	25 Northeast ALS Consortium (NEALS) centers in the USA	Completed; Final CSR
AMX3500-OLE	Evaluation of the Safety, Tolerability, Efficacy and Activity of AMX0035, a Fixed Combination of Phenylbutyrate (PB) and Tauroursodeoxycholic Acid (TUDCA), for Treatment of Amyotrophic Lateral Sclerosis: Open-Label Extension	24 Northeast Amyotrophic Lateral Sclerosis Consortium (NEALS) centers in the USA	Completed; Final CSR
A35-004 (PHOENIX)	A Phase III, Randomized, Double-Blind, Placebo-Controlled, Multicenter Trial to Evaluate the Safety and Efficacy of AMX0035 Versus Placebo for 48-week Treatment of Adult Patients with Amyotrophic Lateral Sclerosis (ALS)	Approximately 55 sites in Europe and the USA are planned to participate in the trial	Recruitment completed synopsis
A35-005	Pharmacokinetic and Pharmacodynamic Study of AMX0035 in Patients With ALS	Norman Fixel Institute for Neurological Diseases	Enrolling; no data available

2.6.2. Clinical pharmacology

2.6.2.1. Pharmacokinetics

AMX0035 is a fixed-dose combination of two small molecules, PB and taurursodiol (also referred to as TUDCA or ursodoxicoltaurine), being evaluated for the treatment of ALS.

AMX0035 is formulated as a powder for oral suspension supplied as sachets, each containing 3 g sodium PB and 1 g TUDCA. The contents of the sachet are mixed with 8 ounces of water and administered orally or via a feeding tube. The recommended daily dose in adults is 1 sachet a day for the first 3 weeks of treatment. After 3 weeks of treatment, the dose is 1 sachet twice a day.

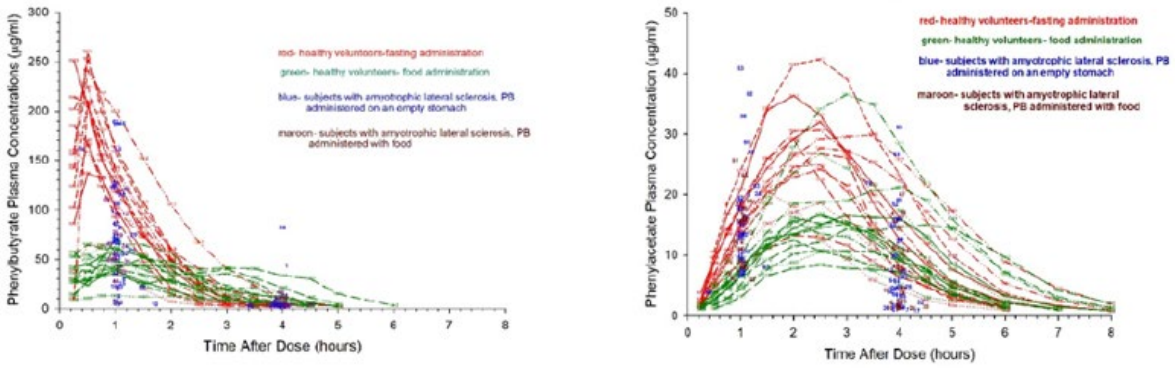
The PK of PB and TUDCA were studied in a phase I study in healthy volunteers in fasted and fed state and in a phase III study in ALS patients (sparse sampling).

Methods

Plasma concentrations of PB and its metabolite PAA and TUDCA and its metabolites UDCA and GUDCA were analysed using LC-MS/MS methods.

A population PK model was built for PB and PAA. In general, the population PK model is adequately describing the observed PB and PAA concentration as parameters were estimated with reasonable precision, and no clear deviations can be observed in the prediction-corrected visual predictive checks. The modelling exercise has been clearly summarised. The plasma concentrations of PB and PAA in the phase I study in healthy volunteers, and the Phase III study in ALS patients are shown in Figure 3.

Figure 3: Plasma-concentration time profiles of phenylbutyrate (left) and phenylacetate (right) on linear scale for patients included in population pharmacokinetic analysis



Data in red represents administration of phenylbutyrate 3000 mg in healthy volunteers under fasting conditions, green is healthy volunteers receiving phenylbutyrate with a high fat meal, blue is ALS patients receiving phenylbutyrate on an empty stomach, and maroon is ALS patients receiving phenylbutyrate with food.

No population PK modelling was performed for TUDCA. It was considered not feasible based on limited plasma concentration data available.

Absorption

The absolute bioavailability of PB and TUDCA was not studied. The bioavailability of TUDCA is known to be high. Approximately 95% of the bile acid pool is recirculated. Furthermore, a mass balance study in rats showed that absorption in rats was at least 90% for PB and TUDCA (see non-clinical section). In ALS patients, steady-state concentrations of TUDCA, UDCA and GUDCA were clearly higher than the endogenous levels.

Bioequivalence studies were not performed. This was not necessary because the differences in composition between the formulations used do not raise concerns regarding bioequivalence.

The food effect was studied in a Phase I study following single-dose administration of one sachet of AMX0035 (3 g PB and 1 g TUDCA) in fasted and fed (high-fat meal) condition according to a cross-over design. Administration of Albrioza in the presence of a high-fat meal resulted in a lower C_{max} (reduced by 75%) and lower AUC (reduced by 55%) of PB. The AUC of TUDCA was increased in fed condition compared to fasted condition (39%). For C_{max}, there was no relevant difference between the fasted and the fed condition. TUDCA was for a major part converted to the metabolites UDCA and GUDCA, in both fasted and fed conditions. AUC of UDCA and GUDCA was slightly higher in fed conditions than in fasted conditions. For the metabolite UDCA, T_{max} was largely increased under the fed condition compared to the fasted condition (16 h vs. 6 h). The exposure to TUDCA was slightly higher in females than in males (up to nearly 2-fold higher), in both fasted and fed conditions. This is not expected to be clinically relevant. Exposure to UDCA and GUDCA was not statistically significantly different between males and females. Exposure to PB in Japanese participants was comparable to those observed in Caucasian participants. Exposure to TUDCA and its metabolites was higher in Japanese participants than in Caucasian participants. However, the safety profiles were similar between the two populations.

Distribution

For PB, the plasma protein percentage from the *in vitro* study in human plasma, 82%, is supported by the plasma protein percentage from literature, 81-98%. The apparent volume of distribution (Vz/F) was 8.4 L in fasting conditions and 21.3 L in fed conditions. Thus, PB has a relatively small Vz/F, compared to total body fluid of 42-47 litre (average 70 kg man).

In vitro plasma protein binding of TUDCA was 99% at 108 µM. It is currently unknown whether this value is representative of the clinical situation because this concentration was approximately 75-fold the clinical C_{max}. The Vz/F in healthy subjects was 1600 L in fasted condition and 1000 L in fed condition, which implies that TUDCA was extensively distributed with a Vz/F far beyond total body water.

Elimination

The involvement of CYP enzymes in the metabolism of PB and TUDCA was investigated using human recombinant CYP enzymes and human liver microsomes. Both PB and TUDCA were not metabolised by CYP enzymes *in vitro*.

PB is mainly converted to PAA in enterocytes and/or hepatocytes. PAA is conjugated with glutamine in enterocytes, hepatocytes and/or the kidney.

TUDCA, UDCA and GUDCA are endogenous compounds belonging to the bile acid pool. Endogenous bile acids are formed mainly in the liver. Primary bile acids are cholic acid (CA) and chenodeoxycholic acid (CDCA). These bile acids are conjugated with glycine or taurine and transported to the gall bladder. Following the intake of food, bile acids are excreted with the bile into the duodenum. A large part of bile acids will be reabsorbed, passively or actively, and transported back to the liver. Another part will be converted into secondary bile acids, such as deoxycholic acid (DCA), lithocholic acid (LCA) and ursodeoxycholic acid (UDCA), by intestinal bacteria. Secondary bile acids can also be absorbed and transported to the liver, where they can be conjugated with glycine or also with taurine (conjugation of UDCA with taurine leads to the formation of TUDCA and conjugation with glycine leads to the formation of GUDCA). In the study in ALS patients, plasma concentrations of TUDCA, UDCA and GUDCA were increased significantly as a result of treatment.

No mass balance study was performed.

The elimination $t_{1/2}$ of PB in healthy volunteers was 0.461 – 0.599 hours. The apparent clearance (Cl/F) was 211 – 410 mL/min. The elimination $t_{1/2}$ of PAA was 0.78 – 0.81 hours. Following oral administration of sodium PB, a majority of the administered compound (approximately 80–100%) is excreted by the kidneys within 24 hours as the conjugation product, PAGN.

The elimination $t_{1/2}$ of TUDCA was 3.4 – 4.3 h. CL/F was 3440 – 4260 mL/min. The elimination $t_{1/2}$ of UDCA and GUDCA was 4.8 – 5.3 and 12.7 – 16.5 h. According to the literature, approximately 95% of the bile acid pool is recirculated and approximately 5% is excreted via the faeces. No figures are given for urinary excretion, but it is expected to be minor (< 5%).

Dose proportionality and time dependencies

No data from the performed studies are available regarding the dose-proportionality of PB and TUDCA because all studies have been performed with the same dosage. According to a publication provided during the assessment, at clinically relevant doses sodium phenylbutyrate exposure increased approximately dose proportional, and limited available data showed a less than proportional increase in exposure for ursodoxicoltaurine with increasing doses.

Given the low $t_{1/2}$ of PB and PAA, no accumulation is expected. Therefore, no further discussion by the applicant on the time dependency of PB and PAA is considered necessary.

Data in ALS patients from week 12 and week 24 at 4 h post-dose, which is approximately similar to T_{max} of TUDCA, indicate no evidence of accumulation of TUDCA or the metabolites UDCA and GUDCA. No

information is available regarding the time of steady-state of TUDCA. However, considering the $t_{1/2}$ of TUDCA, which is approximately 3 – 4 h, it can be considered to occur before week 12.

Special populations

Dosing of PB and TUDCA does not need to be adjusted for mild renal impairment. The effect of moderate and severe renal impairment has not been studied. No subjects with hepatic impairment were studied. Dosing of TUDCA does not need to be adjusted for body weight. Dosing of PB and TUDCA does not need to be adjusted for gender and age ≥ 65 years. The number of subjects older than 75 years is limited (Table 1). Dosing of PB and TUDCA does also not need to be adjusted in the Japanese population. No data was provided in children, which is no issue since Albrioza is not indicated in children.

Table 1: Special population age 65 years and older

	Age 65-74 (Older subjects number /total number)	Age 75-84 (Older subjects number /total number)	Age 85+ (Older subjects number /total number)
Clinical studies A35-002, AMX3500	28/141	5/141	0/141

Pharmacokinetic interaction studies

AMX0035 as victim

It is considered not likely that other medicinal products will influence the disposition of PB and TUDCA, because they are not metabolized by CYP enzymes.

The applicant claims aluminium-based antacids have been shown to adsorb bile acids *in vitro* and may interfere with the absorption of ursodoxicoltaurine. Also, it is mentioned that probenecid should not be used with Albrioza as it is known to inhibit the renal excretion of many organic compounds.

AMX0035 as perpetrator

Cut-off values for intestinal concentrations were within the IC_{50} range of all tested CYP enzymes. This has consequences for CYP3A, because it has pronounced intestinal expression.

In the liver, no inhibition of CYP enzymes by TUDCA is expected because the IC_{50} values for the inhibition of the enzymes were higher than the cut-off value for systemic exposure. Inhibition of CYP2C9 by PB cannot be excluded. Regarding CYP2B6, both inhibition and induction potential has been observed.

No inhibition is expected of the hepatic uptake transporters OATP1B1 and OATP1B3 and of the renal transporters OCT2, OAT3, MATE1 and MATE2-K. Inhibition of P-gp and BCRP by PB and TUDCA cannot be excluded. Inhibition of OAT1 by PB and TUDCA is possible based on an *in vitro* study and an *in vivo* study in rats.

Pharmacokinetics using human biomaterials

See under "Pharmacokinetic interaction studies".

2.6.2.2. Pharmacodynamics

Mechanism of action

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

Sodium PB is an HDAC inhibitor that decreases ER stress through the upregulation of chaperone proteins and acts as a small molecular chaperone. Ursodoxicoltaurine decreases mitochondrial stress by reducing

mitochondrial permeability and increasing the apoptotic threshold of the cell through inhibition of the BAX protein.

Primary and Secondary pharmacology

Primary pharmacology

No information was provided by the applicant.

Secondary pharmacology

The provided literature discusses the role of PB and TUDCA in other disorders. How these molecular effects relate to the pathophysiology of ALS is uncertain.

2.6.3. Discussion on clinical pharmacology

Methods

Plasma concentrations of PB and TUDCA and metabolites were analysed using validated (LC-MS/MS) methods. The maximal storage time of 967 days for PB, and 964 days for PAA was not within the established long-term stability (13 days). During the assessment, the stability of PB and PAA was confirmed up to 440 days. Additional long-term stability data up to 967 days are planned to be generated and it is agreed that this could have been provided during the post-authorisation phase. The selectivity of the analysis of TUDCA, UDCA and GUDCA could not be determined very well because endogenous peaks were also present in many samples. It was not possible to provide results from blank samples, because of the endogenous concentrations already present. To investigate selectivity, "blank" samples were spiked up to the level of the LLOQ and the level of bias was determined. Mean percentage bias and %CV at the LLOQ of TUDCA, UDCA and GUDCA were within 15%.

In the population PK model for PB and PAA, body weight was implemented as an estimated covariate effect instead of the more mechanistically sound allometric theory with fixed exponents of 0.75 for clearance and 1.0 for the volume of distribution parameters. Additionally, bodyweight was only included on model parameters for PAA. The ETA vs. covariate plots indicate that bodyweight could also be a relevant covariate for PB PK. The model update which was provided during the assessment, with fixed allometric scaling exponents (0.75 for clearance and 1.0 for volume of distribution parameters) indeed indicates that it cannot be excluded that bodyweight is an important factor in predicting exposure to PB and PAA as no relevant differences between both models were detected. Nonetheless, it can be agreed with the applicant that remaining within- and between-subject variability in exposure is still high. Therefore, this issue is not further pursued and fixed dosing is considered acceptable. Furthermore, administration of the drug via a feeding tube was excluded as a covariate. During the assessment, population PK showed that absorption of PB via a feeding tube resembles absorption after oral administration in a fasted state. Using linear mixed-effect models, the presence of a feeding tube had no relevant effect on TUDCA PK. A disease effect of ALS (as compared to healthy volunteers) on model parameters for V_{max} and V of PAA was implemented. This disease effect on model parameters could also represent study differences or differences between single-dose data and steady-state data due to limitations of the included data and should therefore be interpreted with caution. Also, the delay modelled using the transit compartment indicates a quick transit time of approximately 5 minutes. Considering this all, the model cannot be used for any future extrapolations and should only be used to estimate individual PK parameters in the included patients in the population PK model, which is mainly due to data limitations and to a minor degree due to modelling decisions.

Absorption

Because the proposed product is a powder that needs to be dissolved and has a different dosage regimen than was used in the study from literature, the applicant was requested to bridge the data from the literature to the proposed product. Providing more literature data, of for example, powders for oral solution was also possible if adequately bridged to the proposed product. During the assessment, some literature was provided, but no bridge was made to the current product. However, it is already known that approximately 80 – 100% of PB is excreted via the kidneys as the metabolite PAGN. This implies that at least approximately 80% of PB is systemically available. TUDCA is a bile acid derivative, and regarding bile acids, it is known that they are, for the major part (approximately 95%) recirculated and, therefore, also systemically available to this extent. Although it would have been informative to know more about the exact bioavailability of PB and TUDCA, it would not significantly have altered the current conclusions regarding this product. This issue was, therefore, not pursued.

Bioequivalence studies were not performed. This was not necessary because the differences in composition between the formulations used do not raise concerns regarding bioequivalence. Upon request, preliminary data are provided for study A35-007. In this study, exposure was compared between Caucasian and Japanese subjects and single dose and multiple dose (7 days). Preliminary data indicate no consistent differences between Caucasian and Japanese subjects, except for higher AUC and C_{max} for TUDCA and UDCA after 7 days in Japanese subjects compared to Caucasian subjects. It was not clear whether the subjects were dosed in the fed or fasted state. After 7 days, no accumulation was observed for PB and metabolites. For TUDCA and metabolites, AUC after 7 days was at least 2 times the value after a single dose. In the protocol, it is indicated that in the morning, breakfast was served after the administration of the drug. However, single-dose C_{max} and AUC were more comparable to the values obtained in fasted condition initially submitted. During the procedure, it was clarified by the applicant that the subjects were served a standardised (not high-fat, high-calorie) breakfast after administration of Albrioza. The study report is expected to be available in Q3 2023. It is agreed that the CSR could have been submitted as soon as possible during the post-authorisation phase.

It was unclear why the applicant chose to recommend taking a meal or snack before Albrioza, given the fact that for PB and PAA, C_{max} and AUC were statistically significantly lower under food conditions, and for TUDCA and metabolites, PK parameters were higher or comparable. The effect of food on the components was thus contrary, although the effect on PB and PAA was larger than on TUDCA and metabolites. Furthermore, in the CENTAUR study, it was optional to eat a snack or meal after taking study medication. Upon request, the applicant agreed to replace the recommendation to take Albrioza directly before a snack or meal by a recommendation to take Albrioza before a meal. This was in principle endorsed, since the advice is only intended to alleviate the bitter taste of Albrioza. However, the applicant was requested to discuss whether the decreased exposure to PB and the increased exposure to TUDCA in the presence of food can be considered clinically relevant taking into account that the ratio between PB and TUDCA will be quite different in fed conditions compared to fasted conditions. This was however considered not clinically relevant because of the large inter-subject variability observed in the studies coupled with a high therapeutic index of AMX0035.

No data were provided regarding dose-proportionality of PB and TUDCA because all studies have been performed with the same dosage. According to a publication provided upon request, at clinically relevant doses sodium phenylbutyrate exposure increased approximately dose proportional, and limited available data showed a less than proportional increase in exposure for ursodoxicoltaurine with increasing doses.

Special populations

Dosing of PB and TUDCA does not need to be adjusted for mild renal impairment. The effect of moderate and severe renal impairment has not been studied. Since there is currently no information regarding the effect of moderate and severe renal impairment, a clinical study is planned to be conducted to study the effect of renal impairment. It is agreed that this could have been submitted during the post-authorisation

phase. No subjects with hepatic impairment were studied. A clinical study is planned to be conducted regarding the effect of hepatic impairment. It is agreed that this could have been submitted during the post-authorisation phase Although there is a trend for an inverse correlation of bodyweight to PB plasma concentration, this was not significant, as body weight explained only a minor part of the variation compared to the within- and between-subject variation. Therefore, dosing of TUDCA does not need to be adjusted for body weight. Dosing of PB and TUDCA does not need to be adjusted for gender and age ≥ 65 years. The number of subjects older than 75 years is limited. Dosing of PB and TUDCA also does not need to be adjusted in the Japanese population. No data were provided in children, which is acceptable, since Albrioza was not intended to be indicated in children.

Pharmacokinetic interaction studies

AMX0035 as victim

In an *in vitro* study submitted during the assessment, PB was not a substrate of OATP1B1, OATP1B3, OCT1, BSEP, P-gp and BCRP. TUDCA was not a substrate of OATP1B1and OCT1. TUDCA was a substrate of OATP1B3 and BSEP. In the newly submitted *in vitro* DDI study, TUDCA was found to be a substrate of P-gp and BCRP, though only at the lowest dose, whereas it was not a substrate of these transporters in a study already submitted. Since the latter study is more recent and Caco-2 cells were used, as recommended in the Guideline on the investigation of drug interactions (CPMP/EWP/560/95/Rev. 1 Corr. 2**), TUDCA is considered a substrate of P-gp and BCRP *in vitro*. The applicant states that an *in vivo* study is planned to investigate the inhibition of P-gp and BCRP by AMX0035. Depending on the outcome of this study, the necessity to conduct an *in vivo* to investigate AMX0035 as a substrate for P-gp and BCRP would have been evaluated. It is considered that AMX0035 is a substrate of P-gp and BCRP *in vitro*. Bile salts often undergo enterohepatic circulation. This can be influenced by antibiotics. It is not possible to give a general advice regarding this effect, because antibiotics may block or enhance enterohepatic circulation and effects are expected to differ between antibiotics and dependent on the numbers of antibiotics used. Since it is not possible to formulate a general advice on the use of antibiotics in relation to the disposition of TUDCA this issue was not pursued.

AMX0035 as perpetrator

Cut-off values for intestinal concentrations were within the IC_{50} range of all tested CYP enzymes. This has consequences for CYP3A, because it has pronounced intestinal expression. Therefore, the need for an *in vivo* study to evaluate the potential inhibition of CYP3A by PB and TUDCA has been requested to discuss, because IC_{50} (between 2467 and 7400 µM for PB and between 533 and 1600 µM for TUDCA) was around or below the cut-off value for intestinal concentrations (6445 µM for PB and 747 µM for TUDCA) for intestinal CYP3A4 (but not for hepatic CYP3A4). During the assessment, instead of providing a discussion on the need of an *in vivo* study, the applicant submitted another *in vitro* study regarding the inhibition of CYP enzymes, in which PB and TUDCA and their metabolites were tested at lower concentrations than in the first found. This study does not address the original question raised. Finally, the applicant stated that an *in vivo* study is planned to investigate inhibition of CYP3A4/5 by AMX0035. It is agreed that this study could have been provided during the post-authorisation phase. In the new *in vitro* DDI study, inhibition of CYP3A by UDCA was observed. Although it is not likely that UDCA will be formed in the intestine at levels high enough to cause inhibition of CYP3A4 (Ki ≥ 25x C_{max} of UDCA), potential inhibition of CYP3A by UDCA will automatically be investigated in the study mentioned above.

In the liver, no inhibition of CYP enzymes by TUDCA is expected, because the IC_{50} values for the inhibition of the enzymes were higher than the cut-off value for systemic exposure. Inhibition of CYP2C9 by PB cannot be excluded because *in vitro*, IC_{50} (≈ 2467 µM) was only a little higher than the cut-off value for systemic concentrations of PB (2262 µM). Hence the Ki value, which should be used according to the Guideline on the Investigation of Drug Interactions (CPMP/EWP/560/95/Rev. 1 Corr. 2), is lower than the cut-off value. Therefore, a discussion on the need for an *in vivo* study was requested. Instead of

providing such a discussion during the assessment, the applicant submitted another *in vitro* study regarding inhibition of CYP enzymes, with PB and TUDCA tested at lower concentrations than in the first study. Finally the applicant referred to R1 values as cut-off point. However, this cut-off point is not used in the EU. According to the European Guideline on the investigation of drug interactions (CPMP/EWP/560/95/Rev. 1 Corr. 2**) and also according to the draft ICH M12 Guideline on drug interaction studies (EMA/CHMP/ICH/652460/2022), enzyme inhibition *in vivo* cannot be excluded if $C_{maxunbound}$ / Ki $\geq$ 0.02. $C_{maxunbound}$ (=45 µM) / Ki (=2467 / 2) = 0.036. Hence, an *in vivo* study was requested to be conducted to investigate inhibition of CYP2C9 by AMX0035. It is agreed that this study could have been submitted during the post-authorisation phase. Based on *in vitro* studies, inhibition of CYP2C9 by AMX0035 may be possible. Regarding CYP2B6, both inhibition and induction potential has been observed. Potential for induction of CYP2B6 has been found for PB, PAGN, TUDCA and UDCA. An *in vivo* study is planned to evaluate the inhibitory and induction effects of AMX0035 on probe substrates of CYP2B6. It is agreed that this study could have been provided during the post-authorisation phase. Meanwhile and based on *in vitro* studies, it is considered that Albrioza has been shown to inhibit CYP2B6 and to induce CYP1A2, CYP2B6, and CYP3A4 isoenzymes.

The absence of the investigation of the inhibitory potential of the major metabolites of PB (PAA) and of TUDCA (UDCA and GUDCA) had to be justified, since they all had AUC larger than one fourth of the AUC of the parent compound. During the assessment, an *in vitro* study was submitted concerning the inhibition of CYP enzymes by PB, TUDCA and their metabolites. Inhibition of some enzymes was observed by PA (CYP1A2, CYP2B6, CYP2C19), UDCA (CYP2B6, CYP2C8, CYP2C9) and GUDCA (CYP2C8). Calculation of the systemic cut-off value for these metabolites is not possible because no protein binding data are available for the metabolites, or only a very wide range is given. However, in these cases, IC50/2 values were sufficiently above Cmax of PA (117 µM), UDCA (1.67 µM) and GUDCA (1.03 µM), assuming that protein binding will be at least 10% and therefore this inhibition is considered not likely to be clinically relevant. PAGN inhibited CYP2C19 with $IC_{50/2}$ = 69.5 µM. No C_{max} is available for PAGN, but if it would be approximately comparable to C_{max} of PA, inhibition of CYP2C19 by PAGN is possible. As the C_{max} of PAGN following multiple doses in Study A35-007 was approximately 143 µM (38 µg/mL), there is a potential for clinical interaction. An *in vivo* study is planned to investigate the inhibition of CYP2C19 by PAGN. It is agreed that this study could have been provided during the post-authorisation phase.

No inhibition by PB and TUDCA is expected of the hepatic uptake transporters OATP1B1 and OATP1B3 and the renal transporters OCT2, OAT3, MATE1 and MATE2-K. Potential inhibition of BSEP was not investigated initially by the applicant. During the assessment, it was found that TUDCA, GUDCA and PA were inhibitors of BSEP *in vitro*. Since no significant liver damage was observed in the clinical studies, this inhibition of BSEP is considered not clinically relevant. Inhibition of P-gp and BCRP by PB and TUDCA cannot be excluded because the cut-off values for intestinal concentrations (6445 µM for PB and 747 µM for TUDCA) were higher than the potential IC_{50} range in C2Bbe1 cells (between 1790 and 5371 µM for PB and between 222 and 667 µM for TUDCA). Moreover, in MDCK cells, the cut-off values were within the IC_{50} range for BCRP. In an *in vitro* study submitted by the applicant during the assessment, no inhibition of BCRP by PA, PAGN, UDCA and GUDCA was found. None of the test substances inhibited BCRP in this study, whereas BCRP was inhibited by PB and TUDCA in the study submitted initially by the applicant. However, in the new study submitted during the assessment, tested concentrations were lower than in the study in the first one. In the study submitted during the assessment, also inhibition of P-gp by UDCA and GUDCA was found. An *in vivo* study is planned to investigate the inhibition of P-gp by PB and TUDCA. It is agreed that this study could have been provided during the post-authorisation. Inhibition of OAT1 by PB and TUDCA is possible based on an *in vitro* study (the cut-off value for systemic exposure of both compounds [2262 µM for PB and 0.711 µM for TUDCA] was higher than the potential IC_{50} range [between 6.76 and 20.3 µM for PB and between 0.0741 and 0.222 µM for TUDCA]) and an *in vivo* study in rats. In an *in vitro* study submitted during the assessment, OAT1 was also inhibited by the metabolites PA and UDCA.

Pharmacodynamics

An overview of the PD properties of PB, TUDCA or fixed-dose combination AMX0035 was not provided.

Although a link to ALS is suggested, the mechanism of action of AMX0035 appears non-specific, acting at a final common pathway toward apoptosis. It was claimed that there is a synergistic activity of PB and TUDCA to reduce neuronal death; however, the non-clinical data have indicated that this is at best additive.

No studies or literature was provided with respect to the primary pharmacology of AMX0035. It is unclear how AMX0035 interacts with disease-specific aspects of ALS. Although PD parameters (e.g. pNF H levels, TSPO uptake in PET) have been included in phase II study AMX3500, their role in the pathophysiology in ALS is unclear. Moreover, the results are inconclusive (see results from phase II study).

The presented information on the PK/PD relationship of PB and TUDCA is poor. No conclusions can be drawn.

2.6.4. Conclusions on clinical pharmacology

There are no major issues regarding PK that can be considered as blocking. However, there are a number of concerns, referring to AMX0035 as substrate for BCRP and P-gp, inhibition of CYP2C9 by AMX0035 and inhibition of BCRP and P-gp by AMX0035 and also regarding long-term stability, clinical studies regarding renal and hepatic impairment and *in vivo* studies regarding AMX0035 as substrate for BCRP, P-gp and OATP1P3, inhibition of CYP3A4/5 and CYP2C19, and inhibition and induction of CYP2B6. Furthermore, the CSR of study A35-007 was still missing at the time of opinion. It is agreed that the planned studies regarding all issues mentioned above could have been submitted during the post-authorisation phase.

2.6.5. Clinical efficacy

The clinical development program for AMX0035 (3g sodium PB/1g TUDCA) for the treatment of ALS comprises a completed phase 2 study AMX3500 (CENTAUR) and an ongoing phase III study A35-004 (PHOENIX) (see Table 2).

Table 2: Overview of clinical studies[1]

Study ID **Phase** **Objective** **No. of study centres** **Study period**	**Design** **Duration**	**Study posology** **Subjects per arm entered/completed** **Study population**	**Efficacy endpoints**
AMX3500 Phase II Safety/efficacy 25 centres (US only) DB phase June 2017 – September 2019 OLE phase March 2018 – March 2021	RD DB PC: 24 weeks OLE: Up to 132 weeks	AMX0035[2]: 89/67 PBO: 48/38 ALS	Rate of decline ALSFRS-R Rate of decline ATLIS Plasma concentration of pNF-H Rate of decline SVC Survival and hospitalization[3]

A35-004 (recruitment completed[4]) Phase III Efficacy/safety 55 sites (US/EU)	RD DB PC 48 weeks DB + OLE	N=664 ALS	Change in slope ALSFRS-R and survival Rate of decline SVC ALSAQ-40 Decline in King's and MiToS Stages Ventilation Free Survival

ALSAQ-40= Amyotrophic Lateral Sclerosis Assessment Questionnaire, ALS= Amyotrophic Lateral Sclerosis, ALSFRS-R = ALS Functional Rating Scale – Revised, AMX0035= sodium phenylbutyrate/ursodoxicoltaurine, ATLIS= Accurate Test of Limb Isometric Strength, DB= double-blind, MiToS= Milano-Torino, PBO= placebo, OLE = open-label extension, PC= placebo-controlled, pNF-H= phosphorylated axonal neurofilament H subunit, RD= randomized, SVC = Slow Vital Capacity

[1]by Clinical Assessor

[2]AMX0035 was given one sachet once daily for the first 3 weeks of the study; if tolerated, the subject was on AMX0035 BID for the remainder of the study.

[3]Survival was defined as death, tracheostomy or permanent assisted ventilation, tracheostomy.

[4]According to the applicant's information submitted to EMA, the recruitment concluded in February 2023.

2.6.5.1. Dose response study(ies)

The applicant performed no dedicated dose-finding study to support the dosing regimen for AMX0035 in patients with ALS.

Both PB and taurursodiol administered individually have been evaluated in subjects with ALS and were found to be safe, well-tolerated, and exhibited preliminary signs of efficacy. PB was evaluated in a 20-week safety and biomarker study in ALS subjects [Cudkowicz 2009]. This was a Phase I dose-escalation trial, and each subject was scheduled to receive PB at an increasing dose from 9 to 21 g/day. A total of 40 subjects were recruited at 8 sites in the US. Twenty-six subjects completed the 20-week treatment phase. Histone acetylation was decreased by approximately 50% in blood buffy-coat specimens at screening and was significantly increased after PB administration. Blood levels of PB and the primary metabolite, PAA, increased with dosage with a plateau between the 3 and 6 grams t.i.d. regimens. While the majority of subjects tolerated higher dosages of PB, the lowest dose (9 g/day), was the most effective at increasing histone acetylation levels in blood. It is not clear why acetylation levels were highest at 9 g/day. However, the author noted that in a study of PB in Huntington's disease, the effects of PB on mRNA expression levels of a 12-gene biomarker set were greatest at the lowest dosages (4 g t.i.d.) with an inverse dose-response [Hogarth 2007]. In this study, tolerability was similar to that reported in other trials of PB in other indications. There were no changes in safety laboratory tests, EKG or vital signs. The most common adverse events (AEs) were those previously reported with PB, including falls, dizziness, diarrhoea, oedema, dry mouth, headache, nausea and rash. A single subject interrupted treatment with PB at the 9 g/day dose for the occurrence of oedema on the foot and under the eye.

Taurursodiol at 1g b.i.d. demonstrated a statistically significant slowing of ALSFRS-R progression rate in a year-long, multi-site, placebo-controlled clinical trial of ALS [Elia 2015]. In this proof-of-principle trial, 34 ALS subjects under treatment with riluzole were randomized to placebo or taurursodiol (1 g b.i.d.) for 54 weeks. The proportion of responders (defined as subjects with >15% improvement in ALSFRS-R slope) was higher under taurursodiol (87%) than under placebo ($P = 0.021$; 43%). At study end, baseline-adjusted ALSFRS-R was significantly higher ($P = 0.007$) in taurursodiol than in placebo groups. A comparison of the slopes of regression analysis showed slower progression in the taurursodiol than in the placebo group ($P < 0.01$). The AE profile and laboratory anomalies were not different between the taurursodiol and placebo cohort in this study. In the small group of 15 subjects treated with taurursodiol, the adverse events were limited to diarrhoea.

For the Phase 2 trial of AMX0035, a dose of 3 grams of PB and 1 gram of taurursodiol twice a day (6 grams PB per day and 2 grams taurursodiol per day) was selected as these doses had previously demonstrated the desired pharmacologic effect with an acceptable safety profile when administered individually.

2.6.5.2. Main study(ies)

The applicant has completed a phase II study (AMX3500) to support the efficacy and safety of AMX0035 in the treatment of ALS in the context of a CMA.

Study AMX3500

Methods

Study AMX3500 is a phase II randomized, double-blind, placebo-controlled study that evaluated AMX0035 in adults with ALS. The study consisted of an initial 24-week, double-blind, placebo-controlled treatment phase with an open-label extension (OLE) phase. The data cut-off for the survival analysis of the OLE was 01 March 2021.

- **Study Participants**

The relevant inclusion criteria were:

1. Male or female, aged 18 to 80 years of age
2. Definite diagnosis of sporadic or familial ALS as defined by the World Federation of Neurology revised El Escorial criteria (i.e., clinical evidence alone by the presence of UMN, as well as LMN, signs of neurodegeneration in at least 3 of 4 regions [i.e., brainstem (bulbar cranial motor neurons), cervical, thoracic, and lumbosacral spinal cord (anterior horn motor neurons)] of the CNS)
3. Less than or equal to 18 months since ALS symptom onset. The date of ALS symptom onset was defined as the date the subject first had symptoms of their disease, e.g., weakness.
4. Slow Vital Capacity (SVC) >60% of the predicted value for gender, height, and age at the Screening Visit
5. Subjects must either not take riluzole or be on a stable dose of riluzole for at least 30 days prior to the Screening Visit. Riluzole-naïve subjects were permitted in the study.
6. Women of child-bearing potential must have agreed to use adequate birth control for the duration of the study and 3 months after the last dose of the study drug. Likewise, men must have agreed to practice contraception for the duration of the study and 3 months after the last dose of the study drug

The relevant exclusion criteria were:

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

7. Pregnant women or women currently breastfeeding
8. History of cholecystectomy
9. Biliary disease which impedes biliary flow including active cholecystitis, primary biliary cirrhosis, sclerosing cholangitis, gallbladder cancer, gallbladder polyps, gangrene of the gallbladder, abscess of the gallbladder.
10. History of Class III/IV heart failure (per New York Heart Association)
11. Severe pancreatic or intestinal disorders that may alter the enterohepatic circulation and absorption of taurursodiol, including biliary infections, pancreatitis and ileal resection
12. Subjects who have cancer with the exception of the following: basal cell carcinoma or successfully treated squamous cell carcinoma of the skin; cervical carcinoma *in situ*; prostatic carcinoma *in situ*; or other malignancies curatively treated and with no evidence of disease recurrence for at least 3 years.
13. Active participation in an ALS clinical trial evaluating an experimental small molecule within 30 days of the Screening visit.
14. Exposure at any time to any cell therapies and gene therapies under investigation for the treatment of subjects with ALS (off-label use or investigational)
15. Exposure to monoclonal antibodies under investigation for the treatment of ALS (offlabel use or investigational) within 90 days from Screening.
16. Implantation of Diaphragm Pacing System (DPS)
17. Exposure to any disallowed medications listed below:
    a. Subjects who participated in the mexiletine trial for ALS within the last 30 days prior to screening were excluded from the trial because it was possible that co-administration of PB and mexiletine would increase a subject's exposure to mexiletine given that, at 20 times the intended clinical concentration (C_{max}), the principal metabolite of PB, phenylacetylacetate, was shown to be inhibitory to the major enzymes responsible for the breakdown of mexiletine, CYP 1A2 and CYP 2D6. Subjects who were using mexiletine at a dosage less than or equal to 300 mg/day for cramps and fasciculations were not excluded,but were to be monitored for mexiletine-associated AEs, reducing the dosage of mexiletine if needed.
    b. The use of antacids containing aluminum hydroxide or aluminum oxide within 2 hours of administration of AMX0035 was also prohibited as they could inhibit absorption of taurursodiol

- **Treatments**

In the double-blind, main study, subjects were randomized to receive either active treatment (AMX0035) or matching placebo administered orally.

For the first 3 weeks of dosing, subjects were instructed to take 1 sachet daily in the morning (i.e., half-dose, equivalent to 3 g PB and 1 g taurursodiol for subjects randomized to AMX0035). If sufficient tolerability was demonstrated after 3 weeks of treatment, subjects were instructed to increase the dose to 2 sachets daily taken as a BID regimen, 1 in the morning and 1 in the evening (i.e., full-dose, equivalent to 6 g PB and 2 g taurursodiol for subjects randomized to AMX0035). This safety feature consisting of intra-patient dose escalation with a 3-week lead-in period was implemented to confirm initial safety of taurursodiol in patients with ALS as the dose regimen of taurursodiol selected for this

trial was higher than the approved doses for taurursodiol in Europe (Tudcabil®) or ursodiol in the US (URSO®) for treatment of patients with primary biliary cirrhosis.

Method of administration

Subjects were instructed to add the contents of the sachet into a cup or other container and then add approximately 8 oz. (1 cup) of room temperature water and stir vigorously. Study medication may have required significant stirring or gentle crushing to dissolve. Study medication was to be taken within 1 hour of mixing into water.

Subjects were alerted to the fact study medication had a strong bitter taste. Subjects were instructed that there were ways to make the taste more palatable.

- **Objectives**

Primary objectives:

1. Confirm the safety and tolerability of a fixed-dose combination of PB and ursodoxicoltaurine in subjects with ALS over a 6-month period
2. Measure the impact of a fixed-dose combination of PB and ursodoxicoltaurine using the slope of progression with the ALSFRS-R.

Secondary objectives:

3. Assess the impact of AMX0035 on the rate of decline of isometric muscle strength, as measured by Accurate Test of Limb Isometric Strength (ATLIS)
4. Assess the impact of AMX0035 on disease progression as measured by SVC decline, time to tracheostomy, and survival
5. Assess the impact of AMX0035 on biomarkers, including phosphorylated axonal neurofilament H subunit (pNF-H) levels and 18 kDa translocator protein (TSPO) uptake
6. Develop concentration-response models of ursodoxicoltaurine and PB at steady-state after administration of AMX0035 sachet twice daily
7. Measure the impact of AMX0035 on survival

- **Outcomes/endpoints**

Primary endpoint

1. The rate of decline (slope of decline) in the total ALSFRS-R score.

Secondary endpoints

2. The impact of AMX0035 on the rate of decline of isometric muscle strength, as measured by the **ATLIS**
3. The impact of AMX0035 on marker of neuronal death, plasma concentration of **pNF-H**
4. The impact of AMX0035 on measure of lung function, as measured by **SVC** decline
5. The impact of AMX0035 on rates of **survival** (defined as death, tracheostomy or Permanent Assisted Ventilation (PAV), tracheostomy), and hospitalization
6. The **concentration-response** model of PB and taurursodiol at steady-state after administration of AMX0035 4 g BID

7. The impact of AMX0035 on TSPO uptake measured **by positron emission tomography (PET) scan**.

The **ALSFRS-R** is a validated measure of clinical function that is correlated with clinical progression and survival in patients with ALS and shows strong internal consistency and construct validity. Initial validity was established by documenting that in ALS patients, change in ALSFRS-R scores correlated with change in muscle strength and lung function (SVC%) over time and predicted survival (Cedarbaum 1999, Kaufmann 2005). With appropriate training, the ALSFRS-R can be administered with high inter-rater reliability and test-retest reliability (Cedarbaum 1999). The ALSFRS-R is a quickly administered (5 minutes) ordinal rating scale (ratings 0 to 4) used to determine patients' assessment of their capability and independence in 12 functional activities relevant to ALS. Higher scores indicate better performance, and the maximum score is 48 points. Based on a survey conducted among ALS experts, it was reported in 2010 that a change of 20% or greater in the slope of the ALSFRS-R would be considered as clinically meaningful by most ALS clinicians. This definition is particularly limited when considering that a 20% change in slope represents different levels of functional change across the scale due to its scoring structure. Additionally, decline in the ALSFRS-R score is often assumed to be linear for the purposes of statistical analysis, but in reality, the scale declines in a curvilinear manner.

The ALSFRS-R can be broken down into 4 domains as described below:

- Bulbar, comprised of 3 questions assessing: speech, salivation, swallowing
- Fine Motor, comprised of 3 questions assessing: handwriting, cutting food and handling utensils (for both patients with and without gastrostomy), dressing and hygiene
- Gross Motor, comprised of 3 questions assessing: turning in bed, walking, climbing stairs
- Breathing, comprised of 3 questions assessing: dyspnea, orthopnea, respiratory Insufficiency

Isometric strength was measured using the **ATLIS** device developed by Dr. Patricia Andres of Massachusetts General Hospital (Andres 2012). The device was specifically designed to alleviate the reproducibility concerns that existed for prior strength measurements such as hand-held dynamometry. The ATLIS device measures isometric strength in 6 upper (right and left grip strength, elbow extension and flexion) and 6 lower (right and left ankle dorsiflexion, knee extension, and knee flexion) extremity muscle groups with a high degree of reproducibility using a fixed load cell and a wireless dynamometer with standard positions, rather than relying on examiner strength (Andres 2012). Two attempts of each manoeuvre were performed during every assessment, adding a third attempt if the first 2 differed by more than 15%. The analysis used the highest score from all attempts of a given manoeuvre at each assessment. If a subject was too weak to perform 1 of the ATLIS limb tests, this was noted in the case report form (CRF), and the strength in this limb was considered to be 0% (i.e., it was not considered a missing data point). Raw values were standardized to percent predicted normal (PPN) strength based on sex, age, weight, and height (Andres 2013).

As degenerating neurons release **pNF-H** into the CSF and, subsequently the blood, elevated plasma levels of pNF-H are presumed to correlate with neuronal injury (Poesen 2019, Henninger 2016, Cudkowicz 2014). For this reason, blood samples were drawn over multiple time points with the intention of generating a longitudinal dataset correlating neurofilament levels to observed clinical outcomes.

Vital capacity was determined using the **upright SVC** method. Three trials were required for each testing session; however, up to 5 trials could be performed if the variability between the highest and second highest SVC was 10% or greater for the first 3 trials. Only the 3 best trials were recorded on the CRF. The highest SVC recorded was utilized for analysis, regardless of the number of trials performed. SVC volumes were standardized to PPN based on age, sex, and height (Knudson 1983).

Survival was defined as death, tracheostomy (irrespective of the reason for tracheostomy, whether respiratory distress or control of mucus secretion) or PAV (defined as more than 22 hours daily of non-invasive mechanical ventilation for more than 1 week/7 days). The date of onset of PAV was the first day of the 7 days. The date of death was collected for subjects in the course of the trial. In addition, the protocol allowed for monitoring of death after a subject elected not to enter the OLE or were terminated at any time during follow-up and information on whether a subject had died could be obtained by the subject's family, clinic notes or utilizing public means such as a reliable internet source such as the Centers for Disease Control and Prevention (CDC) National Death Index (http://www.cdc.gov/nchs/ndi.htm) or the Social Security Death Index (http://ssdmf.info/). In practice, a professional firm, Omnitrace, was contracted to conduct such a search using cutoff dates of 29 February 2020, 20 July 2020, and 01 March 2021 (final analysis) to determine the death status of all subjects.

A subset of consenting subjects (n=9) in the placebo-controlled main phase underwent **MR-PET scans** at the Baseline visit and again between the Week 12 and 21 study visits to evaluate brain uptake of TSPO, which is part of a multimeric protein complex associated with the outer mitochondrial membrane of many cells. Present in peripheral tissues and glia cells (astrocytes and microglia), TSPO is involved in many processes such as apoptosis, regulation of cellular proliferation, immunomodulation and steroidogenesis and may be a useful biomarker of inflammation in patients with ALS (Corcia 2012).

- **Sample size**

Subjects in the Pooled Resource Open-Access ALS Clinical Trials database (Atassi 2014) who had a definite El Escorial Diagnosis of ALS and were <540 days since symptom onset were found to progress considerably faster than the overall ALS population. An initial shared-baseline, mixed-effects analysis using these criteria in a different database (ceftriaxone) with a 2:1 subject randomization between treatment and placebo indicated that approximately 131 subjects followed over 6 months would provide 80% power to detect a 30% treatment effect when tested at a two-sided alpha of 0.1. Covariates were added to improve the model's ability to fit the planned study population and have higher power.

- **Randomisation and Blinding (masking)**

Subjects were randomly assigned in a 2:1 ratio to receive AMX0035 or matching placebo. Sites did not hold inventory for multiple patients on-site, instead, adequate drug supplies for a single patient to complete the study were shipped at the time a successful Screening visit was completed. As such, the order of shipments defined the randomization for the study.

The study was conducted in a double-blind fashion. To maintain the blind, a matched placebo was used as the control for the study. Denatonium benzoate granules and salt were added to the placebo formulation to match the salty, bitter taste of AMX0035.

- **Statistical methods**

According to the applicant, the primary estimand, as requested during the review, of the study was:

> In patients who were within 18-months of symptom onset with UMN and LMN signs in at least three body areas ('definite' ALS) and who would live at least 24-weeks, the difference in the mean rate of functional progression as measured by the ALSFRS-R for patients receiving AMX0035 as compared to a matching placebo over 24-weeks of follow-up regardless of treatment discontinuation.

A supplementary estimand, incorporating mortality in the analysis, was requested as follows:

> In patients who were within 18-months of symptom onset with upper and lower motor neuron signs in at least three body areas ('definite' ALS) and in a population enriched to survive at least

24-weeks, the difference in a composite outcome of mortality and function at 24-weeks of follow-up, wherein deaths are considered equivalent to maximum functional progression.

The Intent-to-Treat (ITT) population included all subjects who received at least 1 dose of study medication. Subjects in the ITT Population were analyzed based on the study medication they received. The modified ITT (mITT) population included all subjects who received at least 1 dose of study medication and had at least 1 post-baseline total ALSFRS-R score, subjects were analyzed based on the study medication they received.

The per-protocol (PP) Population included all mITT subjects who took the assigned study medication per the study protocol and did not have any major protocol deviations, which excluded them from the PP analysis.

The Safety Population included all subjects who received at least 1 dose of study medication. Subjects included in the Safety Population were analyzed based on the actual study medication they received.

The primary population for efficacy analysis was the mITT Population. Unless otherwise specified, efficacy analyses, including analysis of primary and secondary efficacy endpoints, were performed on both the mITT and PP Populations. A *post hoc* analysis also evaluated efficacy outcomes for the ITT population.

The primary efficacy measure for the study was the rate of decline (slope of decline) in the total ALSFRS-R score. A shared-baseline, mixed-effects analysis compared the placebo and active (AMX0035 treatment) arms. Covariates of age, rate of disease progression prior to entering the study (i.e., ΔFS [del-FS]), and rate of disease progression prior to entering the study of the efficacy outcome of interest (other than ALSFRS-R) interacting with time were included in the respective analysis.

Historical analyses have shown that del-FS is a strong predictor of future disease progression since ALS has a linear disease progression (Karanevich 2018). Del-FS is derived from baseline ALSFRS-R score and time since ALS symptom onset and is a measurement of decline in the subject since symptom onset. The del-FS calculation was made at the Baseline visit and calculated as the decrease in ALSFRS-R from 48 divided by the time in months from symptom onset.

The mixed-effects model used, that accounted for both the variance between subjects and the deviation within subjects from their average rate of decline, was as follows:

$$Y_{i,} = \mu + u_i + (\beta_0 + b_i) \times t + \beta_1 \times z_i \times t + \beta_2 \times Age_i \times t + \beta_3 \times DelFS_i \times t + \beta_4 \times DelY_i \times t + \varepsilon_{i,}$$

where:

- i represents the i^{th} subject, i ranges from 1 to the number of subjects in the mITT population;
- t represents the actual time in weeks of each observation, time since baseline assessment;
- $Y_{i,t}$ is the dependent variable observed at time t, i.e., the actual efficacy score at time t
- z is a treatment indicator which is 0 in the control group and 1 in the treatment group;
- u_i is the random intercept for each subject and has an unspecified bivariate normal distribution;
- b_i is the random slope in the efficacy outcome for each subject over time and has an unspecified bivariate normal distribution;
- Age_i x t is the interaction representing the effect of age on progression over time. It is expected that older subjects will decline faster;
- $DelFS_i$ x t is the interaction representing the effect of previous progression measured by ALSFRS-R on progression over time. It is expected that subjects who were progressing quickly since symptom onset will continue to progress quickly;

- $DelY_i$ x t is the interaction representing the effect of previous progression measured by the efficacy outcome of interest (response variable) on progression over time. It is expected that subjects who were progressing quickly since symptom onset will continue to progress quickly;
- μ is the estimated intercept of the efficacy outcome across all subjects;
- β0 is the estimated slope for time;
- β1 is the estimated slope for treatment;
- β2 is the estimated slope for age at baseline (years);
- β3 is the estimated slope for del-FS;
- β4 is the estimated slope for del-efficacy (corresponding to Yi,t); and
- ε_i is the random error which shows the amount by which the observed value differs from its expected value.

In order to confirm linearity, the model described above was modified to include quadratic terms for time in a sensitivity analysis. If the quadratic terms for time were insignificant (p-values >0.10), then linearity was assumed, and the linear primary model was used for analysis.

The difference in active treatment and placebo slope were calculated in addition to a p-value for the comparison and a 95% confidence interval (CI) for the estimated difference. Least-squares (LS) means and standard errors were estimated for active treatment and placebo at each scheduled time point for the mean level of baseline covariates across all subjects included in the analysis.

Retention of function is a calculation to determine how much longer patients on active therapy stayed at a given functional level as compared to patients receiving placebo. For example, if patients in the AMX0035 had the same ALSFRS-R score at the end of the study (24 weeks) as patients at Week 17.9 in the placebo arm, a 6.1-week difference, the retention of function would be calculated as 6.1 weeks divided by 17.9 weeks or an ~34% longer retention of function compared with patients randomized to placebo.

As a **sensitivity analysis**, the primary analysis was repeated using the left-censored values for all ALSFRS-R, ATLIS, and SVC. In this analysis, all values that were censored by an intercurrent event of death and death equivalent events were assumed to be lower than all observed values.

A sensitivity analysis was performed to assess if the results were missing at random (MAR) or missing not at random (MNAR). This model imputed missing data for all subjects who discontinued for any reason and is referred to as the Multiple Imputation Model for MNAR. For this model, the imputed values for the placebo arm were imputed on their linear trajectory (with error), and imputed values for the active arm were imputed on their linear trajectory after subtracting out the difference in average slope between the active and placebo groups.

Two medications of interest could be taken during the clinical trial: edaravone and riluzole. Efficacy outcomes were analyzed by comparing efficacy scores over time between treatment groups while accounting for time on concomitant medications of interest.

ALSFRS-R responders were defined as those ALS patients who met the following criteria: subjects whose actual change from baseline in the ALSFRS-R at Week 18 is less or equal to their own ΔFS. Early drop-outs were included as non-responders (i.e., dead prior to Week 18 or withdrawn from study prior to Week 12).

Survival analyses were performed using a Cox proportional hazards model with covariates of del-FS and age at baseline. There were 3 survival outcomes: 1) death, 2) tracheostomy, and 3) PAV. In addition, a

combined survival analysis was performed where any 1 of the 3 events was considered a failure, and the time for the first occurrence of any of the 3 events analyzed. This combined survival analysis was referred to as time to "Death or Equivalent." For the survival analyses, death of subjects who dropped out of the study, but died within the original study window (i.e., 24 weeks after baseline), were included.

A hierarchical analysis plan was used to **control type I error** for secondary outcomes:

- The impact of AMX0035 on the rate of decline of isometric muscle strength, as measured by the ATLIS;
- The impact of AMX0035 on the marker of neuronal death, the plasma concentration of pNF-H;
- The impact of AMX0035 on the measure of lung function, as measured by SVC decline;
- The impact of AMX0035 on rates of survival (defined as death, tracheostomy or PAV, tracheostomy), and hospitalization
- The concentration-response model of PB and taurursodiol at steady-state after administration of AMX0035 4 g BID;
- The impact of AMX0035 on TSPO uptake measured by positron emission tomography (PET) scan.

OLE analyses

Efficacy analyses have 2 study arms, which are referred to in this CSR as the following:

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

All analyses defined in the original Statistical Analysis Plan (SAP) for the main study were also performed for the OLE. Two groups of subjects were compared, those who had previously been in the placebo group of the main study (RP) and those who received AMX0035 in the main study (RA). The following evaluations were conducted:

1. Slope over the entire duration of the main study and the first 24 weeks of the extension study period was assessed using the original model from the main study SAP, but extended throughout the observation time of the OLE study. This analysis assesses the sustainability of the effect rather than the effect of crossing over from placebo to active treatment. This is called the "Extended Slope Analysis".
2. The first 6 months of active treatment data was pooled for subjects in the RA and RP groups who received treatment during the OLE phase. This pooled group was compared to the placebo arm from the main study. An assessment of the relationship between the covariates and the slope over time was performed to make sure that the range of covariate values for the 2 active groups overlap sufficiently to allow the inclusion of both groups in the same model. The second phase of the active arm could also be included in this model as a third treatment arm resulting in a comparison of these 3 arms: placebo, first 6 months of treatment and second 6 months of treatment. This is called the "Pooled (24-week) Crossover Analysis".
3. The slope during the extension was compared to the slope during the main study to determine whether the change from the double-blind phase to the OLE phase differed between treatment groups. This is called the "Change (Δ placebo slopes) vs Change (Δ AMX0035 slopes) Crossover Analysis".

Within-group comparisons of the difference in slope between the extension phase and the main study were performed for subjects who received placebo to determine whether initiation of treatment with AMX0035 influenced the placebo slope and for subjects who received AMX0035 during the main study to

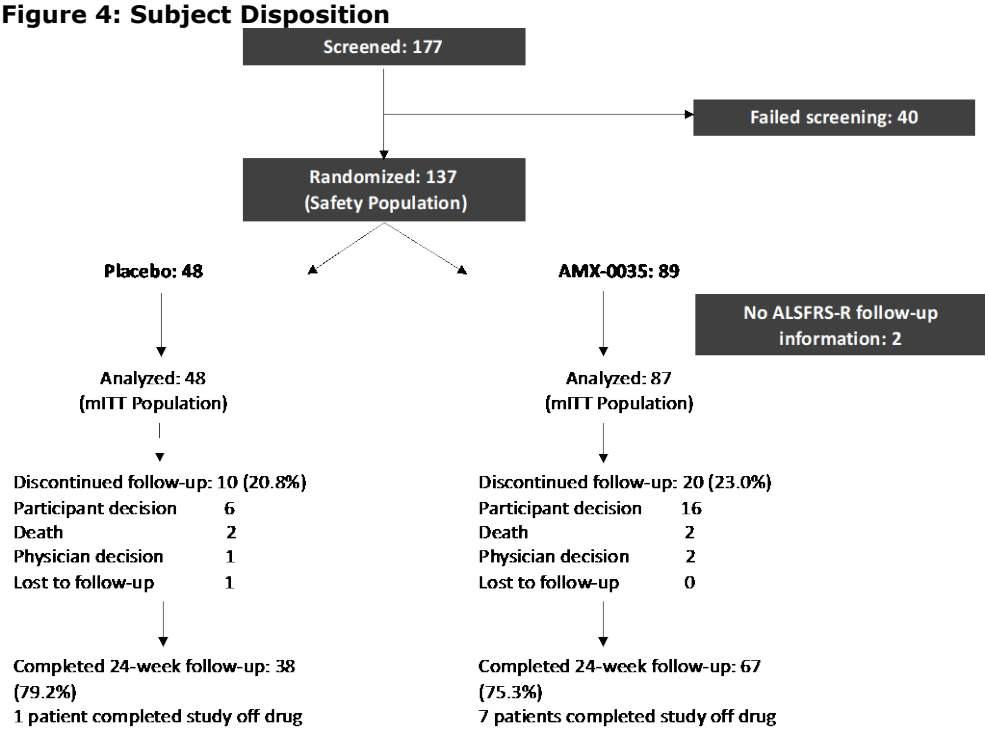
determine robustness and extent of the duration of effect. This is called the "Within Group Crossover Analysis".

Results

- **Participant flow**

A total of 177 subjects with ALS were screened for participation in study AMX3500, and of these, 137 were enrolled, randomized, and received treatment with study medication (i.e., either AMX0035 [89 subjects] or placebo [48 subjects]) at 25 investigational study centres (Figure 4).

Figure 4: Subject Disposition



ALSFRS-R=ALS Functional Rating Scale-Revised; ITT=intent to treat; mITT=modified intent to treat. Note: Death = death or death equivalent (includes tracheostomy or PAV). Some deaths were recorded after subject withdrawal from trial and are not accounted in the reason for discontinuation.

Of the 137 subjects enrolled, 105 (76.6%) completed the 24-week double-blind study. Ninety-seven of the 105 subjects completed the study on study medication, and 8 subjects completed the 24-week double-blind period but discontinued study medication early. The remaining 32 (23.4%) subjects prematurely discontinued the study, and discontinuations were balanced across treatment groups, with 10 (20.8%) and 22 (24.7%) in the placebo and AMX0035 groups, respectively. The most common reason for study discontinuation was subject withdrawal from the study (Table 3).

In the AMX0035 group, 79 (88.8%) subjects were able to increase the dose from 1 to 2 sachets after 3 weeks of treatment, 10 subjects (11.2%) remained on 1 sachet. In the placebo group, 45 subjects (93.8%) were able to increase the dose to 2 sachets, 3 subjects (6.3%) remained on 1 sachet.

Table 3: Subject Disposition – All Populations

	Safety Population			mITT Population			Per Protocol Population		
	Treatment Group								
	Placebo + SOC (N=48)	AMX0035+ SOC (N=89)	Overall (N=137)	Placebo + SOC (N=48)	AMX0035 + SOC (N=87)	Overall (N=135)	Placebo + SOC (N=48)	AMX0035 + SOC (N=86)	Overall (N=134)
Study Disposition (n [%])									
No. of Subjects who Completed the Study	38 (79.2)	67 (75.3)	105 (76.6)	38 (79.2)	67 (77.0)	105 (77.8)	38 (79.2)	67 (77.9)	105 (78.4)
Completed the Study on Study Medication	37 (77.1)	60 (67.4)	97 (70.8)	37 (77.1)	60 (69.0)	97 (71.9)	37 (77.1)	60 (69.8)	97 (72.4)
Discontinued Study Medication Prior to Study Completion	1 (2.1)	7 (7.9)	8 (5.8)	1 (2.1)	7 (8.0)	8 (5.9)	1 (2.1)	7 (8.1)	8 (6.0)
P-value for Group Comparison, Fisher's Exact Test	0.2533			0.2533			0.2533		
No. of Subject who Discontinued the Study	10 (20.8)	22 (24.7)	32 (23.4)	10 (20.8)	20 (23.0)	30 (22.2)	10 (20.8)	19 (22.1)	29 (21.6)
P-value for Group Comparison, Fisher's Exact Test	0.6761			0.8317			>0.9999		
Reason for Study Discontinuation (n[%])									
Withdrawal by Subject	6 (12.5)	17 (19.1)	23 (16.8)	6 (12.5)	16 (18.4)	22 (16.3)	6 (12.5)	15 (17.4)	21 (15.7)
Adverse Event	3 (6.3)	11 (12.4)	14 (10.2)	3 (6.3)	10 (11.5)	13 (9.6)	3 (6.3)	9 (10.5)	12 (9.0)
Disease Progression	2 (4.2)	5 (5.6)	7 (5.1)	2 (4.2)	5 (5.7)	7 (5.2)	2 (4.2)	5 (5.8)	7 (5.2)
Termination of Participation by Subject	1 (2.1)	1 (1.1)	2 (1.5)	1 (2.1)	1 (1.1)	2 (1.5)	1 (2.1)	1 (1.2)	2 (1.5)
Other	0	0	0	0	0	0	0	0	0
Subject Enrolled in Another Trial	0	0	0	0	0	0	0	0	0
Subject Perceived Lack of Efficacy	0	0	0	0	0	0	0	0	0
Travel Difficulties	0	0	0	0	0	0	0	0	0
Death	2 (4.2)	3 (3.4)	5 (3.6)	2 (4.2)	2 (2.3)	4 (3.0)	2 (4.2)	2 (2.3)	4 (3.0)
Subject Died[a]	1 (2.1)	3 (3.4)	4 (2.9)	1 (2.1)	2 (2.3)	3 (2.2)	1 (2.1)	2 (2.3)	3 (2.2)
Subject Met Death Equivalent[b]	1 (2.1)	0	1 (0.7)	1 (2.1)	0	1 (0.7)	1 (2.1)	0	1 (0.7)
Physician Decision	1 (2.1)	2 (2.2)	3 (2.2)	1 (2.1)	2 (2.3)	3 (2.2)	1 (2.1)	2 (2.3)	3 (2.2)
Termination of Participation by Site Investigator	1 (2.1)	2 (2.2)	3 (2.2)	1 (2.1)	2 (2.3)	3 (2.2)	1 (2.1)	2 (2.3)	3 (2.2)
Termination of Participation by Study Sponsor	0	0	0	0	0	0	0	0	0
Lost to Follow-up	1 (2.1)	0	1 (0.7)	1 (2.1)	0	1 (0.7)	1 (2.1)	0	1 (0.7)

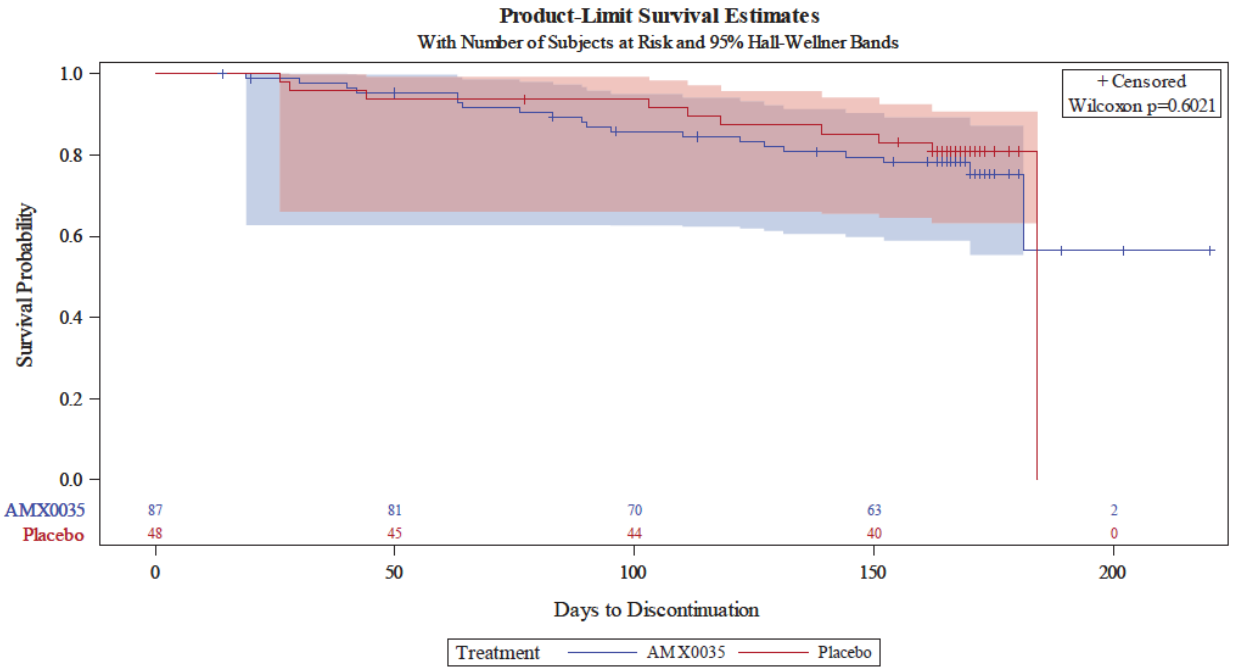
Abbreviations: mITT = modified Intent-to-Treat population; No. = number; SOC = standard of care.

[a] A total of 7 patients (2 who received placebo and 5 who received AMX0035) died during the 24-week study. However, 3 patients (1 who received placebo and 2 who received AMX0035) discontinued participation prior to death; therefore, death is not listed as their reason for discontinuation

[b] Death equivalent defined as requiring tracheostomy or permanent assisted ventilation.

Time to study discontinuation is illustrated in Figure 5 below for discontinuations due to any reason, due to AEs, and due to withdrawal by subject.

Figure 5: Time to Study Discontinuation (All Reasons): Safety Population (N=137)



- **Recruitment**

Date first subject enrolled: 22 June 2017 for double-blind treatment phase: 30 June 2018

Date last subject completed: 25 September 2019

Study AMX3005 included 25 centres in the United States.

- **Conduct of the study**

The initial protocol was finalized on 18 Nov 2016 (Version 1.0). The Protocol was amended 5 times over the course of the study. A summary of substantive changes made to the protocol with each protocol amendment is as follows:

- Protocol Version 2.0 dated 25 Jul 2017:
    - During the baseline visit, instructions related to study drug administration were added to provide strategies to improve tolerance of the bitter taste for the subjects.
- Protocol Version 3.0 dated 20 Oct 2017:
    - To allow concomitant administration of edaravone following its approval in the US
    - Added administration of the ALSFRS-R questionnaire at study visits for the MR-PET sub-study
    - Added an OLE study to follow the 24-week main treatment study
- Protocol Version 4.0 dated 16 May 2018:
    - Added blood draw (at baseline or subject's next visit) for deoxyribonucleic acid (DNA) extraction for genome sequencing
- Protocol Version 5.0 dated 06 Sep 2018:
    - Specified the laboratory tests to be performed for safety assessment during the OLE study

- Modified the definition of an AE in the OLE study so that it was consistent with the definition in the 24-week double-blind study.

- Protocol Version 6.0 dated 11 Jan 2019:
  - Clarified that the one-time blood sample collection for DNA extraction for genomic sequencing was optional and that, if the Baseline visit had passed or the sample had not been collected at Baseline, the sample may have been collected at a subsequent study visit.
  - Expanded the duration of the OLE study (including open-label AMX0035 treatment) from 52 to 132 weeks.
  - Revised the primary outcome measure for the OLE study to confirm the long-term safety of AMX0035 in subjects with ALS over 132 (not 52) weeks.
  - Opened enrollment in the MR-PET sub-study to all investigational study centres (not just NCRI at Massachusetts General Hospital [Boston, MA]).
- Protocol Version 7.0 dated 30 Mar 2020:
  - Updated to include how the drug is dispensed to subject to include the use of the clinical supply chain courier company
  - Updated to include assessment of compliance when remote study visit occurs, and to clarify that missed procedures due to remote study visits are not considered protocol deviations
  - Updated the Schedule of Activities to clarify what procedures should be performed at Week 68 and Week 84
  - Remote study visits were added as an option to the SOA in response to the COVID-19 pandemic
  - Updated section to include the requirements needed for subjects to continue on study drug, in response to the COVID-19 pandemic.

Changes in planned analyses

Changes (prior to unblinding) from the planned statistical analyses outlined in the SAP are summarized below:

- Time to discontinuation overall and by reason were to be analyzed with a Gehan-Wilcoxon test, and the corresponding Kaplan-Meier Plots were displayed. Subjects discontinuing for one of the other reasons were to be censored, and "time to event" was used. However, this analysis was not performed.
- Considering the number of subjects excluded from the PP Population at various time points, summaries of study medication exposure, compliance and tolerability were expanded to include the PP Population, in addition to the planned Safety and mITT Populations.
- The incidence and time to first hospitalization (for placement of feeding tube, infusion port for edaravone, or related to an SAE) were evaluated as a survival analysis as an independent end-point (and also as a combined end-point with survival).
- A summary of Grade 3 and higher AEs by MedDRA system organ class and Preferred Term for the Safety Population was not generated as AEs were not assigned a grade; rather, a summary of AEs by severity, MedDRA system organ class and the preferred term was generated.

- For completeness of review, a summary tabulation of all AEs for subjects who died during the course of the study, or within the 24-week follow-up period, was generated for the Safety Population.

After breaking the study blind, the following *post hoc* analyses were also performed:

The first *post hoc* sensitivity model was a *post hoc* joint rank model in the efficacy (mITT) population that incorporated all survival events into the analysis of function (ALSFRS-R), providing adjusted estimates that accounted for potential bias due to subject death (Berry 2013). The model ranked subjects by time to death and then by the change in ALSFRS-R total score. This ranked score was then analyzed as the outcome of an analysis of the covariance model that included the same covariates as the primary model, but replaced the covariates with ranked covariates.

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

A *post hoc* analysis of the ITT population was performed, including 2 subjects in the active group who did not undergo a post-baseline efficacy assessment and were excluded from the mITT population.

A *post hoc* analysis was also performed to estimate the progression rates of subgroups of patients defined by any use of riluzole, edaravone or a combination of these medications. This analysis was added as a potentially more informative method to analyze concomitant medication effects. The use of these medications was adjusted as an indicator variable to estimate the progression rates of these subgroups. This allowed for a comparison of progression in each of the following subgroups: edaravone only, riluzole only, edaravone or riluzole, and edaravone and riluzole.

Protocol deviations

The percentage of subjects with protocol deviations was similar between treatment groups; 82 (92.1%) of subjects in the AMX0035-treatment group had a total of 279 documented deviations while 43 (89.6%) subjects in the placebo group had a total of 138 documented deviations. Overall, the protocol deviations that occurred were not felt to have a material impact on the scientific integrity of the study or overall report conclusions. The majority of protocol deviations were classified as minor; only 21 deviations in 19 (13.9%) subjects overall were classified as major (12.5% of placebo subjects and 14.6% of AMX0035 subjects).

- **Baseline data**

The subjects in the mITT population of study AMX3500 were predominantly white (94.8%) males (68.9%) with a mean (SD) age at enrolment of 57.5 (9.50) years (Table 4).

Table 4: AMX3500 Demographic and General Baseline Characteristics – All Populations

	ITT Population			**mITT Population**			**Per Protocol Population**		
	Placebo + SOC (N=48)	**AMX0035 + SOC (N=89)**	**Overall (N=137)**	**Placebo + SOC (N=48)**	**AMX0035 + SOC (N=87)**	**Overall (N=135)**	**Placebo + SOC (N=48)**	**AMX0035 + SOC (N=86)**	**Overall (N=134)**
Gender (n [%])									
Male	32 (66.7)	61 (68.5)	93 (67.9)	32 (66.7)	61 (70.1)	93 (68.9)	32 (66.7)	60 (69.8)	92 (68.7)
Female	16 (33.3)	28 (31.5)	44 (32.1)	16 (33.3)	26 (29.9)	42 (31.1)	16 (33.3)	26 (30.2)	42 (31.3)
p-value vs. placebo	0.8495			0.7011			0.7032		

	ITT Population			mITT Population			Per Protocol Population		
	Placebo + SOC (N=48)	**AMX0035 + SOC (N=89)**	**Overall (N=137)**	**Placebo + SOC (N=48)**	**AMX0035 + SOC (N=87)**	**Overall (N=135)**	**Placebo + SOC (N=48)**	**AMX0035 + SOC (N=86)**	**Overall (N=134)**
Age at Enrollment									
n	48	89	137	48	87	135	48	86	134
Mean (SD)	57.3 (7.56)	57.9 (10.57)	57.7 (9.60)	57.3 (7.56)	57.6 (10.45)	57.5 (9.50)	57.3 (7.56)	57.5 (10.50)	57.4 (9.52)
Median	57.5	60.0	59.0	57.5	59.0	59.0	57.5	59.0	59.0
p-value vs. placebo	0.7156			0.8638			0.8879		
Race (n [%[)									
White	46 (95.8)	84 (94.4)	130 (94.9)	46 (95.8)	82 (94.3)	128 (94.8)	46 (95.8)	81 (94.2)	127 (94.8)
Asian	1 (2.1)	2 (2.2)	3 (2.2)	1 (2.1)	2 (2.3)	3 (2.2)	1 (2.1)	2 (2.3)	3 (2.2)
Black or African American	1 (2.1)	2 (2.2)	3 (2.2)	1 (2.1)	2 (2.3)	3 (2.2)	1 (2.1)	2 (2.3)	3 (2.2)
Unknown	0	1 (1.1)	1 (0.7)	0	1 (1.1%)	1 (0.7)	0	1 (1.2)	1 (0.7)
Race Group (n [%[)									
White	46 (95.8)	84 (94.4)	130 (94.9)	46 (95.8)	82 (94.3)	128 (94.8)	46 (95.8)	81 (94.2)	127 (94.8)
Other[a]	2 (4.2)	5 (5.6)	7 (5.1)	2 (4.2)	5 (5.7)	7 (5.2)	2 (4.2)	5 (5.8)	7 (5.2)
p-value vs. placebo	>0.9999			>0.9999			>0.9999		
Ethnicity (n [%[)									
Hispanic or Latino	1 (2.1)	6 (6.7)	7 (5.1)	1 (2.1)	6 (6.9)	7 (5.2)	1 (2.1)	6 (7.0)	7 (5.2)
Not Hispanic or Latino	47 (97.9)	83 (93.3)	130 (94.9)	47 (97.9)	81 (93.1)	128 (94.8)	47 (97.9)	80 (93.0)	127 (94.8)
BMI at Enrollment(kg/m2)									
n	48	89	137	48	87	135	48	86	134
Mean (SD)	26.4 (5.81)	26.9 (4.39)	26.7 (4.92)	26.4 (5.81)	26.9 (4.42)	26.7 (4.94)	26.4 (5.81)	26.9 (4.45)	26.7 (4.96)
Median	25.3	26.8	26.5	25.3	26.8	26.5	25.3	26.8	26.5
p-value vs. placebo	0.5864			0.5546			0.5598		

BMI=body mass index; ITT=intent to treat; mITT=modified intent to treat; SOC=standard of care; Note: Percentages are based on the number of subjects with non-missing data in each treatment group and overall. P-values comparing treatments are presented; T-test was used for quantitative variables and Fisher's exact test was used for qualitative variables.
[a] Other race includes Asian, Black or African American, and Unknown.

Disease characteristics

Baseline disease characteristics for the mITT population in the main phase were consistent with the disease under study and were generally similar for subjects assigned to placebo and AMX0035 (**Table 5**). On average, the time from the onset of the first symptom to the initiation of the study was approximately 13.5 months.

Most subjects (77.0%) were on either edaravone or riluzole at or prior to study entry. More subjects in the placebo group took edaravone at or prior to study entry (active versus placebo: 25.3% vs 50.0%), and fewer subjects in the active group took riluzole at or prior to study entry (active versus placebo: 67.8% vs 77.1%). Time since first exposure to either edaravone or riluzole was comparable between treatment groups.

Baseline scores for efficacy endpoints were similar between groups, with an average ALSFRS-R Total Score of 36.0 at baseline, an average ATLIS total score PPN of 55.8, and an average SVC PPN of 83.7.

Table 5: Baseline Disease Characteristics– mITT population

Statistic	**Placebo + SOC (N=48)**	**AMX0035 +SOC (N=87)**	**Overall (N=135)**
DEL-FS			
N	48	87	135
Mean (SD)	0.926 (0.6012)	0.953 (0.4267)	0.943 (0.4938)
Median	0.760	0.885	0.852
Time Since Onset of ALS Diagnosis (months)			

N	48	87	135
Mean (SD)	6.3 (3.22)	5.9 (3.33)	6.0 (3.29)
Median	6.0	5.3	5.7
Time Since Onset of ALS Symptoms (months)			
N	48	87	135
Mean (SD)	13.6 (3.64)	13.5 (3.83)	13.5 (3.75)
Median	13.3	13.9	13.7
Use of Either Edaravone or Riluzole at or Prior to Study Entry			
Yes	42 (87.5%)	62 (71.3%)	104 (77.0%)
No	6 (12.5%)	25 (28.7%)	31 (23.0%)
Use of Edaravone Only at or Prior to Study Entry			
Yes	5 (10.4%)	3 (3.4%)	8 (5.9%)
No	43 (89.6%)	84 (96.6%)	127 (94.1%)
Use of Riluzole Only at or Prior to Study Entry			
Yes	18 (37.5%)	40 (46.0%)	58 (43.0%)
No	30 62.5%)	47 (54.0%)	77 (57.0%)
Use of Edaravone at or Prior to Study Entry			
Yes	24 (50%)	22 (25.3%)	46 (34.1%)
No	24 (50%)	65 (74.7%)	89 (65.9%)
Use of Riluzole at or Prior to Study Entry			
Yes	18 (37.5%)	40 (46.0%)	58 (43.0%)
No	30 (62.5%)	47 (54.0%)	77 (57.0%)
Use of Both Edaravone and Riluzole at or Prior to Study Entry			
Yes	19 (39.6%)	19 (21.8%)	38 (28.1%)
No	29 (60.4%)	68 (78.2%)	97 (71.9%)
Time Since First Exposure to Edaravone at Baseline (months)			
N	24	22	46
Mean (SD)	3.6 (2.60)	3.5 (3.04)	3.5 (2.79)
Median	3.3	2.6	3.0
Time Since First Exposure to Riluzole at Baseline (months)			
N	37	59	96
Mean (SD)	5.5 (3.28)	5.7 (3.41)	5.6 (3.34)
Median	4.9	5.0	5.0
Family History of ALS			
Yes	7 (14.6%)	9 (10.3%)	16 (11.9%)
No	38 (79.2%)	76 (87.4%)	114 (84.4%)
Unknown	3 (6.3%)	2 (2.3%)	5 (3.7%)
Site of Onset			
Limb	38 (79.2%)	59 (67.8%)	97 (71.9%)
Bulbar	10 (20.8%)	26 (29.9%)	36 (26.7%)
Other	0 (0.0%)	2 (2.3%)	2 (1.5%)
SVC % Predicted			
N	48	87	135
Mean (SD)	83.9 (15.92)	83.6 (18.17)	83.7 (17.35)
Median	84.0	82.0	83.0
ALSFRS-R Breathing			
N	48	87	135
Mean (SD)	11.0 (1.80)	10.6 (1.92)	10.8 (1.88)
Median	12.0	12.0	12.0
ALSFRS-R Bulbar			
N	48	87	135
Mean (SD)	10.0 (2.60)	9.5 (2.40)	9.7 (2.47)
Median	11.0	10.0	10.0
ALFRS-R Fine Motor			
N	48	87	135
Mean (SD)	8.0 (2.63)	8.0 (2.69)	8.0 (2.66)
Median	8.5	8.0	8.0
ALSFRS-R Gross Motor			
N	48	87	135
Mean (SD)	7.6 (2.62)	7.5 (2.84)	7.6 (2.76)
Median	7.5	7.0	7.0
ALSFRS-R Total			
N	48	87	135
Mean (SD)	36.7 (5.08)	35.7 (5.78)	36.0 (5.54)
Median	37.0	36.0	37.0
ATLIS Lower Extremities			

N	48	85	133
Mean (SD)	57.0956 (25.81398)	57.6483 (24.89289)	57.4488 (25.13290)
Median	55.8745	59.0039	57.7393
ATLIS Upper Extremities			
N	47	85	132
Mean (SD)	51.4442 (25.22005)	54.7599 (24.39991)	53.5793 (24.65037)
Median	51.4650	58.0184	55.9429
ATLIS Lower & Upper Extremities			
N	47	84	131
Mean (SD)	53.9242 (20.94439)	56.8294 (20.08198)	55.7871 (20.36320)
Median	52.9920	55.8374	54.5061

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

The majority of subjects in both treatment groups (84 [95.5%] subjects in the AMX0035 group and 46 [95.8%] subjects in the placebo group) had 1 or more ongoing medical history findings at study enrollment, which was not unexpected considering the significant morbidity associated with a definite diagnosis of ALS in the relatively older adult population studied. The most common (those reported in ≥ 12% of subjects overall) were generally similar between treatment groups and largely reflected the manifestations and complications of ALS and age: hypertension (27.2%), anxiety (23.5%), depression (22.1%), insomnia (19.1%), muscle spasms (18.4%), seasonal allergy (16.2%), muscular weakness (14.7%), hyperlipidemia (13.2%), and affect liability (12.5%).

Prior and concomitant medications

Most subjects in both treatment groups (87 [97.8%] in the AMX0035 group and 48 [100%] in the placebo group) also received medications and therapies prior to initiation of the study medication. The most common ones (those reported in ≥15% of subjects overall, by generic medication name) were similar between the 2 treatment groups and consistent with the disease under study. These included riluzole (71.5%) and edaravone (34.3%) for the treatment of ALS; vitamins, not otherwise specified (NOS) (28.5%); acetylsalicylic acid (21.2%) to reduce pain and inflammation; cholecalciferol (19.7%) for vitamin D deficiency; baclofen (19.7%) to reduce muscle symptoms; cyanocobalamin (19.0%) for vitamin B12 deficiency; and ubidecarenone (16.1%), a powerful antioxidant and essential cofactor in mitochondrial oxidative phosphorylation.

- **Numbers analysed**

The modified intent-to-treat population included 135 subjects: 87 subjects in the AMX0035 group and 48 subjects in the placebo group. The intent-to-treat population differs by two more patients in the treatment arm.

- **Outcomes and estimation**

Primary endpoint

The primary efficacy endpoint for the study was the rate (slope) of decline in capability and independence in 12 functional activities relevant to ALS as measured by total **ALSFRS-R questionnaire** scores assessed using a shared-baseline, mixed-effects model in the mITT population. At baseline, the ALSFRS-R scores were 36.7 (0.733) for placebo and 35.7 (0.620) for the AMX0035 groups respectively.

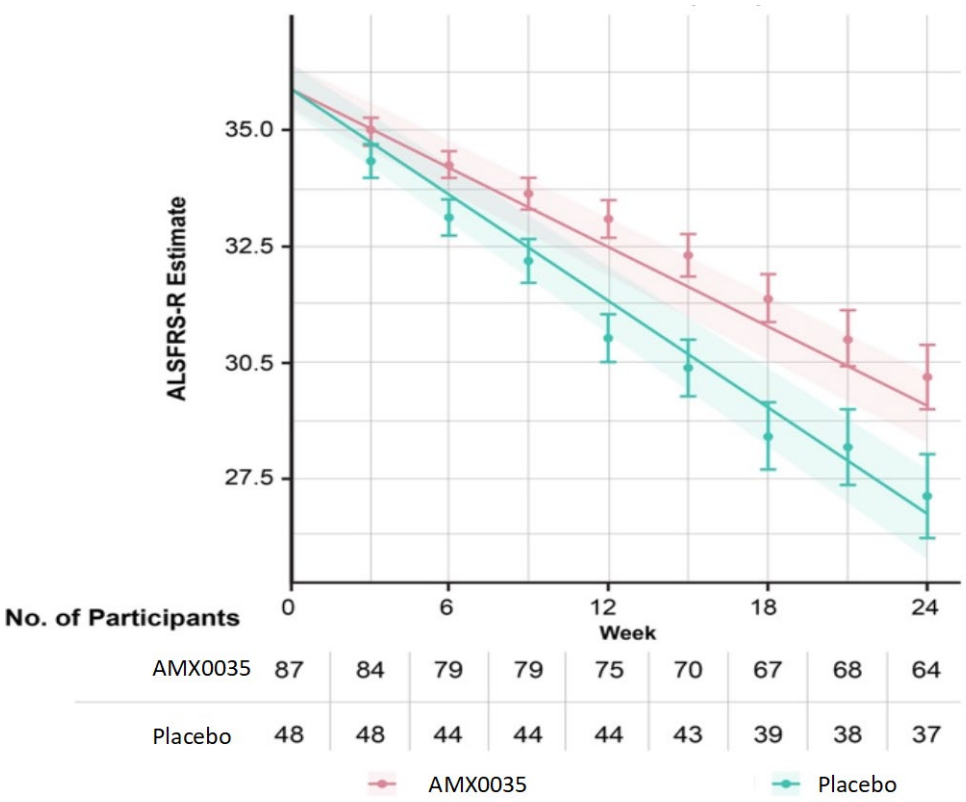
AMX0035 met the prospectively defined primary endpoint and demonstrated a statistically significant slowing of disease progression as measured by the ALSFRS-R total score compared to placebo (p=0.0340) (Table 6, Figure 6).

Table 6: ALSFRS-R Total Score at Week 24 – mITT population

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

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

Figure 6: Estimated Rate of Decline in ALSFRS-R Total Score Over 24 Weeks – mITT population (mean +/- SE)



Overlaid on the estimated slopes from the primary analysis are visit-specific estimates (and standard error bars) from *a post hoc* shared-baseline, repeated-measures mixed model with the same adjustments but categorical time and unstructured covariance among repeated measures

Secondary endpoints

ATLIS

As shown in Table 7, results for the upper, lower, and total ATLIS scores all directionally favoured AMX0035 over placebo, with treatment differences ranging from 2.09 to 4.27 and effect sizes ranging from 12.7% and 25.4%.

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

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

ATLIS = Accurate Test of Limb Isometric Strength; CI = confidence interval; SE = standard error; SOC = standard of care.

pNF-H levels

Plasma levels of pNF-H were assessed as a potential biomarker of neuronal injury. Analysis of the results for the biomarker, pNF-H did not indicate any treatment-related difference between groups. The mean rate of change in the plasma pNF-H concentration was 3.58 pg/mL per month with AMX0035 and -2.34 pg/mL per month with placebo (difference, 5.93 pg/mL per month; 95% CI, -4.41 to 16.26, p=0.2601). Analysis of the results for the biomarker, NF-L did not indicate any treatment-related difference between groups. The mean rate of change in the plasma NF-l concentration was 1.45 pg/mL per month with AMX0035 and 2.04 pg/mL per month with placebo (difference, -0.59 pg/mL per month; 95% CI, -2.30 to 1.13, p=0.5021)

SVC

As shown in Table 8 mean SVC PPN was 5.11 percentage points higher in the AMX0035 group at Week 24 compared to placebo, although the p-value was not significant (nominal p=0.0763).

Table 8: SVC at Week 24 – mITT population

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

CI = confidence interval; SE = standard error; SOC = standard of care; SVC = slow vital capacity

Survival

Single and combined survival analyses were performed using the Cox proportional hazards model for the outcomes of death, death equivalent, and hospitalization (death equivalent was defined as time to death, PAV or tracheostomy). Note that PAV only and tracheostomy only were not analyzed as there was only one event of each in a singular placebo subject during the main phase.

The survival analyses are displayed in Table 9 below. None were nominally statistically significant after only 24 weeks of treatment.

Table 9: Summary of Survival Analyses at Week 24 – mITT population

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

confidence interval; SE = standard error; SOC=standard of care

- **Ancillary analyses**

Sensitivity analyses of the primary endpoint and (key) secondary endpoints

The originally provided primary analysis was not considered appropriate regarding the analysis population (mITT instead of ITT), the analysis model (assumption of linearity), the handling of missing values (implicit MAR assumption) and the handling of deaths. In particular, the estimand that was implicitly targeted was not an appropriate basis for regulatory decision making. Several sensitivity analyses have been provided by the applicant that challenge these assumptions, e.g. testing the linearity assumption. Consequently, the CHMP requested an analysis using the ITT population, not relying on the linearity of time, placebo-based imputation for patients discontinuing and worst-case imputation after death. This analysis, the sensitivity analyses performed by the applicant are shown in Table 10.

Table 10: Sensitivity analyses performed for the ALSFRS-R[1]

mITT versus ITT		
- Changing to all randomised but assuming missing at random	2.32	(0.18,4.47; p=0.034)
Linear progression not assumed		
- Applicant analysis	2.15	(0.17,4.13; p=0.034)
Data is missing not at random		
- Applicant placebo-based imputation placebo-based imputation also after death	1.96	(-0.02,3.94; p=0.052)
CHMP requested sensitivity analysis		
- ITT, no linear progression, placebo based imputation and 0 imputation for death	1.52	(-0.80, 3.84; p=0.20)

[1] made by assessor ALSFRS-R = Amyotrophic Lateral Sclerosis Functional Rating Scale – Revised; ITT = Intent-to-Treat population

There was also no support from the (key) secondary endpoints ATLIS and SVC when analysed in the model without linearity assumption, placebo based imputation for patients discontinuing and worst case imputation after death in the ITT population.

Responder analysis

In this analysis, a response rate of 41% (95% CI: 31%, 52%) was observed in the AMX0035 group compared with a 19% (95% CI: 8%, 30%) response rate in the placebo group (nominal p=0.0076). The odds ratio of 3.06 with 95% CI (1.32, 7.09) indicates that the odds of being a responder on AMX0035 is approximately three times the odds of being a responder on placebo (Table 11). In summary, substantially more patients randomized to AMX0035 compared to placebo appeared to have a substantial response as defined by progression slower than their individual ΔFS.

Table 11: Response Rate by Treatment Group – mITT population

	AMX0035	Placebo	Overall

	(N=87)	(N=48)	
Responder (N,%)	36 (41%)	9 (19%)	45 (33%)
Non-Responder (N,%)	51 (59%)	39 (81%)	90 (67%)
Odds Ratio (95% CI)			3.06 (1.32, 7.09)
p-value (chi-squared)			0.0076

Rate (Slope) of Decline in ALSFRS-R Domains

The rate (slope) of decline in the 4 subscale scores of the ALSFRS-R (i.e., bulbar, fine motor, gross motor, and breathing) was assessed as an exploratory efficacy outcome measure in the placebo-controlled main phase. These subscales were not expected to have sufficient power to detect significant differences; however, there was interest in seeing if the effects of AMX0035 extended across the whole ALSFRS-R scale or were predominantly on specific questions.

The largest difference between the AMX0035 and placebo groups was seen for the fine motor domain of the ALSFRS-R ($p_{nominal}$=0.0148). The other 3 domains also numerically favoured AMX0035 but were not nominally statistically significant (

Table 12). This data suggests that AMX0035 benefit extended across the whole ALSFRS-R scale with a possibility for a largest effect in the fine motor domain.

Table 12: ALSFRS-R Domains at Week 24 – mITT population

Endpoint Time Point	Estimate (SE)		Estimated Difference (SE)	95% CI	p-value
	Placebo+SOC (N=48)	AMX0035+SOC (N=87)			
ALSFRS-R Fine Motor					
Week 24	4.80 (0.379)	5.84 (0.305)	1.04 (0.425)	0.20, 1.87	0.0148
ALSFRS-R Gross Motor					
Week 24	5.05 (0.409)	5.57 (0.342)	0.51 (0.419)	-0.31, 1.34	0.2203
ALSFRS-R Breathing					
Week 24	9.13 (0.373)	9.49 (0.285)	0.36 (0.453)	-0.53, 1.25	0.4306
ALSFRS-R Bulbar					
Week 24	7.68 (0.366)	8.20 (0.320)	0.52 (0.332)	-0.13, 1.17	0.1188

ALSFRS-R = Amyotrophic Lateral Sclerosis Functional Rating Scale – Revised; CI = confidence interval; SE = standard error; SOC = standard of care.

Subgroup analyses

The following subgroup analyses were performed *post hoc* on the change from baseline in ALSFRS-R:

Gender: Although most studies have indicated that gender largely has no effect on ALS outcome, two studies demonstrated that women with ALS may have a shorter lifespan.

Age: Several studies have demonstrated that this is a strong prognostic factor in ALS, with decreasing survival time correlating with increasing age of symptom onset.

Bulbar Onset vs Limb Onset: Bulbar onset of location has also been associated with a worse prognosis versus limb onset and has been suggested to be an independent predictor of ALS outcome.

Pre-baseline Rate of Disease Progression as measured by the DEL-FS (ΔFS) has been cited as another key factor related to prognosis and survival (Kimura et al 2006, Gordon et al. 2006, Labra et al. 2016, Kjældgaard et al. 2021)

ALSFRS-R Baseline Score: The total score and the respiratory sub-score on the ALSFRS-R is also significantly related to outcome (Kaufmann et al. 2005)

% Predicted Slow Vital Capacity (SVC): Many studies published in the literature have found that a lower predicted vital capacity at diagnosis was the single most relevant prognostic factor in ALS.

Levels of Plasma Neurofilament Light: Studies have cited that higher serum NfL concentration is a prognostic factor of death in ALS as early as the time of diagnosis.

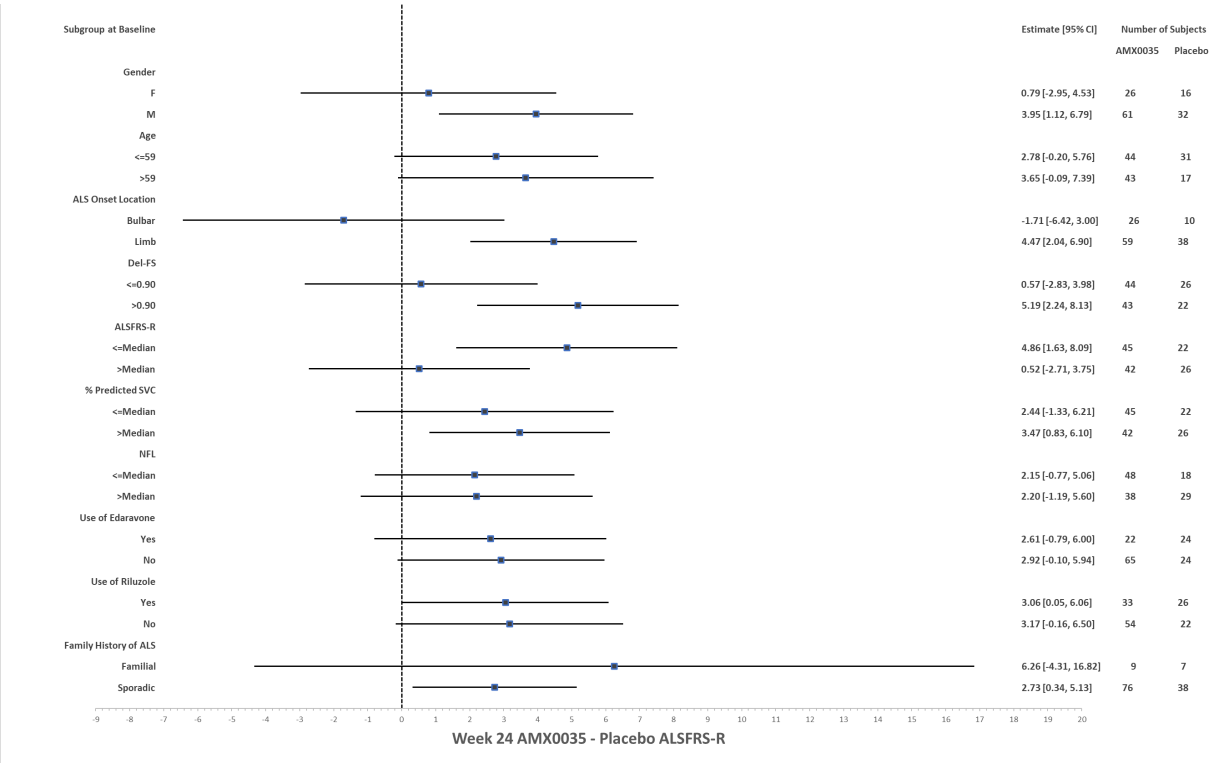
Concomitant Use of Edaravone or Riluzole: Currently these are the only two approved disease-modifying therapies in the US where the CENTAUR trial was conducted.

Familial History of ALS vs Sporadic ALS: Although most studies have not found any differences in outcome between patients with sporadic and those with familial ALS, some mutations may have different effects on the age of onset of symptoms and on the rate of progression of the disease.

The results from the subgroup analyses are shown in

Figure **7** below.

Figure 7: Subgroup Analyses Using Change from Baseline Analysis



- **Summary of main efficacy results**

The following table (Table 13) summarises the efficacy results from the main studies supporting the present application. This summary should be read in conjunction with the discussion on clinical efficacy as well as the benefit-risk assessment (see later sections).

Table 13: Summary of efficacy for AMX3500

Title: AMX3500 (CENTAUR) EVALUATION OF THE SAFETY, TOLERABILITY, EFFICACY AND ACTIVITY OF AMX0035, A FIXED COMBINATION OF PHENYLBUTYRATE (PB) AND TAUROURSODEOXYCHOLIC ACID (TUDCA), FOR TREATMENT OF AMYOTROPHIC LATERAL SCLEROSIS (ALS)			
Study identifier	AMX3500 (CENTAUR): NCT03127514		
Design	Multicentre, randomized, double-blind, placebo-controlled, parallel-group 24-week study evaluating the safety, tolerability, efficacy, pharmacokinetics (PK) and biological activity of AMX0035.		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		24-week Not applicable Up to 132 weeks
Hypothesis	Superiority		
Treatments groups	AMX0035 (study medication) + Standard of Care (SOC)		Randomized, multicentre, 89 subjects, 24-week, administered orally (or via feeding tube) at a dose of 1 sachet twice daily.
	Placebo + SOC		Randomized, multicentre, 48 subjects, 24-week administered orally (or via feeding tube) at a dose of 1 sachet twice daily.
	Primary endpoint	ALSFRS-R	Measure the impact of AMX0035 using the slope of progression with the ALSFRS-R.

	Secondary endpoint	ATLIS	Assess the impact of AMX0035 on the rate of decline of isometric muscle strength, measured by ATLIS.
	Secondary endpoint	Disease progression	Assess the impact of AMX0035 on disease progression measured by SVC decline, time to tracheostomy and survival.
	Secondary endpoint	Survival	Measure the impact of AMX0035 on survival by assessing time to first hospitalization, death, or death equivalent
Database lock	25 November 2019		
Results and Analysis			
Analysis description	**Primary Analysis (Prespecified)**		
Analysis population and time point description	Modified Intent-to-Treat (mITT): All subjects who receive at least one dose of study medication and have at least one post-baseline total ALSFRS-R score. Change from Baseline at 24 weeks		
Descriptive statistics and estimate variability	Treatment group	AMX0035 + SOC	Placebo + SOC
	Number of subjects	87	48
	ALSFRS-R Total Score at Week 24 Estimate Standard Error (SE)	 29.06 0.781	 26.73 0.975
Effect estimate per comparison	ALSFRS-R Total Score at Week 24	Comparison groups	AMX0035+SOC Placebo + SOC
		Week 24 Difference (SE)	2.32 (1.094)
		95% Confidence Interval	0.18, 4.47
		P-value	0.0340
Notes	Subjects in AMX3500 were also on standard of care therapies (88% of subjects on placebo vs 71% of active subjects were on an approved ALS therapy at baseline) as advised by their treating physician, so this benefit is not in comparison to but rather an advance beyond or on top of the current paradigm of care in ALS. Subjects who dropped out had all available baseline and post-baseline data included in the analysis. The main efficacy analysis was a shared-baseline mixed-effect model with ALSFRS-R total score as dependent variable, treatment as fixed effect and age and Del-FS as covariates, implemented using PROC MIXED in SAS®.		

Analysis description	**Secondary analysis (Prespecified)**		
	Total ATLIS (mITT)	Comparison groups	AMX0035 +SOC Placebo + SOC
		Week 24 Difference (SE)	2.82 (1.774)
		95% Confidence Interval	-0.67, 6.31
		P-value	0.1129
	Upper ATLIS (mITT)	Comparison groups	AMX0035 + SOC Placebo + SOC
		Week 24 Difference (SE)	4.27 (2.089)
		95% Confidence Interval	0.16, 8.38
		Nominal P-value	0.0420
	Lower ATLIS (mITT)	Comparison groups	AMX0035 + SOC Placebo + SOC
		Week 24 Difference (SE)	2.09 (2.195)
		95% Confidence Interval	-2.23, 6.41
		Nominal P-value	0.3424
	SVC (mITT)	Comparison groups	AMX0035 + SOC Placebo + SOC
		Week 24 Difference (SE)	5.11 (2.872)
		95% Confidence Interval	-0.54, 10.76
		Nominal P-value	0.0763
	Survival – Death, Death Equivalent, or Hospitalisation (mITT)	Comparison groups	AMX0035+SOC Placebo + SOC
		Hazard Ratio: Active vs. Placebo	0.575
		95% Confidence Interval	0.290, 1.152
		Nominal P-value	0.1122
	Survival – Death or Death Equivalent (mITT)	Comparison groups	AMX0035 + SOC Placebo + SOC
		Hazard Ratio: Active vs. Placebo	0.632
		95% Confidence Interval	0.110, 3.924
		Nominal P-value	0.5960
	Survival – Hospitalisation (mITT)	Comparison groups	AMX0035 + SOC Placebo + SOC
		Hazard Ratio: Active vs. Placebo	0.590
		95% Confidence Interval	0.286, 1.234
		Nominal P-value	0.1530

	Survival – Death Events Only (mITT)	Comparison groups	AMX0035 + SOC Placebo + SOC
		Hazard Ratio: Active vs. Placebo	1.016
		95% Confidence Interval	0.151, 9.753
		Nominal P-value	0.9873
Notes	AMX3500 was statistically powered to observe a change in the slope of the ALSFRS-R and was not powered for secondary outcomes. The hierarchical testing procedure stopped at the first secondary endpoint total ATLIS score. As a consequence, none of the secondary endpoints have been formally evaluated.		

2.6.5.3. Clinical studies in special populations

Not applicable

2.6.5.4. In vitro biomarker test for patient selection for efficacy

Not applicable

2.6.5.5. Analysis performed across trials (pooled analyses and meta-analysis)

Not applicable

2.6.5.6. Supportive study(ies)

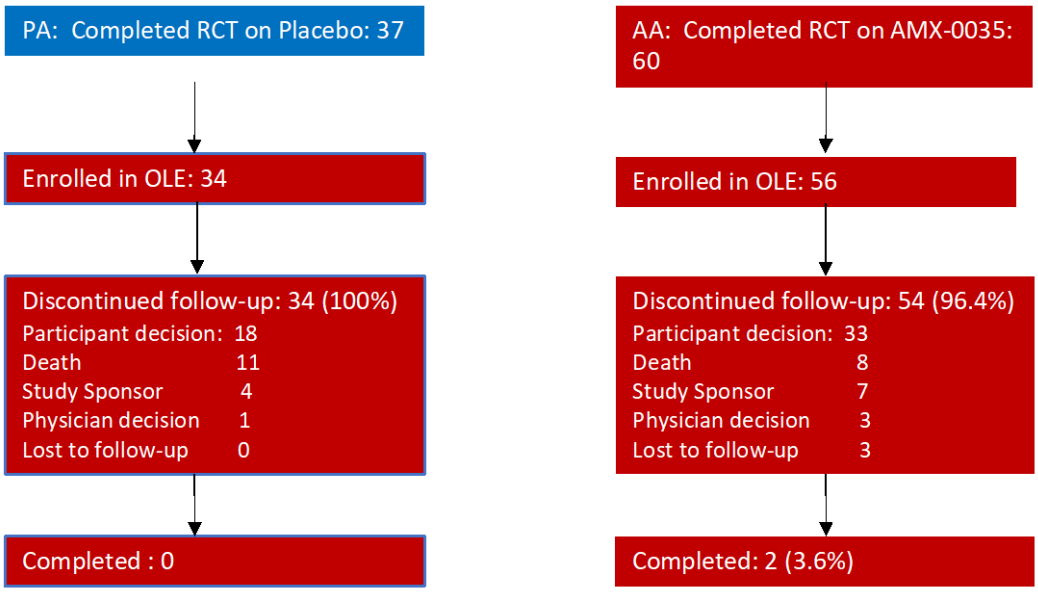
Study AMX3500-OLE was the OLE phase of AMX3500. At the end of the 24-week double-blind treatment phase, subjects had the option to continue into the OLE, where all received active treatment with AMX0035 for up to 132 weeks.

Subject disposition

Ninety-seven of the subjects who completed the main phase completed the study on drug and were therefore eligible for enrollment into the OLE (

Figure 8). In addition, 1 subject who had a brief (approximately 1 week) drug disruption at the very end of the main phase was also permitted to enter the OLE. Of these, 90 (92%) continued into the OLE, 34 of whom had been originally randomized to placebo and 56 to active drug. The longest follow-up was ~40 months after randomization.

Figure 8: OLE Subject Disposition – ITT population



ITT=intent to treat; OLE=open label extension; RCT=randomized clinical trial (weeks 0-24); AA= AA Group of subjects randomized to AMX0035 in the main phase and who stayed on AMX0035 upon enrollment in the open-label extension phase; PA= Group of subjects randomized to placebo in the main phase and who switched to AMX0035 upon enrollment in the open-label extension phase

Rate (Slope) of Decline in Total ALSFRS-R Scores at week 48

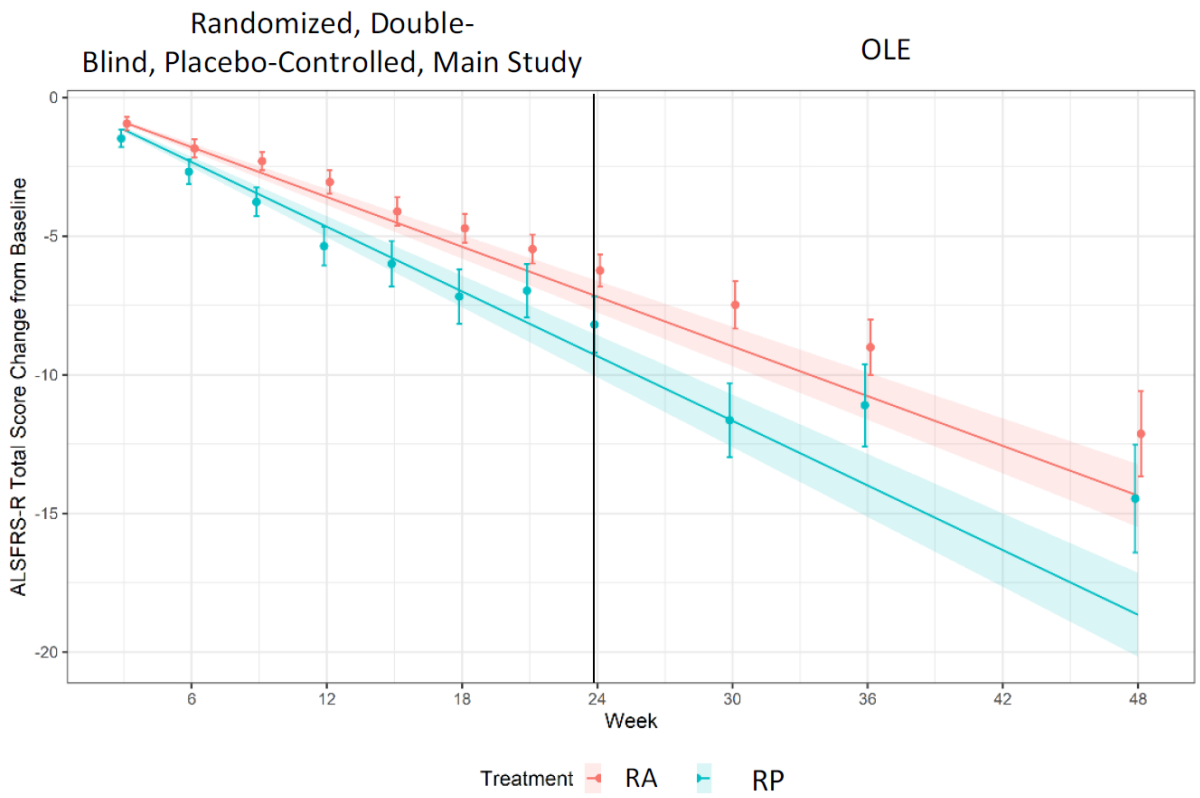
An analysis comparing the difference between the 2 treatment groups (RA [Group of subjects randomized to AMX0035 and stayed on AMX0035 in the open-label extension phase, plus the AMX0035 subjects who did not enter the open-label extension] vs. RP [Group of subjects randomized to placebo in the main phase and switched from placebo to AMX0035 in the open-label extension phase, plus the placebo subjects who did not enter the open-label extension]) in the slope from the main phase baseline through Week 48 (referred to as the Extended Slope Analysis), was nominally statistically significant in favour of the RA arm (Table 14, Figure 9).

Table 14: Extended Slope Efficacy Analysis for ALSFRS-R Total Score – mITT population

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

ALSFRS-R = ALS Functional Rating Scale – Revised; CI = confidence interval; mITT=modified intent to treat; RA=randomized to AMX3500 in the main phase, subjects who continued in OLE received AMX0035 in the OLE; RP=randomized to placebo in the main phase, subjects who continued in OLE received AMX0035 in the OLE; SE=standard error; SOC=standard of care.

Figure 9: Linear MMRM Extended through 48 Weeks of Follow-Up: mITT Population (mean SEM)



OLE=open label extension; RA=randomized to AMX3500 in the main phase, subjects who continued in OLE received AMX0035 in the OLE; RP=randomized to placebo in the main phase, subjects who continued in OLE received AMX0035 in the OLE.
Note: Separate means plotted as points (+/- SEM) and MMRM estimate is plotted as the slope line.

Exploratory survival analysis

The survival endpoint (death or death equivalent) was defined as death, tracheostomy or PAV. Tracheostomy and PAV were recorded prospectively via clinic reports, with censoring at last date of follow-up for those without reported events. The vital status and date of death for all subjects randomized into Study AMX3500 were investigated by OmniTrace. Vital status was obtained as of July 20, 2020 for all but 2 of the 137 subjects, both of whom were non-US residents whose vital status could not be confirmed in US public records or by their corresponding sites; per the SAP, these subjects were censored at the date of last contact with the clinical site. At the time of this analysis, the longest follow-up was 35 months after randomization in AMX3500.

Vital status was also obtained as of March 01, 2021 for the 135 subjects with a known vital status as of July 20, 2020. One patient with missing vital status at July 20, 2020 was now determined to be alive, leading to data for all but one patient. At the time of the March 2021 analysis, the longest follow-up was 40 months after randomization in AMX3500.

The results of the long-term study events analyses using cut off dates of July 20, 2020 and March 1, 2021 are presented in Table 15. At both data cut-off dates, pre-specified analyses in the mITT population of time to death; death or death equivalent; first hospitalization; and time to death, death equivalent or first hospitalization were all nominally statistically significant in favour of the RA group. These analyses were also nominal significant in the ITT analysis except for Time to First Hospitalization using the March 1, 2021 cutoff date.

Of note, as of 01 March 2021, ~69% of patients had died and 83% of patients had an additional event, which included hospitalization and/or tracheostomy.

The 2 groups had similar rates of tracheostomy and PAV events. Nine (10.11%) subjects originally randomized to AMX0035 and 3 (6.3%) subjects originally randomized to placebo experienced these events.

Table 15: Key Study Events: Death, Tracheostomy, and First Hospitalization

	July 20, 2020 Data Cutoff				**March 1, 2021 Data Cutoff**			
	Median Survival Estimate (Months)				**Median Survival Estimate (Months)**			
Population and Outcome	**RA+SOC**	**RP+SOC**	**Hazard Ratio [95%CI]**	**P-Value**	**RA+SOC**	**RP+SOC**	**Hazard Ratio [95%CI]**	**P-Value**
mITT	**N=87**	**N=48**	**-**	**-**	**N=87**	**N=48**	**-**	**-**
Time to First Hospitalization, Death, or Death Equivalent (Original SAP)	**14.8**	**8.9**	**0.532 [0.349, 0.811]**	**0.0034**	**14.8**	**10.0**	**0.615 [0.408, 0.925]**	**0.0196**
Time to First Hospitalization	Not Reached	14.1	0.564 [0.335, 0.949]	0.0311	31.8	14.1	0.595 [0.355, 0.996]	0.0482
Time to Death	25.8	18.9	0.540 [0.330, 0.884]	0.0143	23.5	18.7	0.619 [0.399, 0.960]	0.0324
Time to Death or Death Equivalent	25.8	18.5	0.514 [0.315, 0.837]	0.0074	23.5	17.9	0.597 [0.387, 0.923]	0.0203
ITT	**N=89**	**N=48**	-	-	**N=89**	**N=48**	-	-
Time to death (From Supplemental SAP)	**25.8**	**18.9**	**0.567 [0.348, 0.923]**	**0.0226**	**23.5**	**18.7**	**0.644 [0.416, 0.995]**	**0.0475**
Time to First Hospitalization, Death, or Death Equivalent	14.8	10.0	0.548 [0.360, 0.833]	0.0049	14.8	10.0	0.631 [0.420, 0.947]	0.0264
Time to First Hospitalization	Not Reached	14.1	0.574 [0.342, 0.964]	0.0360	31.8	14.1	0.605 [0.362, 1.011]	0.0552
Time to Death or Death Equivalent	25.8	18.5	0.539 [0.333, 0.874]	0.0122	23.2	17.9	0.621 [0.403, 0.957]	0.0308

Abbreviations: CI = confidence interval; ITT = intent to treat population; mITT=modified intent to treat; RA=randomized to AMX3500 in the main phase, subjects who continued in OLE received AMX0035 in the OLE; RP=randomized to placebo in the main phase, subjects who continued in OLE received AMX0035 in the OLE; SAP = statistical analysis plan; SD=standard deviation; SOC=standard of care. ITT = intent to treat population; mITT = modified intent to treat population

Kaplan-Meier curves for the survival analyses time to death or death equivalent (Figure 10), and time to death (Figure 11) are displayed below.

Figure 10: Cox Proportional Hazards Analysis for Death or Equivalent – mITT population (cut-off: 01 March 2021)

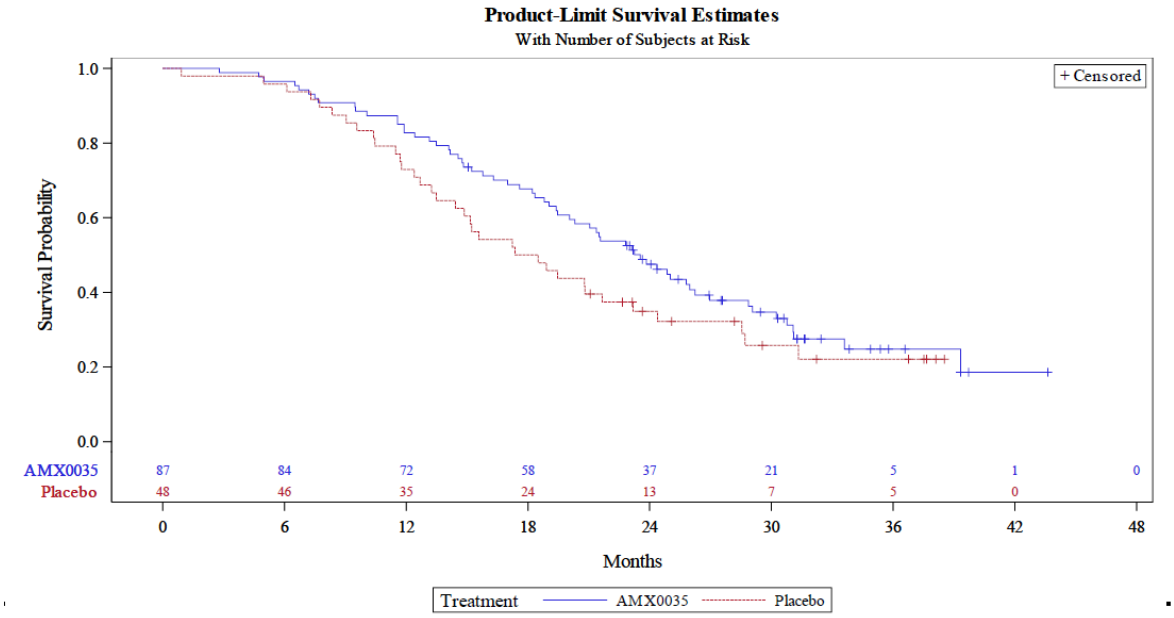
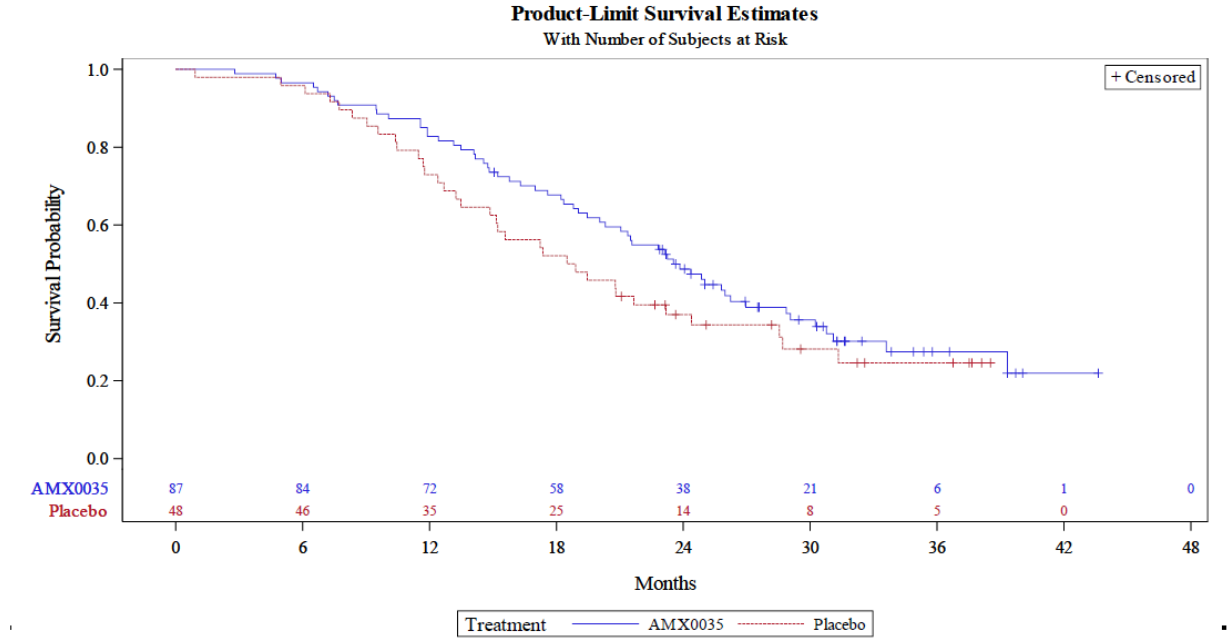


Figure 11: Cox Proportional Hazards Analysis for Death – mITT population (cut-off: 01 March 2021)



2.6.6. Discussion on clinical efficacy

To support a CMA of AMX0035 for the treatment of ALS, results were submitted from the exploratory phase II study AMX3500. A confirmatory phase III study (A35-004) was ongoing during the assessment and at the time of opinion and it was proposed by the applicant as specific obligation for the CMA.

Design and conduct of clinical studies

Study AMX3500 was a phase II proof of concept randomized, double-blind, placebo-controlled study. It consisted of a 24-week double-blind treatment period with an OLE phase, allowing treatment for up to 132 weeks.

The design of the study is considered acceptable to evaluate the efficacy of AMX0035 for the symptomatic treatment of ALS, as described in the EMA Guideline for the Clinical Investigation of Medicinal Products for the treatment of ALS (EMA/531686/2015, Corr.1, hereafter referred to as the EMA ALS Guideline). The duration of the double-blind phase, is considered too short to show an effect on survival, which would support a disease-modifying claim. The EMA ALS guideline states that a study duration of 12-18 months may be sufficient. However, it is also indicated that it is recommended to check during the trial whether the progression rates are in accordance with the assumption. This recommendation could lead to an increase in the sample size or an increase in the study duration. In any case a study of 6 months of duration is clearly insufficient for the purpose of showing benefit in survival.

Target population

The key inclusion criteria with respect to the disease was a definite diagnosis of sporadic or familial ALS as defined by the El Escorial criteria, less than or equal to 18 months since ALS symptom onset and SVC > 60% of the predicted value. This is considered a restricted study population and it might not be representative of the overall ALS population. It was argued by the applicant that definite ALS can be generalised to the whole ALS population because it includes early stage, mid-stage and advanced patients. However, the rate of disease progression is different, and effect modification dependent on the progression rate cannot be excluded. A detailed discussion supporting the potential extrapolation of efficacy, including the impact of disease severity on efficacy, has not been provided. Thus the issue remains that Study AMX3500 included a subpopulation, and it is unclear how the potential benefit of AMX0035 can be extrapolated to a general ALS population. The potential functional benefit (the true effect size at the population level is not known at the time of the opinion) could not be assumed for the general ALS population but only for a subpopulation that is similar to the AMX3500 study population. It is also unclear, how such a population could best be identified in clinical routine practice. Subjects were permitted to take concomitant riluzole, provided the dose was stable. Key exclusion criteria were the presence of tracheostomy, implantation of diaphragm pacing system and exposure to PB, taurursodiol or ursodiol within 3 months prior to the screening visit or planning to use these medications during the course of the study.

Treatment

During the first 3 weeks of treatment, subjects were instructed to take 1 sachet of AMX0035/placebo per day. Following this titration phase subjects were able to increase the dose to 2 sachets daily when tolerated. AMX0035 has a bitter taste, and instructions were provided to make the taste more palatable. The placebo sachets were also designed to mimic the bitter taste to ensure the blind.

According to the applicant, the product is recommended to be taken before a snack and should be administered within 1 hour of combining the contents of the sachet with water. In the study, subjects were allowed 1 hour to complete oral administration of the study drug, i.e. drink the suspension in intervals. However, there is no data available whether a longer dosing interval (> 1 hour) has an impact on the PK profile and efficacy.

Outcomes

The **primary endpoint** was the *rate of decline (slope of decline) in the total ALSFRS-R* score. The **secondary endpoints** were the rate of decline of isometric muscle strength, as measured by the A*TLIS*; the impact on the plasma concentration of *pNF-H*; the rate of *SVC* decline; the rates of survival (*defined as death, tracheostomy or PAV, tracheostomy*), and duration of *hospitalization*; and the impact on TSPO uptake measured by *PET scan*.

The ATLIS is still under significant evaluation at the time of the opinion, i.e. it is not fully validated. Therefore, the contribution of the findings on this endpoint to the overall conclusions of the study are considered very limited.

Vital status (i.e. death) was also monitored of subjects who prematurely discontinued the study. This was achieved by contacting relatives, clinical notes or using US-specific internet sources which list information on death (omnitrace). For all but one patient, vital status could be verified through this method on the data cut-off for analysis.

The study did not include quality-of-life endpoints. Hence, the results on the functional endpoints cannot be contextualised to patient-perceived benefit. For a phase II proof of concept study, this is considered acceptable.

Randomisation & blinding

The randomisation procedure is considered acceptable. Blinding appears to be adequate for investigators. However, it is questioned whether it was so for patients. Although placebo sachets were manufactured to match the salty and bitter taste, a higher percentage of placebo patients predicted they had a placebo compared to active treatment at the exit questionnaire. According to the applicant, self-medication with the components of AMX0035 started after the study start was announced, so this would not have led to patients being enrolled that would recognise the taste.

The study blind was maintained throughout the OLE phase of the study.

Statistical Methods

The sample size calculation uses assumptions of treatment effect based on the Pooled Resource Open-Access ALS Clinical Trials database and analyses of a ceftriaxon database, which is an acceptable approach. However, the calculation was also based on a two-sided alpha of 0.1, which would not be acceptable for a pivotal study. Since the analysis plan used the conventional two-sided alpha of 0.05 and enrolment is finished, and the sample size cannot be changed anymore, this issue was not further pursued during the assessment.

The definition of the study populations for the double-blind phase is considered standard and acceptable. The mITT population was used for the primary analyses. Generally, the ITT population is preferred, especially since the two patients not included in the mITT both died. The analysis using the ITT population resulted in a similar treatment effect, but this was likely due to the fact their missing data was assumed to be random (see discussion on the entire analysis model below).

The primary analysis of the rate of decline (slope) used a shared-baseline, mixed-effects analysis, including age, the estimated rate of disease progression (i.e., ΔFS), and baseline of the efficacy outcome of interest (other than ALSFRS-R) as covariates and interacting with time. This is not considered an appropriate method. The method strongly relies on linearity of disease progression, and although progression has been shown to decrease in a linear fashion over the course of a typical clinical trial, the linearity is disputed over longer periods (Karanevich 2018), as is the case here since the model includes the progression rate from symptom onset to baseline as covariate. Furthermore, the same model was used for secondary endpoints, and it is unclear whether these all behave in a linear fashion. The applicant did perform a sensitivity analysis to test linearity by including a quadratic term in the model; however, this just tests one type of non-linearity. This choice was of influence since, in a *post hoc* analysis using time as a fixed categorical factor, the effect size was smaller.

The primary analysis model also assumes data will be MAR. This is not considered a reliable assumption as it is unlikely that patients that discontinue or die will still experience a treatment effect. Indeed, the sensitivity analysis assuming MNAR, using placebo-based imputation, found a decreased treatment effect compared to the primary analysis. This MNAR imputation is considered more reliable for patients discontinuing. In a *post hoc* analysis using worst-case imputation for patients that died reduced the treatment effect, which was no longer statistically significant.

Therefore, the applicant was requested to perform and present analyses of the primary and secondary endpoints in the double-blind phase using the change from baseline for the ITT population, with time as a fixed categorical factor instead of continuous in the model (i.e., not relying on linearity) and using a placebo based imputation for discontinuations and worst-case imputation in case of death. This showed that all assumptions of the predefined analysis led to more optimistic results. The analysis requested from the applicant is considered more adequate with regard to study population, (non-)linearity and handling of missing data. This conservative analysis was provided, alongside several sensitivity analyses that also tested the assumptions of the applicant's original model (e.g., linearity assumed or not). Results from these analyses indicate that the effect of AMX0035 strongly depends on the analyses method used. In the most optimistic case, i.e., the applicant's original model, the effect estimate on the ALSFRS-R is 2.32 and the difference between study arms statistically significant (p=0.034). In the most conservative estimate, i.e., the CHMP's requested analysis, the effect estimate is smaller and no longer statistically significant (1.52, p=0.20). The additionally provided sensitivity analyses fall somewhere in between these effect estimates. During the assessment, no convincing arguments have been provided that would lead to a different insight on what would be the best analysis. Therefore, it cannot be concluded that the results of the primary findings are robust, considering that the size of the effect and the conclusion of a statistically significant effect depend on the choices made for the primary analysis.

Post hoc, the applicant defined an estimand, describing how death or treatment discontinuation are handled in the predefined analysis. This estimand is not agreed upon, it does not reflect the actual primary analysis as the estimand includes patients that would not die within 24 weeks in the population attribute, while the original primary analysis assumes mortality is a random intercurrent event (using a hypothetical strategy). Later during the assessment, the applicant presented an estimand reflecting a "while alive" strategy, similar as the one previously presented, while the primary analysis appears to be more "as if alive". The second estimand is aligned with the requested analysis seeing mortality as the worst outcome was discussed as well, the applicant phrased this using a composite strategy for the handling of death, while it should be seen as a treatment policy strategy with imputation for death. It is acknowledged that imputation with 0 in case of death may be too conservative, on the other hand, the use of a hypothetical strategy in the primary estimand (as if patients would not die) is considered too optimistic. As the estimand definitions are *post hoc* and what the true estimand would have been, if defined in advance, is a hypothetical discussion, this was not further pursued.

An ALSFRS-R responder was defined as those ALS patients whose actual change from baseline in the ALSFRS-R at week 18 is less or equal to their own ΔFS.

The death or death equivalent endpoint is analysed using Cox regression, which is considered standard and acceptable.

The populations analysed in the open-label phase are acceptable based on initial randomisation. However, since entering the OLE was optional and only about 2/3rd of the patients actually entered the OLE, selection bias cannot be ruled out, making the OLE results exploratory at best. The endpoints of the open-label phase were analysed using the same shared-baseline, mixed-effects analysis, as used in the double-blind phase, and the same problems as mentioned above apply. The assumption of linear progression is even more troublesome since patients switch to active treatment in the OLE. Together with the selection bias, this makes the results very hard to interpret.

A similar problem arises in the survival analysis of the OLE. The initial Cox regression model ignores the switch to active treatment, likely leading to a violation of the proportional hazards assumption. Re-analysis using treatment of a time-dependent covariate and re-analysis using rank preserved structural failure time analysis were provided, both leading to a decreased HR for patients initially randomised to AMX0035 compared to the initial Cox regression model. This supports the original analysis of survival but also shows that the exact extent of survival is unclear. The analyses presented are not easy to

interpret as it cannot be ruled out that selection bias was introduced because entering the long-term extension was voluntary. Furthermore, the reliability of the regression model may be low as the number of patients staying on placebo after six months is small.

The applicant also performed a pooled crossover analysis. Since this combines both a parallel component (the double-blind comparison) and a cross-over component (pre-post comparison of switched placebo patients), the results are hard to interpret because of the selection bias and are considered exploratory.

Multiplicity across primary and secondary endpoints was handled using hierarchical testing. This will preserve the study-wise type I error rate and is acceptable. The OLE study also had a hierarchical testing procedure, but since the OLE is considered part of the double-blind study where the hierarchy ended at the first secondary endpoint, multiplicity is no longer controlled for later endpoints, including those in the OLE.

Conduct of the study

The study protocol was revised multiple times throughout the study, with the most notable changes being the addition of an open-label extension phase (version 3.0, October 2017), allowing concomitant edaravone use following FDA approval version 3.0, October 2017) and an extension of the OLE phase from 52 to 132 weeks (version 6.0 January 2019). The extension of the OLE phase was carried out to ensure that subjects could continue receiving AMX0035 treatment. With respect to concomitant edaravone use, subjects were permitted to start edaravone treatment prior to study enrolment as well as during the study.

Efficacy data and additional analyses

Of the 177 subjects screened, 137 were randomized into the study (89 in AMX0035, 48 in placebo). For an exploratory proof of concept study, the size of the study population is considered acceptable. 105 subjects completed the 24-week double-blind treatment phase (67 in AMX0035, 38 in placebo).

Of the 67 subjects in the AMX0035 group who completed the double-blind portion of the study, 7 subjects (8.0%) were listed as "*discontinued study medication prior to study completion*". Almost all of these subjects prematurely discontinued study medication due to gastrointestinal AEs. Additional analyses have been provided on whether (the removal of) these subjects impacted efficacy. While there appears to be no impact on ALSFRS-R effect size, the most valid analysis method was not used. However, additional analyses are not considered warranted in light of all the issues identified with the overall study.

The study population consisted primarily of white (~95%), middle-aged (~58 years old) males (~68%) with overweight (mean body mass index ~27). Baseline disease characteristics were largely similar, with a slight imbalance in ATLIS upper & lower scores. The study was conducted in the US only, and additional justification was requested. Reference is made by the applicant to the paper of Takei et al. (2017), which compared clinical guidelines and clinical trial data between Japan, the US and the EU. Some differences are observed between the regions concerning patient demographics and clinical practice. While these differences would likely not lead to a different interpretation of efficacy/safety between the regions, the reliability is questioned as most of the data is based on clinical trials and not naturalistic data. Moreover, the issue remains that Study AMX3500 concerned a specific ALS subpopulation. Based on information from patient representatives, as well as news articles it seems that ALS patients are self-medicating with either one or both of the components of AMX0035. As stated before, there are concerns about whether prior exposure to the product could have impacted blinding. Subjects were only excluded from the study if prior exposure to PB or TUDCA occurred in less than 3 months prior to study entry. According to the applicant, self-medication with the components of AMX0035 started after the study start was announced.

24-week double-blind treatment phase

As stated above, there appears to be an effect of AMX0035 on function as measured by the ALSFRS-R at week 24 (2.32, p=0.0340), but the credibility of the effect is questioned due to the model used for analysis. The method relies on several assumptions, which are all questioned. First, linearity of progression is assumed, which, after seeing the graph with the linear estimate and the actual observations, exaggerates the treatment effect. Second, the analysis is performed on the mITT analysis, ignoring two patients included in the ITT population that died before efficacy measurements. Third, missing data due to discontinuations or death were imputed, assuming the data was MAR. Therefore, re-analyses were requested. Based on the CHMP analysis (i.e. combination of the ITT population instead of mITT, categorical time (i.e., not relying on assumptions on linearity), a placebo-based imputation for discontinuations instead of missing at random, and a worst-case imputation (ALSFRS-R score of "0") in case of death), the effect size is smaller, and the endpoint is no longer statistically significant (1.52, p=0.20).

At week 24 there was no statistically significant difference between AMX0035 and placebo in the rate of decline on the total ATLIS score ($p_{nominal} > 0.1129$). The testing hierarchy ends here, and thus aside from the primary endpoint, all remaining endpoints have not been formally tested.

None of the secondary endpoints supports the findings of the primary endpoint. Moreover, there was also no difference shown between the treatment arms on the biomarkers pNF-H ad pNF-L. With the re-analysis of the model and the sensitivity analyses provided, it can be concluded that the effect estimate and determination of statistical significance depends on the choice made for the analysis method. During the assessment, no convincing arguments have been provided that would lead to a different insight on what would be the best analysis. Hence, the robustness and statistical compellingess of the results of study AMX3500 was not demonstrated. Consequently, as the true effect of AMX0035 cannot be estimated, the clinical relevance of the findings cannot be determined.

Open-label extension phase

Of the 97 subjects who completed the 24-week double-blind treatment phase on study drug, 90 entered the OLE phase. The OLE study mITT population consisted of 32 subjects in the placebo > AMX0035 (PA) group and 54 in the AMX0035 > AMX0035 (AA) group. At week 48, there were only 55 subjects left in the study (19 in PA, 36 in AA).

The baseline demographics for the OLE were consistent with those of the double-blind treatment phase. Most of the OLE baseline disease characteristics were consistent with what has been observed in the double-blind treatment phase taking into account ALS disease progression of 6 months. Baseline SVC % predicted, and ALSFRS-R total scores were comparable between the PA and AA arms. This is consistent with the observation that the actual ALSFRSR-R scores nearing the end of the initial 24-week treatment phase are coming together.

An extended slope analysis was performed at week 48 for the rate of decline in ALSFRS-R. The analysis is based on the same model for the double-blind, for which several issues have been highlighted. In addition, the extended slope analyses do not take into account that subjects who initially received placebo switched to active treatment, as well as the selection bias at the start of the OLE. Therefore, the results cannot be adequately interpreted.

Survival

Survival was evaluated at two timepoints: July 20, 2020 and March 1, 2021.

The EMA ALS guideline recommends a composite survival endpoint that evaluates time to death, tracheostomy or permanent ventilation. In the current survival analysis, here is referred to as "time to death or death equivalent". Subjects who died after study discontinuation have also been incorporated in this analysis, as vital status was captured via Omnitrace. Although study cites were encouraged to

report permanent assisted ventilation events after study discontinuation, the risk remains that the data is incomplete as this has not been systematically evaluated, such as all-cause mortality (via Omnitrace). Therefore, overall survival (i.e. time to death), is considered the more relevant evaluation.

At the March 1, 2021 cut-off, the median time to death was 23.5 for the AA group and 18.7 for the PA group (hazard ratio 0.619, $p_{nominal}$ = 0.0324). This suggests a difference in survival of around 5 months between the treatment groups.

There were several uncertainties identified with the performed survival analysis. First, it was questioned whether the proportional hazard assumption holds as subjects initially randomized to placebo switched to active treatment. The CHMP requested an analysis that does not assume a proportional hazard, i.e. inclusion of a time-varying covariate incorporating the treatment switch in the model and confidence interval of the median. These re-analyses were provided and are consistent with the initial findings, i.e. there appears to be a survival effect, and the reported hazard ratio and p-value are more positive compared to the original analysis.

It is known that functional deficits precede survival outcomes in ALS. It is argued that subjects randomized to AMX0035 in the study had a slowing the functional decline (on ALSFRS-R), and this is expected to predict a future delay in time to death. However, the true effect on functional decline cannot be established. ALS symptom progression result from the degeneration of motor neuron degeneration, causing the loss of motor function. It is hypothesized that AMX0035 reduces neuronal and cell death by the PB/TURSO combination via mitigating both ER and oxidative stress, yet an effect on the pNF-H (biomarker indicating neuronal injury) could not be concluded, hampering support via the mechanism of action. Moreover, the survival curve should change in subjects on placebo and switching to active treatment after similar exposure time. This is not the case. Therefore, the plausibility of the survival effect observed remains questionable, as it is not supported by any slowing of functional decline across all the endpoints measured during the 24-week placebo-controlled treatment period. Aside from the plausibility of survival, the question targeted by the survival analysis is unclear. It does not pertain to the effect of 'delayed' treatment, as not all subjects from the main phase continued into the OLE, nor does it target the effect of active treatment vs placebo, as all subjects were on active treatment during the OLE. A difference in survival "regardless of the open-label extension" appears to target a hypothetical estimand strategy, which is not agreed. Assuming a treatment effect, if the open label extension would not have been performed and patients would not have continued with or switched to AMX0035, survival would likely have been different. Therefore, the issue remains what question is targeted and due to the study design, with a voluntary OLE and treatment switch, it was not possible to accurately define it.

Ancillary analyses

The provided responder analysis is hard to interpret. Initially, a responder is defined as any subject with the same pre-treatment rate of progression on the ALSFRS-R or less. In principle, with this definition under the linearity assumption, all placebo subjects should be a responder. As this was not observed in the analysis, the linearity assumption is challenged. An additional responder analysis was provided where a responder was defined as any subject who has less than a 6-point decline on the ALSFRS-R at week 24. This cut-off is based on the assumption that on average, an (untreated) ALS patient experienced 1 point decline on the ALSFRS-R per month. This definition of a responder is not supported as the assumption that a one-point difference on ALSFRS-R is clinically meaningful is not agreed as this difference has not been validated as a minimal clinically important difference in the ALSFRS-R. However, in light of the issues identified with the overall results of study AMX3500, additional responder analyses were not considered warranted.

Subgroup analyses were performed *post hoc* by the applicant. In general, it appears that the effect of AMX0035 was largely consistent across the subgroups. There appears to be some suggestion of effect modification for gender, pre-treatment progression rate, bulbar onset and baseline ALSFRS-R score. Contradictory appears the difference in direction for pre-treatment progression rate and baseline ALSFRS. However, the interpretation of these subgroups is hampered by the large overlapping CIs and the small subgroups. As such, no conclusions can be drawn about whether AMX0035 is more efficacious (or not) in a specific subpopulation evaluated in the study or not.

Additional expert consultation

During the procedure, a SAG Neurology meeting was convened upon request of the CHMP. The following issues were discussed:

1. Does the SAG consider the effect size observed with Albrioza with regard to improvement in the ALSFRS-R score at week 24 as clinically meaningful? Please consider the applicant's analysis as well as the analysis requested by CHMP.

A majority of SAG-N experts agreed that an effect of 2 points (or more) on ALSFRS-R score may be clinically relevant, provided that the estimate is valid or confirmed by another study.

It was mentioned that for some specialists the cut-off for efficacy could be higher (effect of 3.2 for Fournier et al, 2022).

Some SAG-N experts expressed the opinion that imputing a value of 0 for the ALSFRS-R score if the patient dies is not considered as a standard practice.

The SAG-N experts pointed out that the results of the different analyses which were performed (i.e., Applicant's analysis, CHMP requested analysis and FDA' analysis) gave divergent results for both the effect size and the significance level. The CHMP and FDA analyses resulted in an effect lower than 2 points (1.5 and 1.8, respectively) for the ALSFRS-R score, which was not considered as relevant for a majority of experts. The uncertainty about the results was such that that the SAG-N experts considered that the study results cannot be considered as reliable enough and that they should be confirmed in a phase III trial.

From a patient perspective, an opinion was expressed that there was a strong wish from the ALS community for having this drug available, and if the requested confirmatory study supports the effect of the drug, not approving it based on the current data would mean that the access to an efficacious drug for ALS was delayed for current patients. It was also stated that the drug is not a "game changer" and that results are not robust, however this drug still might be an option if used with another drug for ALS, and such an alternative will be needed for the heterogenous ALS population. Thus, although the drug will not be a gamechanger to cure the disease, a conditional approval will be a gamechanger for the ALS community in Europe.

2. Considering the effects on functional endpoints and the study duration of 24 weeks, does the SAG consider the observed effects on survival from the post hoc analyses robust and plausible?

Although a 4-5 month survival difference, if valid, can be considered as a huge effect size for ALS, SAG-N experts were not convinced that the survival data are robust and plausible. The SAG-N experts raised methodological issues including

- that results came from post hoc analyses with no control for multiplicity,
- a small sample size

- and, in particular, the pattern of survival curves in the study does not match with the expected pattern under the assumption that the drug works, considering the large number of patients in the placebo group crossing over to Albrioza after 24 weeks, without any apparent effect on the survival curves.

Further, as the study did not meet the primary endpoint (as per the CHMP analysis), and in the applicant's primary hierarchical analysis the first of the secondary endpoints (including survival) failed to demonstrate a significant difference all other endpoints should be considered as exploratory only.

3. How does the SAG consider the overall strength of evidence for efficacy of Albrioza taking into account the totality of the results, including secondary endpoints (ATLIS, SVC, survival)?

The SAG-N considers that the evidence provided by the applicant should be regarded only as hypothesis generating, because of the lack of support from primary and secondary endpoints.

Most SAG-N experts were not fully convincing about the use of ATLIS for measuring muscle strength in ALS studies. However, the SAG-N experts stated that measuring respiratory function (SVC) is relevant and that not having an effect on this scale questions the efficacy of the drug. On the other hand, one expert expressed that the results go in the right direction and that the lack of efficacy may be explained by the short study duration. Further, some experts highlighted that no effect was seen in biomarkers, while an effect would be expected according to expert opinion.

The SAG-N experts consider that the overall strength of evidence for efficacy of Albrioza is weak, but can be considered hypothesis generating, needing to be confirmed in a phase III trial.

4. Does the SAG consider that the results from study AMX3500 (CENTAUR) in "definite" ALS patients can be extrapolated to the broader ALS population?

Overall, the majority of the SAG-N experts considered that the available results do not allow for a firm conclusion on the extrapolability of the results to the broad ALS population. The uncertainty about the data was highlighted. Additionally, one expert pointed out that based on the eligibility criteria of this study less than 7% of ALS patients population in their respective country-wise registry would be eligible for this treatment.

Additional EMA Methodology Working Party consultation

During the procedure, the EMA Methodology working party meeting was convened upon request of the CHMP. The following issues were discussed:

The statisticians directly involved in the (co)Rapporteurs assessment provided a summary of the methodological issues for discussion. It is noted that there is no divergence between Rapporteur and Co-Rapporteur's statistical assessment. The CHMP request was interpreted as a more in-depth assessment of the methodological considerations and consequences underpinning the major objection (5) related to efficacy.

There was general agreement during the tOEG discussion that the applicant's primary analysis presents several deficiencies:

- the analysis does not include all randomised patients (mITT instead of ITT),
- the analysis model is based on the questionable assumption of linearity over time,
- there is a lack of adequate handling of missing data due to discontinuations and deaths (implicitly assumed to be missing at random).

The Methodology Working Party (MWP) assessment is structured following in the different estimands associated with the primary, secondary and additional analyses that were presented for the primary

efficacy endpoint, the ALSFRS-R, in the main study. Secondly, the (unplanned) additional survival analyses based on the open label extension are addressed.

Primary protocol pre-defined analysis

The predefined analysis of the ALSFRS-R corresponds to a hypothetical strategy for the intercurrent events treatment discontinuation and death. This can (still) be of value for regulatory decision making as part of assessing the totality of evidence, but usually not without supportive analyses that take events such as death adequately into account. This primary predefined analysis essentially compares *the estimated rate of linear decline* between groups, *in the modified ITT population* (excluding subjects that did not start medication or did not have any post baseline assessment of ALSFRS-R). The primary estimand this primary analysis would be aligned to would be: 'what is the difference in rate of decline in the ALSFRS-R score in patients with ALS in the absence of treatment discontinuations and deaths?'. It was statistically significant (p=0.0340), and is a customary analysis for ALSFRS-R (Table 16). Hence, at the time of protocol development could possibly have been a supported primary analysis.

Table 16: ALSFRS-R Total Score at Week 24: mITT

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

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

However, upon usual scrutiny of the model (analysis of residuals, and adding non-linear terms to explore the linearity assumption), the assumption of linearity is not adequately supported by the data. As a sensitivity analysis in the full ITT population (within this same hypothetical strategy, targeting essentially the same estimand, assuming missing at random), an MMRM analysis with visit as categorical factor fitting leads to a slightly smaller treatment difference but with a p-value still below 0.05 (difference between groups 2.15, p=0.0335). Nevertheless, it is noted from the FDA clinical and statistical review that a similar analysis approach in the mITT (MMRM under MAR without linearity assumption) led to a smaller estimated difference and a loss of statistical significance (difference between groups 1.86, p-value 0.0749). Hence, statistical conclusions based on the hypothetical strategy are not considered very robust. Given the (somewhat informal) pre-defined hierarchical evaluation of secondary endpoints, this lack of robustness translates to secondary endpoints, which do not strongly support the effect seen in the primary endpoint.

Alternative estimand strategies

Alternative estimand strategies that aim to include death or death equivalent events such that the associated estimand can be considered more directly following a treatment policy strategy include the (1) joint rank analysis, which is supported for the PHOENIX Study and (2) worst case imputation (for death) and placebo imputation (for discontinuations) in an MMRM model for the change from baseline in ALSFRS-R score (Table 17) and where visit is included as a categorical variable (thus enabling a non-linear time trend). The latter were conducted on request of (co-) rapporteurs, the first on request of FDA (and supported by CHMP for the PHOENIX study) . In an *appropriate joint rank analysis* (i.e., ranking based on multiple imputation for missing values and applied to the ITT population), the p-value for the difference is 0.0785 – with the effect size difficult to interpret clinically. For the MMRM analysis with

imputation of missing data as indicated, it is noted that the specific method of placebo imputation implemented in the *post hoc* analysis was not entirely clear. This is relevant as several approaches would be possible (i.e. jump to reference, copy reference, copy increments in reference…), but the respective assumptions involved for missing data would not be equally acceptable from a regulatory perspective. The results of the analysis presented for the full ITT population lead to a smaller estimated treatment effect (1.52), which is non-significant. This can be considered to target an estimand for the treatment effect assuming all subjects who die are treatment failures (worst case assumption for the change from baseline in the ALSFRS-R score), and those that withdraw from the trial receive no further benefit from randomised treatment (placebo imputation, which assumes these participants can still experience some placebo effect).

Table 17: ALSFRS-R change from baseline - worst case imputation for missing values due to death in the categorical MMRM without linearity assumption - ITT population

Endpoint Time Point	Estimate (95% CI)		Estimated Difference	95% CI	p-value
	Placebo+SOC (N=48)	AMX0035+SOC (N=89)			
Change from baseline ALSFRS-R Total (ITT, without linearity assumption, placebo-based imputation for missing values after discontinuation and worst-case imputation for missing values from discontinuation due to death)					
Week 24	9.32 (-11.18, -7.45)	-7.79 (-9.19, -6.40)	1.52	(-0.80, 3.84)	0.20

CI: Confidence interval, SOC: Standard of care

Hence, a plausible treatment policy estimand strategy does not lead to statistically compelling results; it is associated with p-values (that expresses the strength of evidence) equal or larger than 0.0785. It should be noted, however, that these analyses are all *post hoc* (including the specific implementation of imputation strategies) and are not sensitivity analyses for the pre-defined primary, as they target different estimands.

Survival analyses

The survival analyses based on the OLE part were derived from sweeps of survival status, and were fully *post hoc*. It is suggested to only take the analyses with death as endpoint into primary consideration, as these have a less ambiguous ascertainment of the endpoint. The comparison between groups essentially compares continuous treatment with Albrioza with a delayed start: first 6 months placebo followed by continuous treatment with Albrioza. Given the substantial number of patients not entering the OLE, this comparison is not easy to interpret (e.g., to which patient or study population it actually applies). Any difference observed is (by design) essentially attributed to the difference in treatment during the first six months. Statistically, the Cox proportional hazards analysis in combination with the relatively complete follow-up provides a proper analysis for hypothesis testing. This analysis is fully *post hoc* and with an associated p-value indicative of significance at the nominal 5% level. The statistical model seems to imply a "treatment policy" strategy for handling intercurrent events (it includes all available data regardless of intercurrent events). Nevertheless, the relevance of this strategy can be questioned in the context of the crossover after 6 months (the treatment switch does not reflect any real life treatment policy) and the relative large number of subjects that did not continue into the OLE, making the interpretation of the survival curves difficult after the initial 6 month period. The proportional hazards assumption could be of concern for estimating the absolute size of the treatment difference (in median or mean survival), but results from RMST analysis were consistent with the Cox regression, which is reassuring. The analysis is not, in principle, expected to be biased in favour of the experimental arm. The main issue, therefore, remains the plausibility of the observed treatment effect on survival. The *post*

hoc exploratory nature as well as the clinical relevance of the survival analysis were both acknowledged, making it difficult to settle on the weight to be given to such results.

Reflections

Further alternative analyses, possibly based on other estimands with more or less conservative assumptions (in comparison with predefined and already requested analyses) might be valuable. Indeed, other imputation approaches after discontinuations / deaths could be considered based on different scenarios. For instance, it could be assumed that patients who withdraw may retain some of the benefit accrued during the treatment period. Alternatively, a joint model of change from baseline in ALSFRS-R scores and survival could be estimated, which would account for the association between change from baseline and mortality. Given the diversity of estimands and associated treatment effect estimates and hypotheses tests already presented, such additional *post hoc* analyses will not likely change the primary assessment of efficacy or its statistical robustness.

From regulatory perspective it is worth noting that the FDA also concluded on the lack of statistical robustness of the primary endpoint. Thus, from a statistical perspective, there is no disagreement with EMA Rapporteurs' point of view.

Conclusions

Based on the mostly non-significant sensitivity / supplementary analyses, it can be concluded that the primary analysis results are **not statistically robust**. Given the range of estimands and estimates already investigated, additional estimands and associated (unbiased) estimates for ALSFRS-R as primary endpoint cannot be expected to make the results more robust or compelling.

The need for any additional estimand(s) and associated statistical analysis is therefore primarily driven by clinical judgement for regulatory decision making, and will require clinical input as well as statistical considerations. If considered needed from a clinical perspective, it is advised to define the estimand and analyses as part of the request to the applicant, rather than asking for a proposal.

The survival analysis does not raise particular statistical concerns, but **its interpretability is challenging** in the context of a treatment switch after 6 months.

Additional efficacy data needed in the context of a conditional MA

Study AMX3500 was a phase II proof of concept study performed in a small ALS population. The study protocol was revised several times while the study was already ongoing. The effect estimate and clinical significance for the primary endpoint depends on the analysis method applied. None of the secondary endpoints support the primary endpoint, confirming overall concerns with the robustness of study AMX3500. Moreover, the clinical relevance of the observed effects cannot be evaluated as the true effect of AMX0035 cannot be determined. The observed effect on survival is not considered plausible, as functional loss precedes mortality, and slowing of functional loss cannot be concluded through the methodological issues observed. Moreover, no change is observed in the curve of subjects who switched from placebo to AMX0035 after similar exposure. Overall, the provided data from Study AMX3500 is not considered sufficient to establish a positive benefit risk.

The applicant has proposed the ongoing A35-004 study as SOB. The feasibility of ongoing study A35-004 to provide the confirmatory evidence needed is questionable. With the recent approval of AMX0035 by the FDA, a large proportion of US subjects prematurely discontinued the study. Based on the cut-off of 16 Jan 2023, of the 112 US participants enrolled in the study, 79 discontinued the study prematurely as they had an opportunity to transition to commercial therapy, confirming the potential to impact study conduction. During the procedure, the applicant confirmed that the study concluded the recruitment in February 2023 with 664 subjects and 552 EU participants which is reassuring. To ensure that EU subjects

complete the 48-week placebo-controlled study, the applicant proposes to manage the timing of commercial drug availability in the EU not earlier than the last patient out (Dec 2023/Jan 2024), i.e., not place the medicinal product on the market. This strategy is not considered acceptable from an ethical perspective but also from a regulatory perspective in particular in the frame of a CMA (see criteria above). Later, the applicant clarified that it is anticipated that around half of the randomized subjects will have their week 48 visit before October 2023, with the other half before January 2024. Thus, the study is well under way at the time of the opinion. Based on the provided argumentation, in the hypothetical situation that the product would be commercially available after granting the marketing authorisation, the impact on study participation would be small. Although the timelines presented by the applicant cannot be considered certain, this is considered reassuring.

2.6.7. Conclusions on the clinical efficacy

The CHMP considers that currently, efficacy has not been conclusively demonstrated. The study results are considered to be neither robust nor statistically compelling, following a number of sensitivity analyses, using different assumptions examining the robustness of the observed treatment effects and statistical inferences. The effect estimate for the primary endpoint varies significantly based on the applied analysis method. Thus, the true treatment effect of AMX0035 and its clinical significance cannot be estimated due to the uncertainties highlighted above.

Regarding the generalisability of the results of Study AMX3500, it must be highlighted that it included a subpopulation of ALS patients, and in the absence of an appropriately justified extrapolation exercise, any potential benefit of AMX3500 cannot be reliably generalised to the general ALS population. The potential functional benefit (the true effect size of which could not be estimated by the time of opinion) could not be assumed for the "real life" ALS population, but only for a subpopulation of patients that is similar to the one recruited in the study AMX3500. In addition, it is also unclear, how such a population could best be identified in clinical routine practice.

2.6.8. Clinical safety

2.6.8.1. Patient exposure

To substantiate the safety of AMX0035, the applicant submitted safety data of AMX0035 for the treatment of ALS is primarily based on data from a Phase 2 study (AMX3500) with a completed main phase and a completed OLE phase in adults with ALS. Additional supportive safety data come from a completed study of AMX0035 in healthy volunteers (A35-002) (See Table 18 for study characteristics).

Table 18 : Clinical Studies Contributing to the Safety Evaluation*

Protocol Number/ study status	***Study Design***	***Subject Population***	***Dose & Route of Adminis-tration***	***Number of Subjects Treated***	***Safety assessment***

AMX3500 (main phase) (CENTAUR) Completed	Phase 2, multicenter, randomized, double-blind, placebo controlled 24-week study	Male or female subjects 18 to <80 years of age with a confirmed definite diagnosis of sporadic or familial ALS with ALS symptom onset of ≤18 months, and an SVC of ≥60% of predicted at the Screening Visit	3 grams PB and 1 gram TUDCA or placebo 1 sachet in the morning daily for 3 weeks. If tolerated, dosing was increased to 2 sachets Oral	137 (48 placebo; 89 AMX0035)	Safety was assessed by AEs, vital signs, electrocardiograms, clinical laboratory testing, physical and neurological examinations, the Columbia-Suicide Severity Rating Scale (C-SSRS), as well as the Upper Motor Neuron Burden Scale for subjects in the Magnetic Resonance-Positron Emission Tomography Sub-Study.
AMX3500 (OLE phase) (CENTAUR-OLE)	Open-label long-term (up to 132 weeks) follow-up to the main 24-week study	Subjects who completed the AMX3500 main study	3 grams PB and 1 gram TUDCA No dose escalation Oral	90 (all with AMX0035)	Long-term safety of AMX0035 was evaluated cumulatively through the main phase and the OLE phase.
A35-002 (Phase I)	Phase 1, single-dose, open-label, balanced 2-period crossover, pharmacokinetic, food-effect study	Healthy male or female subjects aged between 40 and 65 years, inclusive	a single oral dose of AMX0035 on 2 occasions, with a washout of no fewer than 4 days between periods	14 (all with AMX0035)	Safety was assessed by AEs, vital signs, ECGs, clinical laboratory testing, and physical examinations. A safety follow-up telephone call was conducted 7 days after the final dose.

* adapted by Assessor. Abbreviations: ALS = amyotrophic lateral sclerosis, OLE = open-label extension; PB = phenylbutyrate; SVC = slow vital capacity

Data cut-off dates for the two studies are as follows:

A35-002 (Phase I): None. Study and analyses complete.

AMX3500 (including OLE phase; Phase II):

- All data available for the randomized phase.
- All data available through 48 weeks of treatment.
- For long-term survival evaluation, a data cut-off of 01 March 2021 was used.

A tabulated overview of the exposure to AMX0035 for study AMX3500, main phase and OLE is provided in Table 19.

Table 19: Extent of exposure to study medication, safety population (Study AMX3500)*

Parameter	Main Phase		OLE Phase for Subjects Who Entered OLE		
	Placebo (N=48)	AMX0035 (N=89)	PA (N=34)	AA (N=56)	All Subjects Who Entered the OLE (N=90)
Duration of Exposure (weeks)					
N	48	89	34	56	90
Mean (SD)	21.5 (5.82)	19.7 (7.89)	32.9 (33.49)	43.7 (36.68)	39.6 (35.71)
Median	23.9	23.9	15.4	31.4	27.7
Q1, Q3	22.8, 24.1	16.3, 24.4	6.1, 51.0	19.5, 60.0	11.6, 59.7
Min, Max	1.0, 25.9	0.6, 31.6	0.4, 112.4	0.6, 134.6	0.4, 134.6
Mean Difference (SE) vs. Placebo	1.8 (1.30)		10.8 (7.72)		NA
Number of Subjects (n [%])					
Increased Dose to 2 Sachets	45 (93.8)	79 (88.8)	NA	NA	NA
Did Not Increase Dose to 2 Sachets	3 (6.3)	10 (11.2)	NA	NA	NA
Drug compliances (%) [1] [2]					
N	47	86	33	55	88
Mean (SD)	90.2 (15.67)	91.8 (15.44)	85.6 (19.23)	88.0 (23.9)	85.9 (21.74)
Median	95.6	96.3	90.7	92.2	92.2
Q1, Q3	89.2, 99.0	88.8, 99.4	83.6, 97.6	78.6, 98.4	81.3, 98.2
Min, Max	7.7, 100.3	36.8, 166.7	0.4 (4.81)		15.0, 172.9
Mean Difference (SE) vs. Placebo	1.6 (2.82)				NA

*adapted by assessor. AA = group of subjects randomized to AMX0035 in the main phase and who stayed on AMX0035 upon enrollment in the OLE phase; NA = not applicable; OLE = open-label extension; PA = group of subjects randomized to placebo in the main phase and who switched to AMX0035 upon enrollment in the OLE phase; SD = standard deviation; SE = standard error.
1 Number of Drug Administrations = Total Sachets Consumed. Sachets Consumed is reported in drug accountability (SDTM.DA).

2 Compliance = Total Sachets Consumed/Total Sachets Required. Sachets Required is also reported in drug accountability (SDTM.DA).

Expressed in patient months, the exposure to AMX0035 was 404 patient months in the main study phase, 821 months in the OLE phase and cumulatively 1236 patient months. Expressed in patient-years, the exposure to AMX0035 in the main phase of the study was 33.7 patient-years, in OLE 68.4 patient-years and cumulatively 103 patient-years.

2.6.8.2. Adverse events

The incidence of Treatment -emergent (TEAEs) per SOC observed during the 24-week placebo-controlled main phase and long-term are presented in Table 20 and Table 21, respectively.

The common TEAEs (≥5% of subjects in any treatment group) observed during both the 24-week placebo-controlled main phase and long-term (cumulatively) are presented in Table 22.

Table 20: Treatment-emergent Adverse Events of Subjects in Either Treatment Group by MedDRA System Organ Class for Double-Blind Phase – Safety Population

MedDRA System Organ Class	Treatment Group(s)		
	Placebo (N=48) n (%)	AMX0035 (N=89) n (%)	Overall (N=137) n (%)
Subjects With at Least 1 TEAE (n [%])	46 (95.8%)	86 (96.6%)	132 (96.4%)
Total Number of TEAEs (n)	318	597	915
Gastrointestinal Disorders	30 (62.5%)	59 (66.3%)	89 (65.0%)
Musculoskeletal and Connective Tissue Disorders	19 (39.6%)	37 (41.6%)	56 (40.9%)
Injury, Poisoning and Procedural Complications	22 (45.8%)	31 (34.8%)	53 (38.7%)
Nervous System Disorders	20 (41.7%)	35 (39.3%)	55 (40.1%)
Infections and Infestations	19 (39.6%)	28 (31.5%)	47 (34.3%)
Respiratory, Thoracic and Mediastinal Disorders	12 (25.0%)	27 (30.3%)	39 (28.5%)
General Disorders and Administration Site Conditions	13 (27.1%)	20 (22.5%)	33 (24.1%)
Investigations	10 (20.8%)	23 (25.8%)	33 (24.1%)
Skin and Subcutaneous Tissue Disorders	7 (14.6%)	17 (19.1%)	24 (17.5%)
Psychiatric Disorders	10 (20.8%)	14 (15.7%)	24 (17.5%)
Renal and Urinary Disorders	8 (16.7%)	11 (12.4%)	19 (13.9%)
Metabolism and Nutrition Disorders	4 (8.3%)	10 (11.2%)	14 (10.2%)
Vascular disorders	3 (6.3%)	9 (10.1%)	12 (8.8%)
Cardiac disorders	0 (0%)	7 (7.9%)	7 (5.1%)
Blood and lymphatic system disorders	2 (4.2%)	4 (4.5%)	6 (4.4%)
Eye disorders	0 (0%)	5 (5.6%)	5 (3.6%)
Reproductive system and breast disorders	3 (6.3%)	2 (2.2%)	5 (3.6%)
Product issues	1 (2.1%)	1 (1.1%)	2 (1.5%)
Surgical and medical procedures	1 (2.1%)	1 (1.1%)	2 (1.5%)
Ear and labyrinth disorders	1 (2.1%)	0 (0%)	1 (0.7%)
Hepatobiliary disorders	0 (0%)	1 (1.1%)	1 (0.7%)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	0 (0%)	1 (1.1%)	1 (0.7%)

Table 21: Treatment-emergent Adverse Events of Subjects in Either Treatment Group by MedDRA System Organ Class for Open-Label Phase – Safety Population

MedDRA System Organ Class	Treatment Group(s)		
	PA + SOC (N=34) n (%)	AA + SOC (N=56) n (%)	Overall (N=90) n (%)
Subjects With at Least 1 TEAE (n [%])	32 (94.1%)	49 (87.5%)	81 (90.0%)
Total Number of TEAEs (n)	145	305	450
Gastrointestinal Disorders	24 (70.6%)	20 (35.7%)	44 (48.9%)
Respiratory, Thoracic and Mediastinal Disorders	13 (38.2%)	20 (35.7%)	33 (36.7%)
Infections and Infestations	9 (26.5%)	17 (30.4%)	26 (28.9%)
Injury, Poisoning and Procedural Complications	6 (17.6%)	20 (35.7%)	26 (28.9%)
Musculoskeletal and Connective Tissue Disorders	5 (14.7%)	19 (33.9%)	24 (26.7%)
Nervous System Disorders	8 (23.5%)	15 (26.8%)	23 (25.6%)
General Disorders and Administration Site Conditions	6 (17.6%)	12 (21.4%)	18 (20.0%)
Investigations	5 (14.7%)	13 (23.2%)	18 (20.0%)
Psychiatric Disorders	2 (5.9%)	13 (23.2%)	15 (16.7%)
Skin and Subcutaneous Tissue Disorders	2 (5.9%)	8 (14.3%)	10 (11.1%)
Renal and Urinary Disorders	2 (5.9%)	7 (12.5%)	9 (10.0%)
Vascular disorders	2 (5.9%)	6 (10.7%)	8 (8.9%)
Metabolism and Nutrition Disorders	2 (5.9%)	5 (8.9%)	7 (7.8%)
Cardiac disorders	2 (5.9%)	4 (7.1%)	6 (6.7%)
Blood and lymphatic system disorders	1 (2.9%)	3 (5.4%)	4 (4.4%)
Product issues	0 (0%)	3 (5.4%)	3 (3.3%)
Eye disorders	0 (0%)	2 (3.6%)	2 (2.2%)

Hepatobiliary disorders	0 (0%)	2 (3.6%)	2 (2.2%)
Ear and labyrinth disorders	0 (0%)	1 (1.8%)	1 (1.1%)
Immune system disorders	1 (2.9%)	0 (0%)	1 (1.1%)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	0 (0%)	1 (1.8%)	1 (1.1%)
Surgical and medical procedures	0 (0%)	1 (1.8%)	1 (1.1%)

Table 22: Treatment-Emergent Adverse Events Occurring in ≥5% of Subjects in Any Group, Safety Population (Study AMX3500)

	Main Phase		**Cumulative from Main Phase Through OLE (Includes Subjects Who Did Not Enter OLE)**			
			By Former Randomized Treatment			**On AMX0035**
MedDRA SOC Preferred Term	**Placebo (N=48) n (%)**	**AMX0035 (N=89) n (%)**	**RP (N=48) n (%)**	**RA (N=89) n (%)**	**Combined (N=137) n (%)**	**(N=123)[a] n (%)**
Subjects With at Least 1 TEAE (n [%])	**46 (95.8)**	**86 (96.6)**	**46 (95.8)**	**89 (100.0)**	**135 (98.5)**	**120 (97.6)**
Number of Distinct TEAEs	318	597	459	901	1360	1039
Gastrointestinal Disorders						
Diarrhoea	8 (16.7)	19 (21.3)	13 (27.1)	26 (29.2)	39 (28.5)	34 (27.6)
Nausea	6 (12.5)	16 (18.0)	12 (25.0)	20 (22.5)	32 (23.4)	27 (22.0)
Constipation	12 (25.0)	12 (13.5)	15 (31.3)	16 (18.0)	31 (22.6)	19 (15.4)
Abdominal pain	4 (8.3)	7 (7.9)	4 (8.3)	8 (9.0)	12 (8.8)	8 (6.5)
Salivary hypersecretion	1 (2.1)	10 (11.2)	2 (4.2)	11 (12.4)	13 (9.5)	13 (10.6)
Vomiting	1 (2.1)	4 (4.5)	3 (6.3)	7 (7.9)	10 (7.3)	9 (7.3)
Dry mouth	4 (8.3)	3 (3.4)	5 (10.4)	3 (3.4)	8 (5.8)	3 (2.4)
Abdominal pain upper	1 (2.1)	4 (4.5)	3 (6.3)	5 (5.6)	8 (5.8)	7 (5.7)
Abdominal discomfort	0	5 (5.6)	0	7 (7.9)	7 (5.1)	7 (5.7)
Abdominal distension	1 (2.1)	4 (4.5)	3 (6.3)	4 (4.5)	7 (5.1)	6 (4.9)
Dysphagia	4 (8.3)	3 (3.4)	5 (10.4)	5 (5.6)	10 (7.3)	6 (4.9)
Musculoskeletal and Connective Tissue Disorders						
Muscular weakness	9 (18.8)	18 (20.2)	12 (25.0)	21 (23.6)	33 (24.1)	24 (19.5)
Back pain	4 (8.3)	5 (5.6)	4 (8.3)	6 (6.7)	10 (7.3)	6 (4.9)
Neck pain	5 (10.4)	2 (2.2)	5 (10.4)	4 (4.5)	9 (6.6)	4 (3.3)
Muscle spasms	3 (6.3)	5 (5.6)	3 (6.3)	6 (6.7)	9 (6.6)	6 (4.9)
Arthralgia	2 (4.2)	5 (5.6)	2 (4.2)	7 (7.9)	9 (6.6)	8 (6.5)
Musculoskeletal pain	2 (4.2)	5 (5.6)	2 (4.2)	6 (6.7)	8 (5.8)	6 (4.9)
Musculoskeletal chest pain	1 (2.1)	4 (4.5)	1 (2.1)	5 (5.6)	6 (4.4)	5 (4.1)
Pain in extremity	0	4 (4.5)	0	5 (5.6)	5 (3.6)	5 (4.1)
Injury, Poisoning and Procedural Complications						
Fall	18 (37.5)	25 (28.1)	19 (39.6)	33 (37.1)	52 (38.0)	39 (31.7)
Contusion	4 (8.3)	5 (5.6)	4 (8.3)	6 (6.7)	10 (7.3)	6 (4.9)
Skin laceration	3 (6.3)	6 (6.7)	4 (8.3)	8 (9.0)	12 (8.8)	9 (7.3%)
Skin abrasion	2 (4.2)	1 (1.1)	3 (6.3)	5 (5.6)	8 (5.8)	6 (4.9)
Stoma site pain	2 (4.2)	3 (3.4)	2 (4.2)	5 (5.6)	7 (5.1)	5 (4.1)
Nervous System Disorders						
Headache	11 (22.9)	13 (14.6)	11 (22.9)	15 (16.9)	26 (19.0)	16 (13.0)
Dizziness	2 (4.2)	9 (10.1)	4 (8.3)	13 (14.6)	17 (12.4)	15 (12.2)
Dysarthria	2 (4.2)	4 (4.5)	2 (4.2)	6 (6.7)	8 (5.8)	6 (4.9)
Syncope	2 (4.2)	1 (1.1)	3 (6.3)	2 (2.2)	5 (3.6)	3 (2.4)
Infections and Infestations						
Viral upper respiratory tract infection	2 (4.2)	10 (11.2)	2 (4.2)	12 (13.5)	14 (10.2)	12 (9.8)
Urinary tract infection	3 (6.3)	5 (5.6)	4 (8.3)	8 (9.0)	12 (8.8)	10 (8.1)
Upper respiratory tract infection	3 (6.3)	4 (4.5)	3 (6.3)	5 (5.6)	8 (5.8)	5 (4.1)
Pneumonia	1 (2.1)	2 (2.2)	3 (6.3)	4 (4.5)	7 (5.1)	6 (4.9)

MedDRA SOC Preferred Term	Main Phase		Cumulative from Main Phase Through OLE (Includes Subjects Who Did Not Enter OLE)			
			By Former Randomized Treatment			On AMX0035
	Placebo (N=48) n (%)	AMX0035 (N=89) n (%)	RP (N=48) n (%)	RA (N=89) n (%)	Combined (N=137) n (%)	(N=123)[a] n (%)
Fungal infection	2 (4.2)	1 (1.1)	3 (6.3)	1 (1.1)	4 (2.9)	3 (2.4)
Respiratory, Thoracic and Mediastinal Disorders						
Dyspnoea	4 (8.3)	9 (10.1)	4 (8.3)	14 (15.7)	18 (13.1)	14 (11.4)
Respiratory failure	4 (8.3)	4 (4.5)	9 (18.8)	10 (11.2)	19 (13.9)	15 (12.2)
Cough	3 (6.3)	5 (5.6)	6 (12.5)	7 (7.9)	13 (9.5)	10 (8.1)
Pneumonia aspiration	0	1 (1.1)	4 (8.3)	1 (1.1)	5 (3.6)	5 (4.1)
Investigations						
Alanine aminotransferase increased	3 (6.3)	4 (4.5)	3 (6.3)	5 (5.6)	8 (5.8)	5 (4.1)
Aspartate aminotransferase increased	3 (6.3)	4 (4.5)	3 (6.3)	4 (4.5)	7 (5.1)	4 (3.3)
Weight decreased	1 (2.1)	5 (5.6)	3 (6.3)	8 (9.0)	11 (8.0)	10 (8.1)
General Disorders and Administration Site Conditions						
Fatigue	3 (6.3)	7 (7.9)	3 (6.3)	9 (10.1)	12 (8.8)	9 (7.3)
Pyrexia	1 (2.1)	4 (4.5)	2 (4.2)	5 (5.6)	7 (5.1)	6 (4.9)
Oedema peripheral	3 (6.3)	2 (2.2)	3 (6.3)	3 (3.4)	6 (4.4)	3 (2.4)
Asthenia	0	5 (5.6)	0	5 (5.6)	5 (3.6)	5 (4.1)
Disease progression	2 (4.2)	0	4 (8.3)	2 (2.2)	6 (4.4)	5 (4.1)
Skin and Subcutaneous Tissue Disorders						
Rash	4 (8.3)	5 (5.6)	4 (8.3)	7 (7.9)	11 (8.0)	8 (6.5)
Psychiatric Disorders						
Anxiety	3 (6.3)	2 (2.2)	3 (6.3)	8 (9.0)	11 (8.0)	8 (6.5)
Affect lability	2 (4.2)	2 (2.2)	3 (6.3)	2 (2.2)	5 (3.6)	3 (2.4)
Insomnia	3 (6.3)	3 (3.4)	3 (6.3)	5 (5.6)	8 (5.8)	5 (4.1)
Depression	1 (2.1)	2 (2.2)	1 (2.1)	5 (5.6)	6 (4.4)	5 (4.1)
Renal and Urinary Disorders						
Proteinuria	2 (4.2)	6 (6.7)	2 (4.2)	7 (7.9)	9 (6.6)	7 (5.7)
Ketonuria	1 (2.1)	4 (4.5)	1 (2.1)	5 (5.6)	6 (4.4)	5 (4.1)
Metabolism and Nutrition Disorders						
Decreased appetite	2 (4.2)	7 (7.9)	3 (6.3)	9 (10.1)	12 (8.8)	10 (8.1)
Vascular Disorders						
Deep vein thrombosis	1 (2.1)	1 (1.1)	1 (2.1)	5 (5.6)	6 (4.4)	5 (4.1)

MedDRA = Medical Dictionary for Regulatory Activities; OLE = open-label extension; RA = group of subjects randomized to AMX0035 and stayed on AMX0035 in the OLE phase, plus the AMX0035 subjects who did not enter the OLE; RP = group of subjects randomized to placebo in the main phase and switched from placebo to AMX0035 in the OLE phase, plus the placebo subjects who did not enter the OLE; SOC = system organ class; TEAE = treatment-emergent adverse event

A The 123 subjects who received AMX0035 is the 137 subjects who entered Study AMX3500 minus the 14 subjects who received placebo in the main phase and did not enter the OLE phase.

Percentages are based on the number of subjects in each treatment group.

2.6.8.3. Serious adverse event/deaths/other significant events

Serious Adverse events

An overview of the severe TEAEs during the main and OLE phase is presented in Table 23.

Table 23: serious treatment-emergent adverse events in the main and OLE Combined Group, Safety Population (Study AMX3500)

MedDRA SOC Preferred Term	Main Phase		Cumulative from Main Phase Through OLE (Includes Subjects Who Did Not Enter OLE)			
			By Former Randomized Treatment			On AMX0035
	Placebo (N=48) n (%)	AMX0035 (N=89) n (%)	RP (N=48) n (%)	RA (N=89) n (%)	Combined (N=137) n (%)	(N=123) n (%)
Subjects with at least 1 SAE	8 (16.7)	11 (12.4)	18 (37.5)	27 (30.3)	45 (32.8)	40 (32.5)
Respiratory, Thoracic and Mediastinal Disorders						
Respiratory failure	3 (6.3)	2 (2.2)	8 (16.7)	8 (9.0)	16 (11.7)	13 (10.6)
Pneumonia aspiration	0	0	4 (8.3)	0	4 (2.9)	4 (3.3)
Acute respiratory failure	0	0	1 (2.1)	0	1 (0.7)	1 (0.8)
Dyspnoea	0	1 (1.1)	0	1 (1.1)	1 (0.7)	1 (0.8)
Pulmonary embolism	1 (2.1)	0	1 (2.1%)	1 (1.1)	2 (1.5)	1 (0.8)
Hypoxia	0	0	0	1 (1.1)	1 (0.7)	1 (0.8)
Pleural effusion	0	0	1 (2.1)	0	1 (0.7)	1 (0.8)
Pneumothorax	0	0	1 (2.1)	0	1 (0.7)	1 (0.8)
Respiratory arrest[a]	0	1 (1.1)	0	1 (1.1)	1 (0.7)	1 (0.8)
Infections and Infestations						
Bacteraemia	1 (2.1)	1 (1.1)	1 (2.1)	1 (1.1)	2 (1.5)	1 (0.8)
Catheter site infection	1 (2.1)	0	1 (2.1)	1 (1.1)	2 (1.5)	1 (0.8)
Pneumonia	0	1 (1.1)	1 (2.1)	1 (1.1)	2 (1.5)	2 (1.6)
Diverticulitis	0	0	1 (2.1)	0	1 (0.7)	1 (0.8)
Gastroenteritis	0	0	0	1 (1.1)	1 (0.7)	1 (0.8)
Implant site cellulitis	0	1 (1.1)	0	1 (1.1)	1 (0.7)	1 (0.8)
Medical device site infection	0	0	1 (2.1)	0	1 (0.7)	1 (0.8)

Deaths

Seven subjects died during the main phase of the study, and the incidence was similar between the AMX0035 group (5 subjects [5.6%]) and placebo group (2 subjects [4.2%]) (Table 24). The majority of deaths in the main phase (5 of 7) were from respiratory failure/arrest (3 subjects in the AMX0035 and 2 subjects in the placebo group). Other causes of death in the AMX0035 group included subdural hematoma, and diverticular perforation. None of the deaths was assessed as study medication related (i.e., possibly, probably, or definitely related) by the Investigator.

Table 24: Treatment-Emergent Adverse Events with Fatal Outcomes, Safety Population (Study AMX3500)

MedDRA SOC Preferred Term	Main Phase		Cumulative from Main Phase Through OLE (Includes Subjects Who Did Not Enter OLE)			
			By Former Randomized Treatment			On AMX0035
	Placebo (N=48) n (%)	AMX0035 (N=89) n (%)	RP (N=48) n (%)	RA (N=89) n (%)	Combined (N=137) n (%)	(N=123) n (%)
Subjects with a fatal TEAE	2 (4.2)	5 (5.6)	11 (22.9)	11 (12.4)	22 (16.1)	20 (16.3)
Respiratory, Thoracic and Mediastinal Disorders						
Respiratory failure	2 (4.2)	2 (2.2)	7 (14.6)	7 (7.9)	14 (10.2)	12 (9.8)
Pneumonia aspiration	0	0	1 (2.1)	0	1 (0.7)	1 (0.8)
Respiratory arrest	0	1 (1.1)	0	1 (1.1)	1 (0.7)	1 (0.8)
General disorders and administration site conditions						
Disease progression	0	0	1 (2.1)	1 (1.1)	2 (1.5)	2 (1.6)
Cardiac Disorders						
Cardiac arrest	0	0	1 (2.1)	0	1 (0.7)	1 (0.8)

MedDRA SOC Preferred Term	Main Phase		Cumulative from Main Phase Through OLE (Includes Subjects Who Did Not Enter OLE)			
			By Former Randomized Treatment			On AMX0035
	Placebo (N=48) n (%)	AMX0035 (N=89) n (%)	RP (N=48) n (%)	RA (N=89) n (%)	Combined (N=137) n (%)	(N=123) n (%)
Infections and Infestations						
Diverticular perforation	0	1 (1.1)	0	1 (1.1)	1 (0.7)	1 (0.8)
Injury, Poisoning and Procedural Complications						
Subdural haematoma	0	1 (1.1)	0	1 (1.1)	1 (0.7)	1 (0.8)
Nervous System Disorders						
Amyotrophic lateral sclerosis	0	0	1 (2.1)	0	1 (0.7)	1 (0.8)

MedDRA = Medical Dictionary for Regulatory Activities; OLE = open-label extension; RA = group of subjects randomized to AMX0035 and stayed on AMX0035 in the OLE phase, plus the AMX0035 subjects who did not enter the OLE; RP = group of subjects randomized to placebo in the main phase and switched from placebo to AMX0035 in the OLE phase, plus the placebo subjects who did not enter the OLE; SOC = system organ class; TEAE = treatment-emergent adverse event
Percentages are based on the number of subjects in each treatment group.

Adverse events of special interest

Neurological events

In the double-blind part of Study AMX3500, 20 (41.7%) subjects in the placebo group and 35 (39.3%) subjects in the AMX0035 group experienced a nervous system disorder. Three (6.3%) placebo subjects and 8 (9.0%) AMX0035 subjects experienced neurological TEAEs that were moderate or severe. Headache, dizziness and dysarthria occurred in ≥ 4 AMX0035 subjects, however, of these, only dizziness occurred more frequently in AMX0035 vs. placebo treated subjects (10.1% vs. 4.2%). No clear imbalance between treatment groups was derived regarding moderate or severe neurological TEAE by PT. An overview of neurological TEAEs considered related is presented in **Table *25***.

Table 25: Neurological TEAEs related to study drug during Study AMX3500

MedDRA System Organ Class Preferred Term	Placebo (N=48) n (%)	AMX0035 (N=89) n (%)	Overall (N=137) n (%)
Nervous system disorders	7 (14.6)	17 (19.1)	24 (17.5)
Headache	4 (8.3)	7 (7.9)	11 (8.0)
Dizziness	1 (2.1)	5 (5.6)	6 (4.4)
Dysgeusia	1 (2.1)	3 (3.4)	4 (2.9)
Somnolence	0	3 (3.4)	3 (2.2)
Migraine	0	1 (1.1)	1 (0.7)
Muscle contractions involuntary	0	1 (1.1)	1 (0.7)
Paraesthesia	0	1 (1.1)	1 (0.7)
Restless legs syndrome	1 (2.1)	0	1 (0.7)

Of note, TEAEs in the nervous system disorders SOC including headache, dizziness but also dysgeusia were also frequently reported in the placebo group. Further, the analysis of the most common TEAEs by 3 week intervals and SOC indicated no increase in the incidence of TEAEs in the nervous system disorders SOC with longer treatment duration, but rather the opposite. Therefore, comparative data derived from the placebo-controlled study phase are considered most informative.

Cardiac events

Cardiac events were considered of special interest as inhibition of hERG channels occurred at PBA and PAA concentrations 560-fold and 56-fold, respectively, the unbound UCD concentrations in adults. QTc prolongation up to 25 ms in the first 2 hours was seen with oral glycerol phenylbutyrate doses of 4 g/kg in unrestrained Cynomolgus monkeys (AusPAR 2016).

In the Cardiac Disorders System Organ Class, TEAEs were reported in only subjects who were receiving AMX0035 (n=13, 10.6%). The most common event was tachycardia occurring in 5 subjects (4.1%) followed by atrial fibrillation and palpitations, each reported in 2 subjects (1.6%), and atrioventricular block first degree, bundle branch block left, cardiac arrest and sinus tachycardia each reported in 1 subject (0.8%).

A detailed review of cardiac events in the entire study (main phase and OLE) by an expert cardiologist that included a review of subject medical history, concomitant medications, study medication administration history, results of all recorded ECGs, and narratives, concluded that the incidence of cardiac events in the 137 ALS subjects, many of whom were elderly, was low. The review further concluded that AMX0035 did not appear to be responsible for any serious cardiac events and was unlikely the cause of any minor cardiac events.

Pulmonary events

Pulmonary events were considered of special interest as this was the leading cause of death.

Aspiration pneumonia was reported in 5 subjects all while receiving AMX0035. None of the events was considered to be related to study medication, and it should be noted that these events are common in ALS. Three of the events were severe in intensity. Two subjects experienced acute events of aspiration pneumonia that ultimately led to death. Neither event was considered related to AMX0035.

Cumulatively, a total of 19 treatment-emergent events of respiratory failure have been reported. Fourteen events of respiratory failure were fatal, and none were assessed as related to the study drug.

Cumulatively, aspiration pneumonia was reported in 5 subjects all while receiving AMX0035. 1 subject in the main study part (with no subject in the placebo group) and 4 subjects during OLE part (all in the PA group).

2.6.8.4. Laboratory findings

Haematology, chemistry and urinalysis

Overall, in Study AMX3500 (main phase and the OLE phase for the PA and AA groups), there were no clinically relevant trends for changes over time in either treatment group in any haematology, chemistry, or urinalysis parameter. At the individual subject level, clinically significant laboratory abnormalities were reported; however, none resulted in premature discontinuation of the study drug.

The graphical presentation of mean changes from baseline in haematology laboratory values during study AMX3500 main phase is indicative of some (seemingly transient) lowering in mean erythrocytes, haematrocrit and haemoglobin, respectively in the AMX0035 group (which is in contrast to the placebo group). For erythrocytes, absolute changes from baseline to both weeks 6 and 12 were 0.1 X 10/L with standard deviations of 0.23 and 0.26 X 10/L respectively, with similarly small numerical differences from baseline to weeks 6 and 12 for haemoglobin and haematocrit. However, for the categorical variables, the incidence of subjects with decreased shifts in any of these parameters was generally comparable between the AMX0035 and placebo group throughout the main study phase.

More specifically, in the main phase of the study clinically significant laboratory abnormalities reported in $\geq$ 2 subjects in the AMX0035 group compared with the placebo group include:

- Crystal urine present (4 [4.5%] subjects in the AMX0035 group vs 0 subjects in the placebo group)
- Blood creatinine increased (2 [2.2%] subjects in the AMX0035 group vs 0 subjects in the placebo group), and
- Transaminases increased (2 [2.2%] subjects in the AMX0035 group vs 0 subjects in the placebo group)

In the OLE phase of the study, according to information displaying individual clinically significant abnormal laboratory values reported as TEAEs, the only PT reported in > 1 subject in any (PA, AA) treatment group were ALT increased (5.4% AA vs. 0% PA), AST increased (3.6% vs. 0%), and blood creatinine increased (3.6% vs. 0%).

Vital signs

As vital signs the applicant assessed respiratory rate, systolic/diastolic blood pressure, heart rate, body temperature and body weight. Clinically significant abnormal vital sign values were reported in the main phase, namely decreased weight. This was reported in more subjects who received AMX0035 (5 [5.6%]) than placebo (1 [2.1%]).

2.6.8.5. In vitro biomarker test for patient selection for safety

Not applicable

2.6.8.6. Safety in special populations

Intrinsic and extrinsic Factors

The sample sizes in the treatment groups in Study AMX3500 (main phase and OLE phase) were small and according to the applicant precluded subgroup analyses based on extrinsic factors. Nevertheless, safety analyses by gender and concomitant treatment with riluzole and edaravone, respectively were requested which did not clearly identify any clinically important differences. Based on available limited data, the AE profile in patients ≤65 and >65 years of age is comparable.

Use in Pregnancy and Lactation

There are no adequate studies of AMX0035 in pregnant women. Subjects or partners of male subjects should not become pregnant during treatment or 30 days after stopping treatment. If a female subject becomes pregnant, treatment with AMX0035 must be discontinued immediately.

It is not known whether AMX0035 is excreted in human milk. Caution should be exercised; therefore, no subject should nurse an infant while taking AMX0035.

Overdose

There have been no reported experiences involving an overdose of AMX0035, PB, or taurursodiol. In the event of an overdose, treatment should be discontinued immediately, and supportive measures implemented.

The AEs reportedly associated with high levels of phenylacetate (PAA, the primary metabolite of PB) have most commonly included nausea, headache, emesis, fatigue, weakness, lethargy, somnolence, dizziness, slurred speech, memory loss, confusion, and disorientation. Except for the symptoms of Kussmaul respiration, metabolic acidosis, cerebral oedema, and coma associated with a fatal overdose

of sodium phenylacetate/sodium benzoate, these symptoms have been reported as rapidly reversible with reduced dosing or interruption of dosing.

Drug Abuse

The dependence potential of AMX0035 has not been studied but is unlikely based on its mechanism of action.

Withdrawal and Rebound

The effects of withdrawal and rebound after discontinuation of AMX0035 have not been studied.

The impact of AMX0035 on the ability to drive, and operate machinery, or impairment of mental ability has not been studied. AMX0035 is associated with dizziness and somnolence, which may impair the ability to drive and use machines.

2.6.8.7. Immunological events

No information provided

2.6.8.8. Safety related to drug-drug interactions and other interactions

The applicant indicates that no human PK DDI studies were conducted. PB is metabolized in the liver, ultimately to phenylacetylglutamine, and then excreted by the kidney. Taurursodiol is transformed into the liver to ursodiol, and glycoursodeoxycholic acid, and is excreted in majority in faeces. The 2 drugs do not overlap in cytochrome P450 interactions or excretion pathways and, thus, are not anticipated to exhibit a DDI.

Drugs that might impair bile acid processing were not allowed during the study.

2.6.8.9. Discontinuation due to adverse events

During the phase II study adverse events led to drug interruption, dose reduction and withdrawal. See Table 26 for an overview.

Table 26: summary of adverse events requiring actions taken in the main and OLE Combined Group, Safety Population (Study AMX3500)*

	Main Phase		**Cumulative from Main Phase Through OLE (Includes Subjects Who Did Not Enter OLE)**			
			By Former Randomized Treatment			**On AMX0035**
MedDRA SOC Preferred Term	**Placebo (N=48) n (%)**	**AMX0035 (N=89) n (%)**	**RP (N=48) n (%)**	**RA (N=89) n (%)**	**Combined (N=137) n (%)**	**(N=123) n (%)**
Drug Interrupted						
Number Of Subjects With Reported Adverse Event	5 (10.4)	12 (13.5)	12 (25.0)	19 (21.3)	31 (22.6)	27 (22.0)
Number Of Distinct Adverse Events	5	31	17	53	70	66
Dose reduced						
Number Of Subjects With Reported Adverse Event	0	4 (4.5)	1 (2.1)	5 (5.6)	6 (4.4)	6 (4.9)
Number Of Distinct Adverse Events	0	8	4 (8.3)	3 (3.4)	7 (5.1)	6 (4.9)

MedDRA SOC Preferred Term	Main Phase		Cumulative from Main Phase Through OLE (Includes Subjects Who Did Not Enter OLE)			
			By Former Randomized Treatment			On AMX0035
	Placebo (N=48) n (%)	AMX0035 (N=89) n (%)	RP (N=48) n (%)	RA (N=89) n (%)	Combined (N=137) n (%)	(N=123) n (%)
Drug Withdrawn						
Number Of Subjects With Reported Adverse Event	5 (10.4)	18 (20.2)	20 (41.7)	30 (33.7)	50 (36.5)	45 (36.6)
Number Of Distinct Adverse Events	7	26	38	41	79	72

*by assessor Source: AMX3500 CSR Table 14.3.1.5 Part 2-5, and SCS Table T14.3.1.5 Part 2-5

TEAEs leading to study drug discontinuation is presented below (Table 27).

Table 27: Treatment-Emergent Adverse Events Leading to Study Drug Discontinuation in More than 1 Subject in the OLE Combined Group, Safety Population (Study AMX3500)

MedDRA SOC Preferred Term	Main Phase		Cumulative from Main Phase Through OLE (Includes Subjects Who Did Not Enter OLE)			
			By Former Randomized Treatment			On AMX0035
	Placebo (N=48) n (%)	AMX0035 (N=89) n (%)	RP (N=48) n (%)	RA (N=89) n (%)	Combined (N=137) n (%)	(N=123) n (%)
Subjects With at Least 1 TEAE Leading to Study Drug Discontinuation (n [%])	**5 (10.4)**	**18 (20.2)**	**20 (41.7)**	**30 (33.7)**	**50 (36.5)**	**45 (36.6)**
Gastrointestinal Disorders						
Diarrhoea	0	5 (5.6)	3 (6.3)	5 (5.6)	8 (5.8)	8 (6.5)
Nausea	1 (2.1)	1 (1.1)	4 (8.3)	3 (3.4)	7 (5.1)	6 (4.9)
Vomiting	0	0	2 (4.2)	2 (2.2)	4 (2.9)	4 (3.3)
Abdominal discomfort	0	0	0	2 (2.2)	2 (1.5)	2 (1.6)
Abdominal pain upper	0	2 (2.2)	0	2 (2.2)	2 (1.5)	2 (1.6)
Respiratory, thoracic and mediastinal disorders						
Respiratory failure	3 (6.3)	0	6 (12.5)	4 (4.5)	10 (7.3)	7 (5.7)
Pneumonia aspiration	0	0	2 (4.2)	0	2 (1.5)	2 (1.6)
Nervous system disorders						
Dysgeusia	0	2 (2.2)	1 (2.1)	2 (2.2)	3 (2.2)	3 (2.4)
General disorders and administration site conditions						
Disease progression	0	0	1 (2.1)	2 (2.2)	3 (2.2)	3 (2.4)
Infections and infestations						
Diverticulitis	0	1 (1.1)	1 (2.1)	1 (1.1)	2 (1.5)	2 (1.6)
Investigations						
Weight decreased	0	1 (1.1)	2 (4.2)	1 (1.1)	3 (2.2)	3 (2.4)
Metabolism and nutrition disorders						
Decreased appetite	0	1 (1.1)	1 (2.1)	1 (1.1)	2 (1.5)	2 (1.6)

Abbreviations: MedDRA = Medical Dictionary for Regulatory Activities; OLE = open-label extension; RA = group of subjects randomized to AMX0035 and stayed on AMX0035 in the OLE phase, plus the AMX0035 subjects who did not enter the OLE; RP = group of subjects randomized to placebo in the main phase and switched from placebo to AMX0035 in the OLE phase, plus the placebo subjects who did not enter the OLE; SOC = system organ class; TEAE = treatment-emergent adverse event

2.6.8.10. Post marketing experience

Not applicable

2.6.9. Discussion on clinical safety

The safety data include data collected in study AMX3500 (consisting of a main placebo-controlled phase and an open-label extension phase), with some additional data in healthy volunteers for cardiac function analysis.

The median exposure to AMX0035 in the main phase was 24 weeks, and in the OLE approximately 31 weeks. Thus, the available long-term safety data are restricted to a little more than 1 year. From the presented exposure data, it can be deduced that 123 ALS subjects overall were treated with AMX0035, and the maximum exposure duration exceeds 3 years. Approximately 44 subjects were treated for longer than 1 year; however, only a few subjects have been treated for longer than 2 years. Expressed in patient-years, the exposure to AMX in the main phase of the study was 33.7 patient-years, in OLE 68.4 patient-years and cumulatively 103 patient-years.

The most common AEs, i.e. adverse events reported with a frequency of > 10% main phase, are diarrhoea (8 (16.7%) vs. 19 (21.3%)), nausea (6 (12.5%) vs. 16 (18.0%)), constipation (12 (25.0%) vs. 12 (13.5%)), salivary hypersecretion (1 (2.1%) vs. 10 (11.2%)), muscular weakness (9 (18.8%) vs. 18 (20.2%)), neck pain (5 (10.4%) vs. 2 (2.2%), fall (18 (37.5%) vs. 25 (28.1%)), headache (11 (22.9%) vs. 12 (14.6), dizziness (2 (4.2%) vs. 9 (10.1%)), viral upper respiratory tract infection (2 (4.2%) vs. 10 (11.2%), dyspnoea (4 (8.3%) vs. 9 (10.1%)), for placebo and AMX0035 respectively. Based on the provided data on the duration of adverse events during the main phase of the study, there are no notable differences between the placebo and AMX0035 group in the duration of common treatment-emergent adverse events in overall.

The provided data on adverse events causally related to AMX0035 by dose (1 sachet or 2 sachets) suggests a dose-response in most adverse events, specifically diarrhoea, constipation, nausea, abdominal discomfort, abdominal pain upper, flatulence, dizziness, proteinuria, ketonuria and crystal urine present. Overall, throughout the entire study, there was no difference between the dose subgroups in the duration of the adverse events. Dose reductions were applied in some cases which also resulted in relief of the adverse event. However, dose reductions cannot be recommended, as the efficacy of lower dosing is unknown. The majority of patients were able to follow the 2 sachet dosing throughout the study, albeit some with temporary interruptions or dose reductions due to adverse events.

Serious adverse events & death

Severe AEs and SAEs were generally considered isolated cases (occurring in 1 subject), apart from respiratory depression and deep vein thrombosis. Most serious and severe TEAEs are either isolated cases or related to disease progression and reported in both placebo and AMX0035-treated groups. It is difficult to disentangle the disease progression from effects related to AMX0035 as there are uncertainties regarding the exposure, particularly as the events led to withdrawal from the trial.

In order to disentangle TEAEs related to AMX0035 from those related to the underlying disease, the safety profile derived from the placebo-controlled study phase is considered of particular importance. In this context, it is considered reassuring that overall, no imbalance to the disadvantage of AMX0035 was identified with regard to death (i.e. similar incidence in AMX0035 and placebo group during the main phase; lower incidence for subjects treated for a longer period with AMX0035 (in RA vs. RP and in AA vs. PA group, respectively). Further, the incidence of SAEs was somewhat lower in the AMX0035 vs. placebo group during the main study phase (12.4% vs. 16.7%) and the incidence of SAEs was not higher

in subjects treated for a longer vs. those treated for a shorter period, i.e., occurred in 18 (32.1%) AA vs. subjects 13 (38.2%) PA subjects.

In addition, apart from one SAE of nephrolithiasis considered related by the investigator in each, the AMX0035 and the placebo group, no further SAE or death was considered treatment-related by the investigator during the whole study. Further discussion of a potential contributory role of AMX0035 to two of the deaths cases (subdural haematoma and a fatal diverticular perforation) in the AMX0035 group during the main study phase was requested. Based on the information provided by the applicant, a causal relationship of these death cases cannot be established. Apart from these two deaths, the AE with a fatal outcome included respiratory failure, respiratory arrest, disease progression, ALS, cardiac arrest, all of which are considered related to the underlying condition ALS or disease progression and not related to AMX0035 treatment.

One SAE was device dislocation, which could be related to feeding tube placement in the advanced stages of the disease. When looking at the summary of all adverse events, the following events were reported related to device issues: device dislocation (1 [2.1%] vs. 2 [2.2%] in placebo and AMX0035), device malfunction (1 [1.1%] in AMX0035) and device occlusion (1 [1.1%] in AMX0035). In patients using a feeding tube, AEs related to the device (e.g., device dislocation, pain due to peg placement) occurred in 37.5% (6/16) of the patients in the AMX0035 group as compared to 33.3% (4/12) of the patients in the placebo group; thus the incidence is similar. Device occlusion occurred in a patient in the AMX0035 group concerned a port a catheter and is thus not considered relevant. Based on a single event on feeding tube malfunction in the AMX0035 group, nothing can be concluded on the relationship to AMX0035.

Adverse events of special interest

Neurological adverse events were of interest as this was not only affected by ALS disease progression but also by PB and its active metabolite. In the double-blind part of Study AMX3500, 20 (41.7%) subjects in the placebo group and 35 (39.3%) subjects in the AMX0035 group experienced a nervous system disorder. Three (6.3%) placebo subjects and 8 (9.0%) AMX0035 subjects experienced neurological TEAEs that were moderate or severe. Headache, dizziness and dysarthria occurred in $\geq$ 4 AMX0035 subjects; however, of these, only dizziness occurred more frequently in AMX0035 vs. placebo-treated subjects (10.1% vs. 4.2%). No clear imbalance between treatment groups was derived regarding moderate or severe neurological TEAE by PT. Furthermore, in most cases, these neurological adverse events did not lead to drug discontinuation or permanent drug withdrawal. At present, dizziness and somnolence have been identified as common ADRs in the nervous system disorders SOC. Long-term data are still limited; however, the incidence of AEs in the SOC nervous system disorders was lower in the OLE phase as compared to the main phase (26.8% vs. 39.3% in the AMX00035 arm, respectively), suggesting that the incidence does not increase over time, which is reassuring. The neurological AE rate per patient-year was higher in the AMX group as compared to the placebo group during the main phase of the study; however, the event rate is low (1.666) and driven by headache and dizziness. From these, dizziness is considered related to AMX0035 treatment. Looking at cumulative AMX0035 exposure, the event rate per patient-year is 0.823. In light of the data above, taking into consideration the available non-clinical data and the fact that patients continued treatment in most cases, it is considered that monitoring of neurological AEs could be considered sufficient. No serious neurological safety concern is derived from the provided clinical data.

Cardiac events were considered of special interest due to the reporting in the AMX0035 group exclusively. A thorough QTc study was not performed. It is acknowledged that ALS may also cause cardiac events; thus, it may be difficult to disentangle which events are caused by AMX0035 and which are due to ALS disease progression. Considering the patient population, it could be agreed with the conclusion drawn by the expert cardiologist in the review of cardiac events of the entire study AMX0035 (main phase and OLE), that the overall incidence of cardiac TEAEs (TECAEs) and ECG abnormalities (TEEAs) appears to

be still low; some of these latter events were (plausibly) considered invalid events. In contrast to the conclusion of the reviewing cardiologist, there was an imbalance concerning cardiac AEs and ECG abnormalities with AMX0035 vs. placebo. No severe TECAEs/TEEAs were considered related to treatment; however, a contributory role to some of the events of less clinical significance with AMX0035 could not be excluded. Nevertheless, all 7 subjects with cardiac events during the main study phase had other plausible causes and, except for one SAE (considered unrelated to treatment), cardiac AEs were self-limited and without reported sequelae. Concerning ECG findings no clear safety signal can be derived from the ECG analyses performed over the study. Exposure-response modelling of ECG was performed on a Phase I study wherein no clinically relevant QTcF effect was found within observed plasma concentrations, including those of the main metabolite PAA. However, the informational value is limited due to the following considerations: small sample size, dose not above intended dose, it is not fully clear whether PK in patients is completely identical to those seen in HV. From the currently available clinical data with Albrioza no clear cardiac safety risk can be identified beyond potential AEs caused by the sodium contained in Albrioza. In contrast, in the other approved indications, PB has been associated with syncope (common, see SmPC Ammonaps), arrhythmia (uncommon, see SmPC Ammonaps) and ventricular arrhythmia (uncommon, see SmPC Ravicti, containing the prodrug glycerol phenylbutyrate), respectively, which could be due to the higher recommended daily doses of Ammonaps and Ravicti, respectively. Apart from potential sodium-related warnings (concerning Ammonaps), no warnings and precautions referring to cardiac safety are labelled in the SmPC for Ammonaps or Ravicti, respectively. The provided limited integrated risk assessment on QT prolongation and other cardiac risks, compiled based on non-clinical and clinical data as well as literature, points towards low risk of QT prolongation and other ECG changes.

Cumulatively, aspiration pneumonia was reported in 5 subjects all while receiving AMX0035, only 1 subject experienced aspiration pneumonia during the main study phase (in the AMX0035 group, vs. no subject in the placebo group), and 4 subjects experienced aspiration pneumonia during OLE part, all of which were in the PA group. These numbers are not indicative of a contributory role of AMX0035 to aspiration pneumonia.

Gastrointestinal disorders were not considered of special interest by the applicant. However, based on the provided safety analyses, these events appear to be related to AMX0035. These events have also led to withdrawal from the study in some cases within 3 weeks as subjects were not able to tolerate the gastrointestinal AEs. Moreover, some events were also severe in nature. However, it is reassuring that the incidence is highest at the start of the treatment and that there is no considerable difference between placebo and AMX0035 in the intensity and duration (mean or median) of these events. However, 7 patients did discontinue treatment permanently due to gastrointestinal AE/weight loss and in 4 patients, treatment was temporarily interrupted. It is also not considered negligible that 6 patients with a gastrointestinal AEs also reported weight decrease; however, only in one case did this lead to permanent drug withdrawal. Furthermore, as stated earlier, even though the incidence diminishes over time, many patients continue to suffer from GI AEs or experience these events even after long-term treatment.

Most patients were using 2 sachets of AMX0035 at the time of the AE leading to treatment interruption. Data regarding used dose on re-start of treatment shows a variable pattern, some patients started on reduced dose (1 sachet) without never increasing back to 2 sachets, while others started on one sachet later increasing to two and some re-started treatment with 2 sachets. Only 2 patients in the AMX0035 group had recurrence of the same AE after restart of AMX0035, one with reduced dose and one with continuing 2 sachets. One of these patients discontinued treatment later but for other AEs.

Altogether the provided data regarding temporary drug interruption, re-start of treatment and recurrence of AEs do not support giving any specific recommendation regarding treatment interruption or dose reduction. Several established adverse drug reactions for PB and TUDCA are not proposed to be labelled for AMX0035, which is acceptable due to higher doses of PB during treatment with Ammonaps/Ravicti

as well as differences regarding the targeted patient populations (including the underlying) disease. Rash (ADR) occurred in 2 (2.2%) AMX0035 subjects; in addition, according to information in the study report, *rash erythematous* (ADR) occurred in one further AMX0035 subject (vs. no placebo subject each); however, no event of rash led to dose reductions or study drug withdrawal. Further, rash irrespective of causality, occurred with numerically lower frequency in the AMX0035 (5.6%) vs. the placebo group (8.3%). There is thus currently insufficient evidence to label rash as an ADR of Albrioza. Hot flush/flushing was reported in 4 (4.5%) AMX0035 vs. no placebo subjects during the main study phase, was considered related in the majority of subjects and hot flush is labelled ADR of Ravicti. Hot flush is proposed to be labelled as an ADR of Albrioza with a frequency 'common' which is agreed. Asthenia is proposed to be added to be labelled as ADR of Albrioza based on the following findings: Asthenia occurred in 5 (5.6%) AMX0035 vs. no placebo subjects and was considered possibly related in one subject. In another subject, severe asthenia was not attributed to investigational medical product; however, the event led to the withdrawal of it. In one subject, asthenia was plausibly caused by the AE of diarrhoea. In contrast to nausea, *vomiting* is not proposed to be labelled. In the majority of subjects with TEAEs of vomiting during either part of the CENTAUR study, confounding factors for vomiting were present, or the course of the event also with regard to start and potential withdrawal of AMX0035 points against a causal relationship with AMX0035, '*Throat irritation'* related to treatment was reported in 2 (2.2%) AMX0035 subjects during the main study part and also one (2.9%) PA subject during OLE study part; however, in two of the three cases, the symptoms could have been caused or worsened by the consume of sour juice, and two cases resolved despite unchanged drug intake, while in the third case study drug was (only) temporarily interrupted. Laryngospasm (mild, considered probably related by the investigator) was reported in a single subject, concomitantly with events indicative of ALS progression, including dysphonia. Laryngospasm has been reported in the literature as an underreported symptom of ALS, which was very frequently accompanied by dyspnea (Gotesman et al. 2022). Thus, a causal relationship between the reported events of throat irritation and laryngospasm with AMX0035 cannot be established.

Laboratory findings

No unexpected laboratory findings were reported, apart from weight decrease which could be related to gastrointestinal AEs and subjects in the AMX0035 group experienced proteinuria and ketonuria. No clinical data in patients with moderate to severe renal impairment or hepatic impairment (all severity) is available. The need for dosing recommendation in these patient populations from a safety perspective is unclear, taking into account that the dose-response relationship in AEs remains to be addressed.

The applicant has further discussed the findings regarding hematology laboratory values and transaminases, considering that PB has been associated with anaemia and increased transaminases.

The graphical presentation of mean changes from baseline in haematology laboratory values during study AMX3500 main phase is indicative of some (seemingly transient) lowering in mean erythrocytes, hematocrit and haemoglobin, respectively in the AMX0035 group (which is in contrast to the placebo group). However, the extent of the transient mean decreases in erythrocytes, haemoglobin and haematocrit, respectively, found in the AMX0035 group was merely small, and no consistent trend was found with regard to the incidence of subjects with decreased shifts in these parameters. In addition, during the main study part, clinically significant decreases in erythrocytes, haemoglobin or haematocrit, respectively, were not reported in the AMX0035 group, and no TEAE of anaemia was reported.

There appears a trend towards a higher incidence of transaminases increased, and AST increased considered related (during the main study phase). In detail, the ADR of ALT increased occurred with similar incidence: 2 (2.2%) AMX0035 vs. 1 (2.1%) placebo subjects; however, the ADR AST increased, and the ADR Transaminases increased were reported in 2 (2.2%) AMX0035 vs. no placebo subjects each. Further, there is a trend towards a higher incidence of TEAEs of transaminases increased during the OLE study part in subjects with longer AMX0035 exposure (i.e., in the AA group compared to the PA group):

with ALT increased, AST increased, and transaminases increased in 5.4%, 3.6% and 1.8% of AA subjects vs. 0%, 0% and 2.9% of PA subjects, respectively. In both treatment groups, the incidences of respective ADR were the same as the given TEAE incidences. However, the respective numbers and differences were low. It is further acknowledged that the numbers of TEAEs of transaminases/ALT/AST increased irrespective of causality were rather low during both study parts, with overall no apparent imbalance across treatment groups during the main study phase. In addition, shifts to increased ALT and AST, respectively were comparable in the AMX0035 and placebo group during the main study part and between the PA and AA group during the OLE study part.

Safety in special populations

From a clinical perspective, the discussion regarding safety related to DDI is considered insufficient. The use of AMX0035 in elderly subjects is considered relevant given the underlying disease. The mean age in the study was 59 years, with a limited number of subjects above the age of 65 years. The comparison of data in subjects aged ≤65 and > 65 years of age suggests a similar safety profile of AMX0035 in these subgroups.

Discontinuation due to adverse events

A total of 87 (63.5%) subjects experienced AEs requiring AMX0035 interruption (31 (22.6%) subjects; 12 (24%) in RP group and 19 (2.3%) in RA group), AMX0035 reduction (6(4.4%) subjects; 1(2.1%) in RP group and 5 (5.6%) in RA group) or discontinued AMX0035 (50 (36.5%); 20 (41.7%) in the RP group and 30 (33.7%) in the RA group). The mean duration of drug interruption was much longer in the AMX0035 group as compared to the placebo group, 19.5 vs. 8 days, however, it should be noted that there were more events in the AMX0035 group (31 vs 5). Nevertheless, the duration of the AE was in some cases, also clearly longer in the AMX0035 arm as compared to placebo. In general, the AE duration was a day or two shorter than the drug interruption.

The main reasons for discontinuations were gastrointestinal disorders and respiratory disorders. Gastrointestinal disorders were reported to lead to withdrawal in 22 (16.1%) of the subjects (9 (18.8%) in the RP group and 14 (15.7%) in the RA group. For respiratory disorders, this occurred in 12 (8.7%) of the subjects, of which 8 (16.7%) were in the RP group and 4 (4.5%) in the RA group.

As stated previously, data regarding used dose on re-start of treatment shows a variable pattern. Based on the provided data on drug interruption, dose reduction and drug discontinuation, taking into account the unknown efficacy of reduced dosing, no specific advice on risk mitigation through these measures can be recommended.

2.6.10. Conclusions on the clinical safety

The safety profile of AMX0035 is predominantly characterized by gastrointestinal AEs. While it is reassuring that the incidence of these events is the highest at the start of the treatment, and that there is no considerable difference between placebo and AMX0035 in the intensity and their duration, treatment discontinuations (temporary or permanent) did occur. Some patients also continued to suffer from gastrointestinal AEs or experienced these events even after long-term treatment.

Other common AEs are neurological, such as dizziness, somnolence, and fatigue. In most cases, these adverse events did not lead to drug discontinuation or permanent drug withdrawal. While long-term data are still limited, it is reassuring that the incidence of neurological AEs does not seem to increase over time.

2.7. Risk Management Plan

The CHMP, having considered the data submitted in the application was of the opinion that due to the concerns identified with this application, the risk management plan cannot be agreed at this stage.

2.8. Pharmacovigilance

2.8.1. Pharmacovigilance system

The CHMP and PRAC, having considered the data submitted by the applicant, were of the opinion that, due to the concerns identified with this application, as above outlined, the pharmacovigilance system summary cannot be agreed at this stage.

2.8.2. Periodic Safety Update Reports submission requirements

Not applicable

2.9. Product information

In light of the negative recommendation, a satisfactory summary of product characteristics, labelling and package leaflet cannot be agreed at this stage.

2.9.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

With a median survival of approximately 2 years from diagnosis, ALS is a universally fatal neurodegenerative disease marked by rapid loss of motor function due to degeneration of motor neurons in the CNS. Affected patients eventually require assistance with activities of daily living, with subsequent progression leading to, complete paralysis, and increasingly compromised respiratory function resulting in respiratory failure, the leading cause of death in ALS.

The applicant is seeking conditional approval of Albrioza for the treatment of ALS.

3.1.2. Available therapies and unmet medical need

There is currently one approved medicine for ALS treatment in Europe: riluzole, which blocks glutamatergic neurotransmission in the CNS. Unfortunately, this product only showed a modest survival benefit (~3-6 months) but has not slowed any physical decline.

Despite the availability of riluzole, a high unmet medical need remains for patients with ALS who face rapid morbidity and mortality even with this therapy.

3.1.3. Main clinical studies

The application is based on the results from a single pivotal study (AMX3500). Study AMX3500 was a phase II randomized, double-blind, placebo-controlled study which evaluated the fixed-dose combination AMX0035 (3g sodium PB / 1g TUDCA) in patients with ALS, in the United States. The study included a 24-week double-blind treatment period. Upon completion of this period, subjects could enter the OLE phase of the study, where subjects on placebo switched to active treatment.

Subjects aged 18 to 80 years of age with a definite diagnosis of sporadic or familial ALS, as defined by the El Escorial criteria, with less than or equal to 18 months since ALS symptom onset were eligible to enter into the study. In addition, subjects had to have a SVC of >60% of the predicted value for gender, height and age. Subjects were permitted to use riluzole and/or edaravone.

There were 137 subjects randomized into the study: 89 to AMX0035 and 48 to placebo. During the initial 3 weeks of treatment, subjects were instructed to take 1 sachet of the study drug daily. If well-tolerated, subjects could increase their dose to the recommended dosing of 2 sachets daily, corresponding to a total daily dose of 6g PB/2g TUDCA in the AMX0035 group.

The primary endpoint was the rate of decline in the total ALSFRS-R score at week 24.

Secondary endpoints included the rate of decline in ATLIS; the impact on plasma concentration of pNF-H; the rate of decline in SVC, and the impact on survival (defined as death, tracheostomy or permanent artificial ventilation).

3.2. Favourable effects

The estimated total ALSFRS-R scores at week 24 were 29.06 for the AMX0035 group and 26.71 for the placebo group. The estimated difference between the groups was 2.32, which was statistically significant in favour of AMX0035 (p= 0.0340).

Numerical improvements with AMX0035, were observed, for

- the estimated total ATLIS scores; 39.08 for the AMX0035 group and 36.26 for the placebo group, with an estimated difference of 2.82 at week 24,
- the SVC % predicted; 66.17 for AMX0035 and 61.06 for placebo, with an estimated difference of 5.11 at week 24,
- the estimated percentage of the event of death or death equivalent; 2.8 for the AMX0035 group and 4.4 for the placebo group (hazard ratio 0.632) at week 24. For death events, this was 2.6 for subjects treated with AMX0035 and 2.6 for subjects treated with placebo (hazard ratio 1.016).

Survival was also captured retrospectively at two-time points in the OLE phase of the study. At the most recent cut-off (March 1, 2021), the median survival estimate for death or death equivalent was 23.5 months for subjects who received AMX0035 throughout the study and 17.9 months for subjects initially

randomized to placebo (hazard ratio 0.597). For time to death, the median survival estimates were 23.5 and 18.7 (hazard ratio 0.619) for subjects treated continuously with AMX0035 and those who switched to AMX0035 in the OLE, respectively.

3.3. Uncertainties and limitations about favourable effects

Based on the applicant's original analysis, the single pivotal study met its primary efficacy objective by showing a statistically significant difference in favour of AMX0035 in the rate of decline in the ALSFRS-R. However, the CHMP concluded that efficacy had not been conclusively demonstrated, as results were neither robust nor statistically compelling following a number of sensitivity analyses using different assumptions examining the robustness of the observed treatment effects and statistical inferences. In the most optimistic case, i.e., the applicant's original analysis, the effect was 2.32 and the difference between treatment arms was statistically significant (p=0.034). In the most conservative case, i.e., the CHMP's requested re-analysis using ITT with various imputation methods (no linear progression, placebo based imputation for drop-outs and 0 imputation for death), the effect size is smaller, and the primary endpoint is no longer statistically significant (1.52, p=0.20). Additional sensitivity analyses fall somewhere in between these estimates (e.g., placebo based imputation for death: 1.96, p=0.052). This indicates that the effect estimate and clinical significance for the primary endpoint varies according to the applied method for analysis. Thus, the true treatment effect of AMX0035 cannot be reliably estimated due to the uncertainties highlighted above. The results, therefore, are neither robust nor statistically compelling. No convincing argumentation has been provided to indicate what would be the best analysis. As the true treatment effect of AMX0035 cannot be determined due to the statistical uncertainties highlighted above, clinical relevance of the effects cannot be assessed. Moreover, there is no support of any of the secondary efficacy endpoints, e.g., ATLIS, biomarker pNF-H and SVC neither in terms of statistical significance or effect size. The study did not include any quality-of-life outcomes, which could have helped to contextualize clinical meaningfulness.

The key secondary endpoint ATLIS is currently not fully validated at the time of the opinion. Therefore, the contribution of the findings on this endpoint to the overall conclusions of the study is considered very limited.

Post hoc analyses performed over the placebo-controlled + OLE period suggest that AMX0035 had an effect on survival. The requested re-analysis (Cox regression model) appears to be consistent with the initial findings and the reported HR and p-value are more positive compared to the original analysis. However, this needs to be considered in the context of a study failing to provide robust evidence of efficacy for the primary endpoint based on an appropriate analysis.

Furthermore, the plausibility of the presented survival effect observed remains questionable. It would be expected that the survival curve should change in subjects initially randomised to placebo after having switched to active treatment after similar exposure time. However, no favourable effect in this regard could be observed.

Finally, the lack of support from the analysis of the main biomarker pNF-Hs lowers the evidentiary value of the downstream results. ALS symptom progression results from motor neuron degeneration, causing the loss of motor function. It is hypothesized that AMX0035 reduces neuronal cell death by the PB/TURSO combination via mitigating both ER- and oxidative stress. However, neither the results on the biomarker pNF-H (biomarker indicating neuronal injury), nor those on functional decline provide convincing support for the proposed mechanism of action.

AMX0035 is a fixed-dose combination consisting of PB and TURSO. The rationale for combining PB and TURSO based on the assumed mechanism of action can be followed. It can be agreed that the totality of non-clinical investigations suggests a trend in favour of the PB/TURSO combination over treatment

with the individual compounds, indicating a potential additive effect of the fixed-dose combination. A synergistic benefit was not convincingly demonstrated from a non-clinical perspective. Notably, some data still suggest that the combination PB/TURSO is not more effective than TURSO alone. No additional clinical data have been provided by the applicant to support the fixed-dose combination. It can be concluded that the effect of the combination of PB and TURSO is at best additive based on the provided data. This is considered acceptable considering that neither of the active substances have established efficacy in the target population.

3.4. Unfavourable effects

A total of 137 subjects were available for safety analysis during the main phase of study AMX3500 of which 48 were treated with placebo and 89 with AMX0035. During this phase, the mean exposure was 21.5 weeks (Q1-Q3: 22.8 ;24.1) in the placebo group and 19.7 weeks (16.3; 24.4) in the AMX0035 group.

A total of 34/48 subjects from the former placebo group switched to AMX0035 in the OLE phase. For subjects initially randomized to AMX0035, 56/89 subjects rolled over into the OLE phase. The median exposure in the OLE phase was 15.4 weeks in placebo > AMX0035 (RP) and 31 weeks in AMX0035 > AMX0035 (RA). Expressed in patient-years, the exposure to AMX in the main phase of the study was 33.7 patient-years, in OLE 68.4 patient-years and cumulatively 103 patient-years.

The most common adverse events, i.e. adverse events reported with a frequency of >10% main phase are diarrhoea (8 (16.7%) vs. 19 (21.3%)), nausea (6 (12.5%) vs. 16 (18.0%)), constipation (12 (25.0%) vs. 12 (13.5%)), salivary hypersecretion (1 (2.1%) vs. 10(11.2%)), muscular weakness (9 (18.8%) vs. 18 (20.2%)), neck pain (5 (10.4%) vs. 2 (2.2%), fall (18 (37.5%) vs. 25 (28.1%)), headache (11 (22.9%) vs. 12 (14.6), dizziness (2 (4.2%) vs. 9 (10.1%)), viral upper respiratory tract infection (2 (4.2%) vs. 10 (11.2%), dyspnoea (4 (8.3%) vs. 9 (10.1%)), for placebo and AMX0035 respectively.

During the main phase, AEs leading to treatment interruption were reported in 5 (10.4%) vs. 12 (13.5%) subjects, dose reduction in 0 vs. 4 (4.5%) subjects or drug withdrawal in 5 (10.4%) vs. 18 (20.2%) of the subjects in the placebo and AMX0035 group, respectively. Reasons for discontinuations were primarily gastrointestinal disorders (9 (6.6%) subjects of which (1 (2.1%) in placebo and 8 (15.7%) in AMX0035) and respiratory disorders (11 (8.7%) subjects of which 3 (6.3%) in placebo and 8 (16.7%) AMX0035). Across the study, events of gastrointestinal disorders were reported in 21 (13.8%) subjects (of which 9 (18.8%) in the RP and 14 (%) in the RA group) and respiratory disorders in 12 subjects (of which 8 (16.7%) in the RP and 4 (4.5%) in the RA group) and were the main reasons for discontinuation during both phases of the study.

The provided data on AEs causally related to AMX0035 suggests a dose-response relationship in most AEs. The majority of patients were able to follow the 2 sachet dose throughout the study, albeit some with temporary interruptions or dose reductions due to AEs. Data regarding the used dose on re-start of treatment and treatment continuation thereafter shows a variable pattern. Altogether based on the provided data regarding temporary drug interruption, re-start of treatment and recurrence of AEs, and taking into account the unknown efficacy of reduced dose, no specific advice on risk mitigation through these measures can be recommended.

At least one SAE was reported in 8 (16.7%) placebo subjects and 11 (12.4) AMX0035 subjects during the main study part, and in 18 (37.5%) RP and 27 (30.3 %) RA subjects during the overall study, respectively, of which the most frequent were in the SOC respiratory, thoracic and mediastinal disorders. The most frequent SAE by PT was respiratory failure, which occurred in 3 (6.3%) placebo and 2 (2.2%) AMX0035 subjects during the main and in 8 (16.7%) RP and 8 (9%) RA subjects during the overall study,

respectively. Apart from one SAE of nephrolithiasis considered related by the investigator in each, the AMX0035 and the placebo group, no further SAE or death was considered treatment-related by the investigator during the main and OLE study part. This can be agreed. Seven subjects died during the main phase of the study with an incidence of 5 subjects [5.6%] AMX0035 group and 2 subjects [4.2%] placebo group. The cause of death was respiratory failure in 3 subjects in the AMX0035 and 2 subjects in the placebo group. Other causes of death in the AMX0035 group included subdural hematoma, and diverticular perforation. Cumulatively (main and OLE phase) a total of 22 (16.1%) of the subjects died. Fourteen (10.2%) due to respiratory failure.

Considering the totality of non-clinical and clinical data and the existing knowledge on PB and TURSO as individual components, the risk for QT prolongation and other cardiac ECG changes is considered low.

The comparison of available, limited data in subjects aged ≤65 and > 65 years of age suggests similar safety profile of AMX0035 in these subgroups.

3.5. Uncertainties and limitations about unfavourable effects

Three PB-related impurities have not been structurally identified as requested and, therefore, not qualified for genotoxicity. During the procedure, the applicant identified (preliminary data) the likely root cause for the formation of some unidentified impurities. However, the actual analytical data have not been provided nor have the impurities been structurally characterised. It is agreed that the applicant could have addressed these uncertainties during the post-authorisation phase.

The use of AMX0035 in elderly subjects is considered relevant given the underlying disease. While the comparison of data in subjects aged ≤65 and > 65 years of age suggests a similar safety profile of AMX0035 in these subgroups, a limited number of subjects above the age of 65 years was included in the study.

No clinical data in patients with moderate to severe renal impairment or hepatic impairment (all severity) is available. The need for dosing recommendation in these patient populations from a safety perspective is unclear, taking into account that the dose-response relationship in AEs was not addressed by the time of opinion.

3.6. Effects Table

Table 28: Effects Table for Albrioza for the treatment of ALS

Effect	Short Description	Unit	AMX0035	Placebo	Uncertainties/ Strength of evidence	References
Favourable Effects						

Effect	Short Description	Unit	AMX0035	Placebo	Uncertainties/ Strength of evidence	References
ALSFRS-R	the rate (slope) of decline in the total ALSFRS-R score at week 24	Estimate (SE)	29.06 (0.781)	26.73 (0.975)	**SoE**: Estimated difference: 2.32 (1.094) vs. placebo p < 0.0340 **Unc:** (I) Effect is not robust. In sensitivity analyses using different assumptions the treatment effect becomes smaller and stat. significance is lost. (smallest estimated difference 1.52, p=0.20) (II) Clinical relevance questioned and effect not supported by any of the secondary endpoints. (III) The sequential testing procedure already stopped at the first secondary endpoint (ATLIS, p= 0.1129)	Study AMX3500 (double-blind phase)
ATLIS	The rate (slope) of decline in the total ATLIS score at week 24	Estimate (SE)	39.08 (1.990)	36.26 (2.224)	**Unc:** Estimated difference: 2.82 (1.774) vs. placebo p = 0.1129 The endpoint is not validated in ALS	Study AMX3500 (double-blind phase)
SVC	The rate (slope) of SVC decline at week 24	Estimate (SE)	66.17 (2.327)	61.06 (2.812)	**Unc:** Estimated difference: 5.11 (2.872) Nominal p = 0.0763	Study AMX3500 (double-blind phase)
Survival	Time to death or death equivalent	Median survival estimate (months)	23.5	17.9	**SoE:** Hazard ratio: 0.597 (95% CI 0.387, 0.923) Nominal p = 0.0203 **Unc**: (I) No difference between placebo/AMX0035 observed in double-blind treatment period (nominal p > 0.5960). (II) Not formally tested;	Study AMX3500 (double-blind phase; open-label phase) [March 1, 2021 cut-off]
	Time to death	Median survivale estimate (months)	23.5	18.7	**SoE** Hazard ratio: 0.619 (95% CI 0.399, 0.960 Nominal p =0.0324 **Unc**: (I) No difference between placebo/AMX0035 observed in double-blind treatment period (nominal p > 0.9873). (II) Not formally tested. (III) Plausibility of survival benefit questioned. Delay in separation of curves late in the study and no benefit for subjects who switched from placebo to active treatment in OLE. (III) A slowing of functional loss is not observed, which is expected to precede survival.	Study AMX3500 (double-blind phase; open-label phase) [March 1, 2021 cut-off]
Unfavourable Effects						
Gastrointestinal disorders	Adverse event	N (%)	59 (66.3)	30 (62.5)	**Unc**: (I) SOC common reason for study drug discontinuation, temporary suspension or dose reduction. (II) Unclear at what dose (i.e. 1 or 2 sachets) event occurred and whether could be effectively mitigated with dose reduction or temporary discontinuation.	Study AMX3500 (double-blind phase)

ALSFRS-R= Amyotrophic Lateral Sclerosis Functional Rating Scale – Revised, AMX0035= 3g sodium phenylbutyrate / 1g ursodoxicoltaurine, ATLIS= Accurate Test of Limb Isometric Strength, OLE= open-label extension PB= phenylbutyrate SE= standard error, SoE= Strength of Evidence, SVC= Slow Vital Capacity, Unc= Uncertainty

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

The benefit of AMX0035 for the treatment of ALS cannot be determined based on the results of the study AMX3500 (CENTAUR). Efficacy has not been shown conclusively, as results were neither robust nor statistically compelling following a number of sensitivity analyses using different assumptions examining the robustness of the observed treatment effects and statistical inferences. In addition, secondary endpoints failed to show clear differences between AMX0035 and placebo. Consequently, a slowing of functional loss, which normally precedes death, cannot be concluded upon. While an effect on survival is observed, the data from study AMX3500 lack a data-driven, plausible interpretation. Evaluation of survival is based on a *post hoc* analysis following a primary analysis that did not show a statistically significant effect when using the preferred statistical assumptions. In addition, it would be expected that the survival curve should change in subjects initially randomised to placebo after having switched to active treatment after similar exposure time. However, no favourable effect in this regard could be observed. Finally, the mechanism of action is hypothetical and not supported by results on the biomarker pNF-H (biomarker indicating neuronal injury).

On February 15 2023, a SAG Neurology meeting was convened upon request of the CHMP. The uncertainty about the data was highlighted by the participants. This uncertainty was such that the SAG-N experts concluded that the study results cannot be considered reliable enough, and could only be considered as hypothesis generating, with a need of confirmation in a phase III trial. The SAG-N experts also questioned the extrapolability of the results towards a broad ALS population.

On May 24 2023, the applicant was invited for an oral explanation in front of the CHMP in order to address the major objection of the efficacy not being demonstrated for the treatment of patients with ALS. During the oral explanation, the applicant reiterated their position that the available data do allow for a conclusion of a positive benefit risk balance of Albrioza, based on a manageable safety profile and a claim for a demonstrated efficacy on functional decline and survival outcomes. In particular, the applicant claimed that their assumptions for the primary efficacy analysis (mITT, linear decline of ALSFRS-R, discontinuations and death assumed to follow a MAR pattern of missingness) are valid based on the research question. Thus, the estimated difference of 2.32 points in the ALSFRS-R rate of decline favouring Albrioza would be considered a valid estimate of the treatment effect versus placebo. Further, the applicant claimed that multiple sensitivity analyses testing some of these assumptions (ITT, non-linear, MNAR for discontinuations) provided results consistent with their primary result. As per the imputation of 0 in the ALSFRS-R for deaths, the applicant claimed that this method was not appropriate as patients who died during the CENTAUR RCT retained functional capacity in their last evaluation (average 26.2 points for 5 out of 7 patients with available measure before dying). The CHMP took into consideration the applicant's position and reiterated that the primary efficacy population should be based on the ITT population, in particular with the views that 2 patients died before the study conclusion. Further, the linear pattern of ALSFRS-R scale decline was questioned, based on available data suggesting a non-linear decay. Finally, the MAR assumption implied that patients who discontinue or die would still experience a treatment effect, which is not realistic. Therefore, the CHMP maintained the position that discontinuations should be handled assuming MNAR, using placebo-based imputation. With regard to, the imputation of 0 as value in the ALSFRS-R scale for patients who died during the RCT may be considered as a conservative approach for handing these intercurrent events but is not completely unrealistic since all functions are indeed lost at the time of death. However, statistical significance was

also lost when using placebo-imputation for death. In any way, the same time it was stated that the applicant´s approach was considered too optimistic. Overall, the CHMP retained their position that results were neither robust nor statistically compelling following a number of sensitivity analyses using different assumptions examining the robustness of the observed treatment effects and statistical inference, such that the true effect of AMX0035 cannot be estimated. The applicant claimed that the secondary endpoints support the primary endpoint. It needs to be noted that the secondary endpoints were tested in a hierarchical manner to control the alpha error. Despite not being validated, change in ATLIS was included as the first secondary endpoint. As it did not meet the criterion of $p<0.05$, no formal testing was possible for the remaining secondary endpoints. More importantly, the CHMP noted that overall, the secondary efficacy analyses suffered from the same methodological limitations mentioned for the primary endpoint (mITT population, linearity and MAR assumption). The applicant reiterated that Albrioza treatment resulted in an overall survival benefit of around 4-5 months in the ITT population. However, based on the pattern of the curves, esp. after the switch from placebo to AMX0035, and the results on other tests (ALSFRS-S and pNLH) the reliability of the results is still questioned. The applicant claimed that Albrioza was safe and well-tolerated in the CENTAUR trial. The CHMP agreed that the safety profile can be considered manageable and that the available data -even if limited in the long-term -do not identify serious safety concerns. Finally, the applicant presented the status of PHOENIX RCT to justify their position that comprehensive data would be available during the post-CMA phase. The anticipated completion of PHONENIX RCT is January 2024. The commercial availability (in case of CMA) would also be January 2024 (around 4 months after EC decision). Based on this information, the CHMP was reassured that the impact of commercial availability of Albrioza on PHOENIX RCT completion would be limited.

The safety profile of AMX0035 is predominantly characterized by gastrointestinal AEs. While it was reassuring that the incidence is highest at the start of the treatment and that there is no considerable difference between placebo and AMX0035 in the intensity and duration of these events, some patients did discontinue treatment permanently or temporarily due to gastrointestinal AEs/weight loss. Many patients also continue to suffer from gastrointestinal AEs or experience these events even after long-term treatment. Other common AES are neurological, such as dizziness, somnolence, and fatigue.

AMX0035 is a fixed-dose combination consisting of PB and TURSO. It can be concluded that the effect of the combination of PB and TURSO is at best additive based on the non-clinical data. This is considered acceptable considering that neither of the active substances have established efficacy in the target population.

3.7.2. Balance of benefits and risks

A benefit of AMX0035 for the treatment of ALS cannot be determined based on the results of the single pivotal study AMX3500 (CENTAUR) performed in an ALS subpopulation. Efficacy has not been convincingly shown, as results were neither robust nor statistically compelling following a number of sensitivity analyses using different assumptions to examine the robustness of the observed treatment effects and statistical inference. Likewise, the results on the secondary endpoints failed to show a clear difference between AMX0035 and placebo. As the true effect of AMX0035 cannot be determined, clinical relevance cannot be assessed. While an effect on survival is observed, its plausibility is questioned. The absence of support to the hypothetical mechanism of action from biomarkers, further lowers the confidence in the presence of efficacy. The totality of available evidence does not support the conclusion of a positive benefit/risk of Albrioza for the treatment of ALS.

The safety profile of AMX0035 is overall acceptable.

Study AMX3500 included a subpopulation of ALS. It is unclear how such a population could best be identified in clinical practice. In addition, extrapolation of a treatment benefit, if shown, to the general ALS population applied for has not been conclusively justified.

3.7.3. Additional considerations on the benefit-risk balance

Conditional Marketing Authorisation

As comprehensive data on the product are not available as discussed above, a conditional marketing authorisation was requested by the applicant in the initial submission.

The criteria for granting a CMA are as follows:

1. The benefit-risk balance of the medicine is positive;
2. It is likely that the applicant will be able to provide comprehensive data post-authorisation;
3. Unmet medical needs will be addressed;
4. The benefits to public health of the immediate availability outweigh the risks inherent in the fact that additional data are still required.

Study AMX3500 was a phase II proof of concept study performed in a small ALS population. The effect estimate and clinical significance for the primary endpoint varies, based on the analysis method applied and none of the secondary endpoints supports the primary endpoint, confirming overall concerns with the robustness of study AMX3500. The observed effect on survival is not considered plausible, as functional loss usually precedes death but slowing of functional loss cannot be concluded because of the methodological issues identified. Moreover, no change is observed in the curve of subjects who switched from placebo to AMX0035 after similar exposure. Overall, the data from Study AMX3500 is not considered sufficient to demonstrate efficacy of AMX0035 and thus establish a positive benefit risk balance of Albrioza in the treatment of ALS. Thus, the first criterion is not met.

The protocol for study A35-004, intended as SOB for the CMA, has been provided. Study A35-004 is an ongoing 48-week randomized, double-blind, placebo-controlled study evaluating the efficacy and safety of AMX0035 for the treatment of ALS. As per the data cut-off 16 January 2023, 637 subjects were enrolled in the study. Of the 112 US subjects enrolled, 79 discontinued the study prematurely, as the applicant actively transitioned subjects to commercial product. To ensure that EU subjects complete the 48-week study, the applicant proposes to delay marketing of the drug until the last patient out (date Dec 2023/Jan 2024) in the whole EU. As of February 2023, the study has completed recruitment including 644 participants (522 from EU). Approximately 87 participants have completed the 48-weeks of treatment as of 14 April 2023. It is anticipated that around half of the randomized subjects will have their week 48 visit before October 2023, with the other half before January 2024. Thus, the study is well under way. Based on the provided argumentation, in the hypothetical situation that the product would be commercially available after EC approval, the impact on study participation would be small. Although the timelines presented by the applicant cannot be considered certain, this is considered reassuring. While the study aims to evaluate an effect on function (ALSFRS-R) as well as survival, it does not include any measurement of muscle strength. However, as the study is already underway, it is acknowledged that (major) changes to the protocol are generally not supported. Overall it is considered that the applicant would have been able to provide comprehensive data post-authorisation. Thus, the second criterion would have been met.

With regards to the third and fourth criteria, the CHMP considers that, if a positive benefit – risk balance would have been concluded, these two criteria would have been met based on the undisputed medical need for ALS and the fact that overall is agreed that the safety profile is manageable and thus, it could

have been agreed that the benefits to public health of the immediate availability of the medicinal product would have outweighed the risks inherent in the fact that additional data are still required.

The CHMP considers that the product cannot be recommended for a conditional marketing authorisation as the benefit-risk balance is negative (as discussed).

Third party interventions

The CHMP received, during the assessment of this application, 6 correspondences from 6 ALS associations (hereinafter referred to as "third parties") expressing the third parties' views about the efficacy and safety profile of Albrioza and the unmet medical need of ALS patients. The applicant provided observations to three of the correspondences.

The CHMP considered those interventions in the context of its assessment and concluded that the observations put forward by the ALS associations and Applicant's reply to 2 of them were already known by CHMP, and as such had no impact on the CHMP assessment or its conclusions.

3.8. Conclusions

The overall benefit/risk balance of Albrioza is negative.

4. Recommendations

Based on the CHMP review of data on quality, safety and efficacy for Albrioza in the treatment of ALS, the CHMP considers by consensus the efficacy of the above-mentioned medicinal product is not sufficiently demonstrated and, therefore recommends the refusal of the granting of the conditional marketing authorisation for the above-mentioned medicinal product. The CHMP considers that:

- Efficacy has not been shown, as results were neither robust nor statistically compelling following a number of sensitivity analyses using different assumptions examining the robustness of the observed treatment effects and statistical inferences. Consequently, a slowing of functional loss has not been conclusively demonstrated.
- In addition, there is no convincing support of any of the secondary efficacy endpoints, including the biomarker pNF-H (biomarker indicating neuronal injury), which would have been expected to support the proposed mechanism of action, further increasing the uncertainty.
- Finally, the survival data are not considered robust, the plausibility is questioned and hampered by several methodological issues, e.g., inherent limitations of a single pivotal trial, post-hoc data-collection and analyses without control for multiplicity.

Since efficacy of Albrioza has not been sufficiently shown, a favourable benefit/risk ratio cannot be concluded and, consequently, a conditional marketing authorization cannot be granted.

Due to the aforementioned concerns a satisfactory summary of product characteristics, labelling, package leaflet, pharmacovigilance system, risk management plan and post-authorisation measures to address other concerns as outlined in the list of outstanding issues cannot be agreed at this stage.

5. Re-examination of the CHMP opinion of 22 June 2023

Following the CHMP conclusion that Albrioza was not approvable, the applicant submitted detailed grounds for the re-examination of the grounds for refusal.

5.1. Detailed grounds for re-examination submitted by the applicant

5.1.1. Ground #1

Efficacy has not been shown, as results were neither robust nor statistically compelling following a number of sensitivity analyses using different assumptions examining the robustness of the observed treatment effects and statistical inferences. Consequently, a slowing of functional loss has not been conclusively demonstrated.

Applicants position on the first ground for re-examination

The applicant does not accept the validity of this ground for refusal that the study results were not robust or statistically compelling. Consistent with the accepted regulatory standard, the data based on the CENTAUR study meets the pre-specified primary analysis in demonstrating clinically meaningful and statistically significant slower functional decline than placebo as measured by the ALSFRS-R score over a period of 24 weeks. As detailed below, a series of sensitivity analyses further established the credibility and robustness of this finding under different assumptions or scenarios.

Study design for CENTAUR

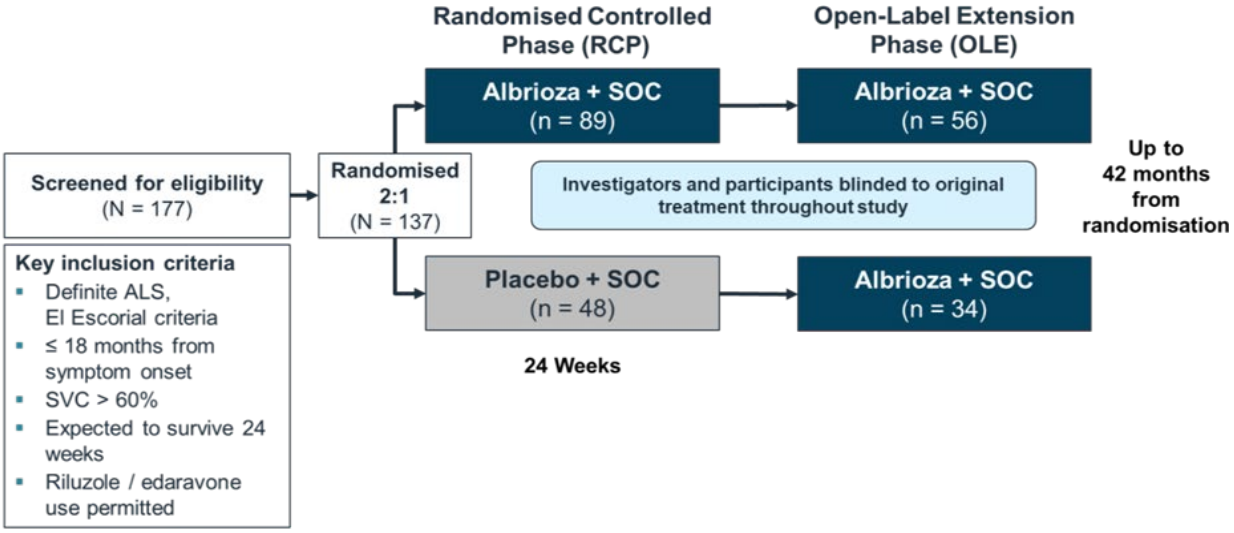
Albrioza (AMX0035) was studied in a 24-week randomised, placebo-controlled study (Study AMX3500 CENTAUR) in 137 participants living with ALS (Figure 12). The goal of the 24-week randomised phase was to assess the rate of functional progression in people living with ALS as measured by the ALSFRS-R. After completion of the 24- week randomised phase, participants were followed for up to an additional 3.5 years in an OLE phase (CENTAUR OLE). Throughout the OLE, blinding to the initial randomisation in the 24-week study was maintained.

The CENTAUR study was designed in collaboration with leaders in the ALS field and conducted at high quality centers in the US through the NEALS network, the largest ALS trial consortium in the world.

The primary statistical model has been used in past ALS clinical trials and was developed by an expert statistician in the field of ALS.

Baseline demographic and disease characteristics were generally similar between the Albrioza and placebo groups.

Figure 12: CENTAUR – Study Design

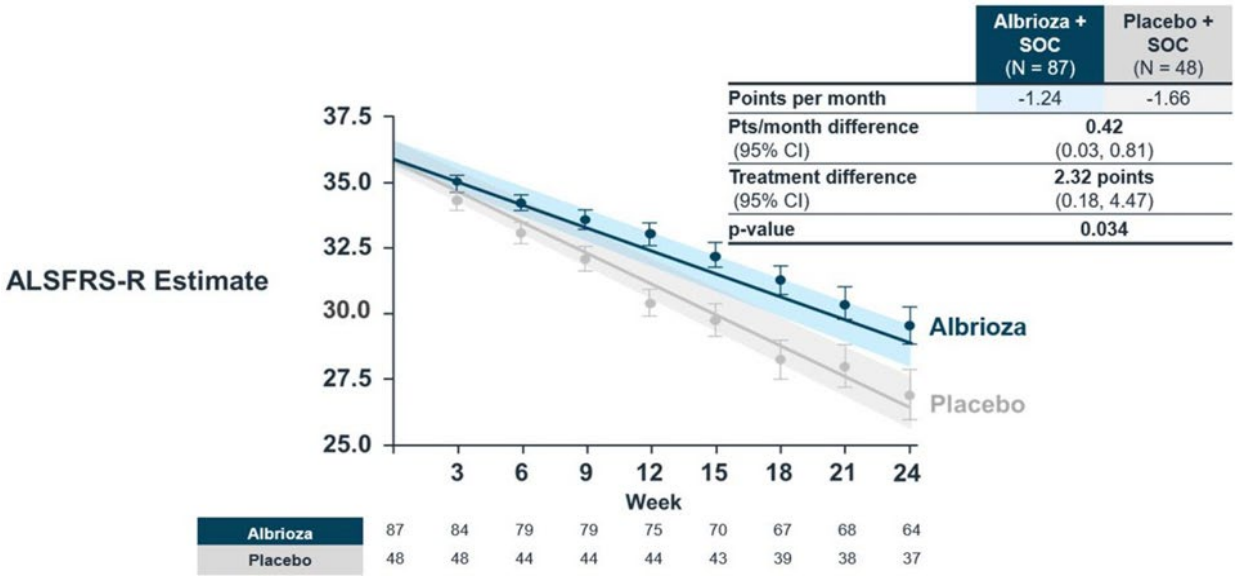


Definite ALS, El Escorial criteria: meets criteria for ALS diagnosis and shows ALS upper and lower motor neuron symptoms in ≥ 3 body regions

Prespecified primary outcome

CENTAUR met its pre-specified primary outcome showing a statistically significant and clinically meaningful slowing in ALS functional decline for Albrioza vs. placebo (2.32-point treatment difference over 24 weeks, p=0.034; Figure 13). The majority of experts participating in the SAG-N on Albrioza agreed that a true effect size of 2 points (or more) on the ALSFRS-R score may be clinically relevant. The pre-specified, primary analysis was conducted using a linear mixed effects model – one of the standard models in the field of ALS often described as a classical model to analyse ALS studies (van Eijk et al., 2018).

Figure 13 : Primary Endpoint - Rate of Decline in ALSFRS-R Total Score Over 24 Weeks



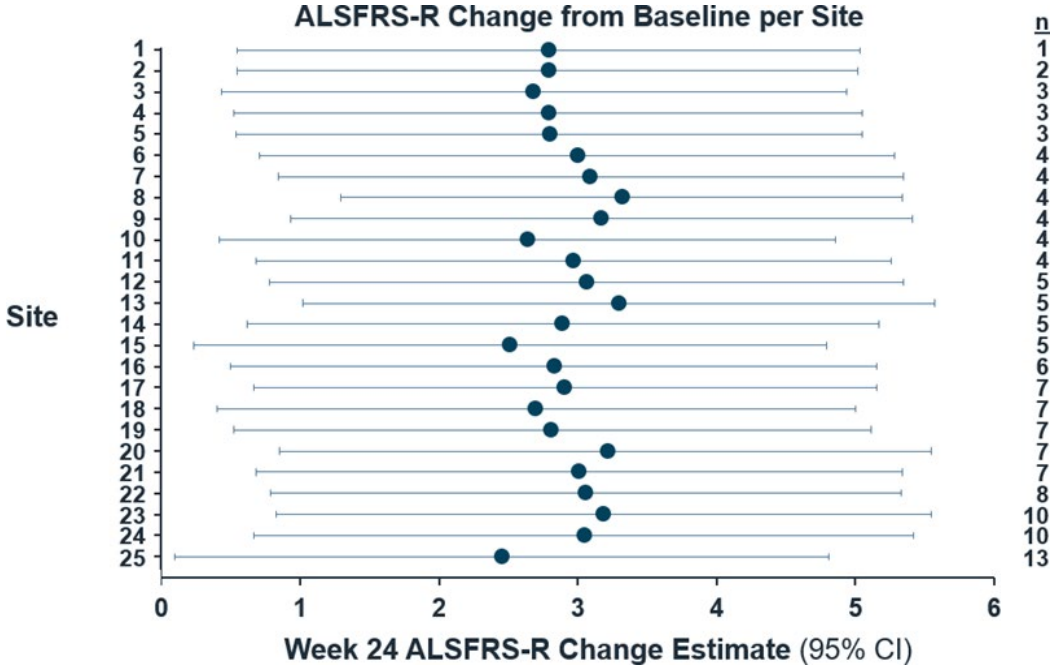
CI: confidence interval, SOC: standard of care.

Sensitivity analyses show that the primary outcome is robust

The primary outcome was found to be robust across all prespecified as well as regulatory and journal-requested sensitivity analyses.

First, the treatment effect was consistent across all 25 centers in the study, and no one study site dominated the magnitude of treatment effect (Figure 14).

Figure 14 : ALSFRS-R Total Score Change from Baseline at Week 24 'Leave-One-Out'* Sensitivity to Determine if any Individual Site Drives Observed Effect



* Change from baseline model was run 25 times, each time removing 1 of the sites from the analysis.

The applicant prespecified the primary model which was a shared-baseline continuous time (i.e. linear) mixed effects model in the mITT population. The applicant supports this model as an appropriate model for several reasons.

1. ALS is rapidly progressing and therefore the exact day on which a visit occurs is useful information for statistical modeling and provides added precision. Making a categorial rather than continuous assumption for time does not account for the study day on which visits occur and therefore may obscure important information on progression rate.

2. The outcome of interest was the rate of progression of ALSFRS-R and this cannot be estimated for participants who only have a baseline value and no follow-up value. The population of interest for analysis was therefore the mITT population which included all participants with at least two ALSFRS-R measurements.

3. The primary model, which fits a slope to every participant regardless of dropout, is a common statistical approach and is considered a classical approach in ALS (van Eijk et al., 2018)

For these reasons, the selected primary model was reasonable and appropriate for this study.

The CHMP requested that the applicant perform analyses evaluating the following aspects in the primary model: using the ITT population instead of mITT, not relying on linearity and using categorical time, using a placebo-based imputation for discontinuations instead of missing at random, and using a worst-case imputation (ALSFRS-R score of "0") in case of death.

The first three requested analyses assess the robustness of the observed treatment effect on functional progression and will be addressed first, along with other similarly requested or pre- specified sensitivity analyses. The fourth request aligns to a well-researched topic in ALS – assessing efficacy based on a composite endpoint of function and survival – and will be addressed separately.

Regarding the robustness of the observed treatment effect when evaluating functional progression alone, results from analyses requested by the CHMP and additional sensitivity analyses are presented in Table 29.

Table 29: Pre-specified and CHMP-Requested Sensitivity Analyses Evaluating Assumptions and Robustness of Primary Endpoint Model

Reason for Analysis	ALSFRS-R Analysis	Difference (95% CI); % slowing	p-value
Requested by FDA and NEJM editor	Change from baseline instead of shared baseline assumption	**2.9 (0.7, 5.2) 32%**	**0.010**
Performed variety of concomitant medication sensitivity analyses as common clinician question	**Adjust for baseline use of riluzole** (on riluzole)	**2.9** (0.7, 5.1) **32%**	**0.011**
	Adjust for baseline use of edaravone (on edaravone)	**2.6** (0.4, 4.8) **28%**	**0.023**
	Adjust for baseline use of edaravone (not on edaravone)	**2.5** (0.3, 4.6) **27%**	**0.027**
Pre-specified sensitivity analysis	Time dependent covariate for riluzole use	**2.3** (0.2, 4.5) **25%**	**0.033**
Pre-specified model for Primary Outcome	Shared baseline, linear, mixed effects model	**2.3** (0.2, 4.5) **25%**	**0.034**
Requested by CHMP and *NEJM* editor	Use ITT population instead of mITT	**2.3** (0.2, 4.5) **25%**	**0.034**
Answer common clinician questions	**Adjust for baseline use of riluzole** (not on riluzole)	**2.3** (0.1, 4.5) **25%**	**0.041**
Requested by CHMP and FDA	Remove assumption of linearity	**2.2** (0.2, 4.1) **24%**	**0.034**
Pre-specified sensitivity analysis	Time-dependent covariate for edaravone use	**2.2** (-0.1, 4.4) **24%**	**0.056**
Requested by CHMP	ITT, no linearity, placebo-based imputation	**2.0** (-0.02, 3.9) **22%**	**0.052**
Pre-specified sensitivity; requested by CHMP	Placebo-based imputation for missing data instead of missing at random	**1.9** (0.1, 3.7) **21%**	**0.043**

CI: confidence interval, NEJM: New England Journal of Medicine.

The CHMP assessment report concludes that "the effect of Albrioza strongly depends on the analyses method used." The sensitivity analyses, as shown above in Table 29, demonstrate the opposite conclusion. While the effect sizes and p-values for pre-specified and CHMP-requested analyses do shift slightly depending on assumption, all remain fairly consistent, and the vast majority of methods result in ALSFRS-R differences greater than 2 points and p values below 0.05. The consistency is demonstrated regardless of the assumption of:

- Use of a change-from-baseline model instead of the prespecified primary model,
- Use of concomitant medications,
- Use of the ITT or mITT population,
- Assuming linearity or not,
- Use of a placebo-based imputation for missing data.

Importantly, this robust demonstration of ALSFRS-R differences of 2 or more points aligns with what the majority of experts at the SAG-N considered clinically meaningful – a 2-point (or greater) difference on the ALSFRS-R provided that the estimate is valid.

The sensitivity analyses, both those pre-specified and those requested by the CHMP, therefore support the robustness of the primary outcome, a statistically significant and clinically meaningful slowing of disease progression.

CHMP requested analysis with worst-case imputation (ALSFRS-R score of "0") in case of death

The CHMP also questioned measuring functional progression independent of deaths, and therefore requested a "worst-case imputation" (ALSFRS-R score of "0") in case of death. In shorter duration trials, few deaths are expected (Cudkowicz and Shefner, 2022) and measuring each outcome separately (functional progression and mortality) is common practice in ALS (Van Eijk et al, 2018). The question of

combining the ALFRS-R and survival into a single outcome is additionally a well-researched topic in ALS, and a brief overview of that research will be provided below.

First, however, two points should be noted that are in support of measuring function independently of survival:

1. There were very few deaths in the 24-week portion of the trial, and they were balanced across groups (5/89 [5.6%] for Albrioza; 2/48 [4.2%] for placebo). Details on the cause of death are provided in the CENTAUR CSR.
2. Over long-term follow-up as more deaths accumulated, there was an ITT overall survival benefit. This will be further shared in Ground for Re-examination 3.

These results support the assumption that it was appropriate in this 24-week study to measure function and survival independently.

Combining function and survival into a single endpoint requires different statistical methodology as it is combining a continuous outcome (function) and a binary outcome (survival) into one outcome. While substituting a "0" into the ALSFRS-R for a death may seem like a solution, it has long been found by the ALS field and biostatistical experts to lead to erroneous conclusions for the estimation of treatment effects on functional progression as measured by the ALSFRS-R (Proudfoot et al., 2016, Van Eijk et al., 2022a). There are several reasons this methodology is incorrect, including that:

- Inserting an ALSFRS-R score of zero does not reflect actual disease trajectory.
- It is inappropriately using the clinical tool; no physician would use the ALSFRS-R to assess death.
- It inappropriately biases the ALSFRS-R, and simulations show that it can lead to wrong conclusions (Van Eijk et al., 2022a)

Experts participating in the Feb 2023 SAG Neurology meeting expressed the same opinion.

The state-of-the-art model for assessing functional progression and mortality in ALS uses a joint-modelling approach (Van Eijk et al., 2022b), and was developed by Prof. Ruben van Eijk, and ALS physician and biostatistician at University Medical Center at Utrecht, with Dr. Kit Roes, Professor of Biostatistics at Radboud University Medical Center and the current Chair of the Methodology Working Party for the CHMP. The model described above has been named Mortality Adjusted Progression in the ALS field.

Mortality Adjusted Progression to account for deaths

The MAP joint-model estimates the relationship between treatment, functional loss and mortality.

The MAP joint-model incorporates longitudinal ALSFRS-R data using the mixed effects model and survival data using the Cox Proportional Hazards model; a Maximum Likelihood Estimation (MLE) is then obtained by simultaneously maximizing the combined likelihood function of the ALSFRS-R score and the Cox model. This method adopts a composite strategy to include death through jointly optimizing the longitudinal and survival parameters, therefore allowing the adjustment for mortality while functional benefits remain the estimand of interest, without the need to make further assumptions on ALSFRS-R values after death or reducing data dimensionality. For a detailed description of the MAP analytical model, reference is made to Van Eijk et al, 2018.

For the CENTAUR study, the MAP joint-model finds ALSFRS-R differences between the Albrioza and placebo arms at 24-weeks of:

- **2.37 points (95% CI 0.17-4.57, p=0.035)**

This result closely aligns with the prespecified analysis. While this model was conducted *post hoc*, it supports the primary outcome finding and suggests that the treatment benefit observed by the pre-specified primary outcome was not biased due to the impact of deaths. However, as noted before, this may not be surprising given that there were few deaths in the 24-week study and that these were balanced across study arms.

Conclusion on ground for re-examination 1

The applicant believes the data show that the Albrioza treatment effect demonstrated in CENTAUR were clinically meaningful, robust across sensitivities, and show a clear benefit on functional progression.

CENTAUR met its pre-specified primary outcome as measured by the rate of progression of the ALSFRS-R total score showing a significant and meaningful benefit (Figure 13).

The primary model and assumptions were reasonable and standard within the ALS field. The effect sizes and p-values for pre-specified and CHMP-requested sensitivity analyses all remain fairly consistent, with ALSFRS-R difference in almost all cases >2 points and $p<0.05$ (Table 29).

These consistent ALSFRS-R differences of 2 or more points align with what the majority of experts at the SAG-N considered clinically meaningful.

The pre-specified primary outcome was not biased due to the impact of deaths. Mortality Adjusted Progression, the current state-of-the-art method to address this question, demonstrates a similar treatment effect size and statistical significance as the prespecified, primary outcome.

As demonstrated, the applicant believes the functional outcome of CENTAUR is robust.

CHMP position on the first ground for re-examination

The first ground for refusal is due to concern that efficacy has not been shown, as results were neither robust nor statistically compelling following a number of sensitivity analyses using different assumptions examining the robustness of the observed treatment effects and statistical inferences. Consequently, a slowing of functional loss has not been conclusively demonstrated.

The applicant disagrees with this assessment and claims that its pre-specified primary outcome shows a statistically significant and clinically meaningful slowing in ALS functional decline for Albrioza vs. placebo (2.32-point treatment difference over 24 weeks, $p=0.034$). It is also claimed that the prespecified primary analysis used a standard statistical approach and that the robustness of the primary endpoint data was demonstrated via all pre-specified sensitivity analyses. In addition, the joint-modelling approach that combines the ALSFRS-R and death into a single endpoint was presented.

The applicant's pre-specified primary analysis was questioned by the CHMP from four aspects. First, the use of mITT population instead of the ITT population was questioned. The mITT population excluded two subjects, both from the experimental arm and both died. Excluding subjects based on events occurring after randomisation can introduce bias in the analysis, especially if the reason for exclusion is death. Secondly, the primary analysis of the rate of decline (slope) used a shared-baseline, mixed-effects analysis, including age, the estimated rate of disease progression (i.e., ΔFS), and baseline of the efficacy outcome of interest (other than ALSFRS-R) as covariates and interacting with time. This is not considered an appropriate method. The method strongly relies on linearity of disease progression, and although progression has been shown to decrease in a linear fashion over the course of a typical clinical trial, the linearity is disputed over longer periods (Karanevich 2018), as is the case here since the model includes the progression rate from symptom onset to baseline as covariate. Furthermore, the primary analysis model also assumed data to be MAR. This is not considered a reliable assumption as it is unlikely that patients that discontinue or die will still experience a treatment effect. It should be noted that there was a fairly marked imbalance in the number of treatment withdrawals with 5 patients in the placebo group

(10.4%) vs 18 in the AMX0035 (20.2%) stopping treatment prematurely. Lastly, handling death in the same way as other missing data was not agreed.

Therefore, the applicant was requested to perform and present an analysis of the primary endpoint using the change from baseline for the ITT population, with time as a fixed categorical factor instead of continuous in the model (i.e. not relying on linearity) and using a placebo based imputation for discontinuations and worst-case imputation (ALSFRS-R score of "0") in case of death, hereafter called "CHMP requested analysis".

The applicant further claims that the primary outcome was found to be robust across all prespecified as well as regulatory and journal-requested sensitivity analyses. This is not agreed. The applicant presents data in the Table 29 misinterpreting the CHMP request and presenting only selective data analyses supporting the applicant's claims and omitting the one which was requested by the CHMP, thus, precluding the fair assessment of the available data. The applicant has here chosen to show analyses addressing one of the issues raised by the CHMP at a time, as well as CHMP request including ITT population, no linearity and placebo-based imputation of missing values, but not worst-case imputation in case of death (value 0). This variation of CHMP analysis (including 3 requests) resulted in difference of ALSFRS-R score of 2.0 (p=0.052). The analysis – as requested by CHMP - addressing all four issues simultaneously has been omitted.

The analysis requested by the CHMP taking into account all four aspects as described above was presented by Applicant during the procedure. In this analysis the point estimate was 1.52 (p=0.20) (Table 30).

Table 30: ALSFRS-R Change from baseline – Worst-case imputation for missing values due to death in the categorical MMRM without linearity assumption – ITT population

Endpoint Time Point	Estimate (95% CI)		Estimated Difference	95% CI	p-value
	Placebo+SOC (N=48)	AMX0035+SOC (N=89)			
Change from baseline ALSFRS-R Total (ITT, without linearity assumption, placebo-based imputation for missing values after discontinuation and worst-case imputation for missing values from discontinuation due to death)					
Week 24	9.32 (-11.18, -7.45)	-7.79 (-9.19, -6.40)	1.52	(-0.80, 3.84)	0.20

CI: Confidence interval, SOC: Standard of care

Thus, the primary efficacy endpoint is not considered robust since the CHMP requested analysis with a reasonably conservative approach for missing data and less dependent of model assumptions results in a different conclusion than the primary analysis.

The applicants' arguments against the CHMP requested analysis are not accepted. The issues with the analysis still remains as stated above. The analysis of the primary endpoint using the change from baseline for the ITT population, with time as a fixed categorical factor instead of continuous in the model (i.e., not relying on linearity) and using a placebo-based imputation for discontinuations and worst-case imputation (ALSFRS-R score of "0") in case of death is still the preferred analysis because it considers the ITT population, does not rely on a linearity assumption and does not rely on the MAR assumption that is considered an unrealistic pattern of missingness.

The applicant presents the arguments that the CHMP requested a "worst-case imputation" (ALSFRS-R score of "0") in case of death is not appropriate since it does not reflect actual disease trajectory, it is inappropriate using this clinical tool to assess death and that it inappropriately biases the ALSFRS-R. While it is agreed that it could potentially bias ALSFRS-R scale due to very sudden jump in score to 0 in

case of death, it should be pointed out that it reflects the reality. Actually, the ALSFRS-R score 0 does not necessarily mean that a patient is dead. If one would use placebo-based imputation for death cases (as for missing data), it may result in overestimating the treatment effect, as patients being alive (and given placebo) probably have a better ALSFRS-R score than those who have passed away. While the disease progression per se might be more gradual, typically worsening approximately 1 point per month, the death reflects an outcome which may occur anytime during the disease and the function at that time point will be equal to zero thus setting the final point to the disease progression trajectory. Imputation of worst efficacy score possible in case of death is not an uncommon way to handle death in conservative sensitivity analyses. In case of robust data, clinically relevant and statistically significant results are not lost.

The applicant's claim that 2 points difference observed on the ALSFRS-R scale could be considered clinically meaningful is agreed if it would have been clearly demonstrated. However, since the CHMP preferred sensitivity analysis is not supporting the applicant's primary analysis it is not considered sufficiently reliable. Further, the AMX0035 inclusion criteria (definite ALS, fast progress but anticipated to live for at least 6 months) restricted the studied population to a subgroup not being a general sample of the ALS affected patients and thus, it is unclear how any potential benefit of AMX0035 could be extrapolated to a general ALS population. It is also unclear how this subpopulation could best be identified in clinical routine practice.

The Mortality Adjusted Progression (MAP) model presented by the applicant here is another *post hoc* analysis, not planned for in the protocol or SAP, with no control for type I error, and without full detailed description of how the model was implemented. Hence, it is not considered to add value to the discussion at this point.

Point not resolved.

5.1.2. Ground #2

In addition, there is no convincing support of any of the secondary efficacy endpoints, including the biomarker pNF-H (biomarker indicating neuronal injury), which would have been expected to support the proposed mechanism of action, further increasing the uncertainty.

Applicant's position on the second ground for re-examination

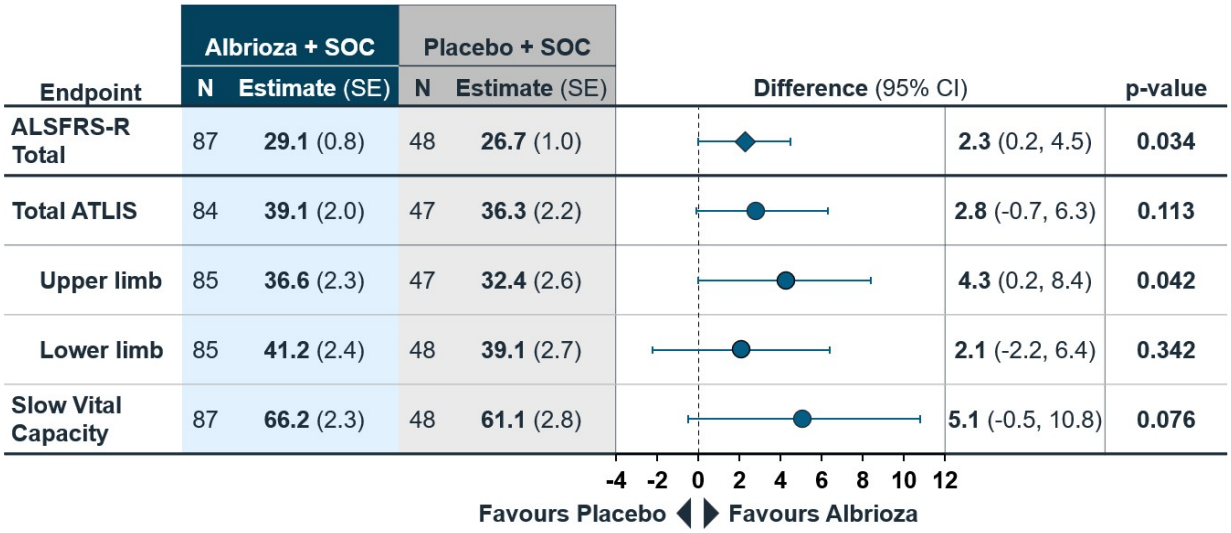
Contrary to the CHMP's position, the trends of the secondary outcomes are supportive of the primary analysis in favour of treatment effect related to Albrioza on slowing the rate of disease progression and while biomarkers are still emerging in ALS, there is biomarker support available for the treatment benefit.

Secondary efficacy outcomes in the 24-week main phase of CENTAUR included the rate of decline in isometric muscle strength as measured by the Accurate Test of Limb Isometric Strength (ATLIS) device, the rate of decline in the SVC, and the decline in plasma levels of the pNF-H as a potential biomarker of motor neuron degeneration.

ATLIS and SVC

CENTAUR was only powered to detect differences on the primary outcome, as there was limited information on ATLIS natural history (CENTAUR was the first interventional study to use ATLIS) and SVC is a notoriously variable endpoint in ALS due to the challenges of patients making a firm lip seal. Regardless, all clinical secondary endpoints for muscle strength (ATLIS) and respiratory function (SVC) favoured Albrioza, further supporting the primary endpoint findings (Figure 15).

Figure 15 : Secondary Clinical Endpoints – Week 24 ATLIS and SVC



CI: confidence interval, SE: standard error, SOC: standard of care, SVC: slow vital capacity.

While not nominally significant, meaningful numerical effect-sizes consistent with the primary outcome finding were observed. For example, in the CENTAUR study, participants randomised to Albrioza progressed 23.1% slower on SVC than the placebo arm narrowly missing statistical significance (p=0.076). This finding is similar to the finding that participants progressed 25% slower on the ALSFRS-R (p=0.034). By the end of the 24-weeks randomised period, participants in the AMX0035 arm were observed to have an absolute slow vital capacity (lung capacity) 5.1 absolute percentage points higher than those randomised to placebo (Figure 15).

In the CENTAUR study, some participants were unable to complete ATLIS assessments (for example due to an inability to transfer from wheelchair, incapable of maintaining orthogonal starting position, foot-drop), and there was more missing data on this outcome (36.5% of participants could not complete ATLIS assessment at study end) than for the ALSFRS-R or SVC; however, nominally significant differences were still observed on upper limb function as measured by ATLIS with a 20.2% slower progression rate in the AMX0035 arm versus placebo, again similar to the effect size noted on the ALSFRS-R.

In summary, these key secondary outcomes in the randomised phase of the CENTAUR study provided similar effect sizes and should therefore be considered supportive of the primary outcome.

Neurofilament in ALS

Neurofilament is a biomarker of interest in ALS and was therefore included as an endpoint in the CENTAUR study. The primary support of neurofilament as a disease biomarker is derived from the observation of generally elevated levels in people with ALS and a correlation to disease progression, although the correlation is highly variable (Lu et al., 2015).

However, to date it is not clear that neurofilaments have been treatment-sensitive. Riluzole, the only EMA-approved treatment for ALS, does not appear to affect neurofilament levels (Esselin et al., 2022). Neither does edaravone, approved for the treatment of ALS in the United States, Canada, and Japan (Berry et al., 2022). In other neurodegenerative diseases, there have also been mixed data on therapeutic impact on neurofilaments. For example, in spinal muscular atrophy (SMA), the EMA-approved antisense oligonucleotide treatment nusinersen was associated with a substantial reduction in neurofilament levels, but to the contrary the EMA-approved gene therapy onasemnogene abeparvovec was associated with increased neurofilament levels (Alves et al., 2021). In Alzheimer's, two recent drug

candidates, lecanemab and donanemab, have both met their prespecified clinical outcomes without any effect on neurofilament (Van Dyck et al., 2023, Pontecorvo et al., 2022).

Thus, while neurofilaments continue to be a biomarker of interest in ALS and other neurodegenerative diseases, there is still much to learn. At a minimum, it is clear that neurofilaments cannot be the only important biomarker in ALS or other neurodegenerative diseases. Additional plasma samples were taken in the CENTAUR study to further study potential biomarkers.

Other biomarkers supporting CNS target engagement by Albrioza

The applicant sought to investigate plasma samples stored from CENTAUR for further biomarker evidence. Of highest interest was YKL-40 (also known as chitinase-3-like protein 1 [CHI3L1]) given recent publications finding correlation of YKL-40 to disease progression, survival and severity in ALS (Masrori et al., 2022, Vu et al., 2020, Dreger et al., 2022, Gaur et al., 2023). Importantly, publications have found that YKL-40 appears to provide prediction of disease progression and survival in ALS *independently* of neurofilament (Masrori et al., 2022, Gaur et al., 2023).

YKL-40 was analysed on all available samples from CENTAUR (Bowser at al., 2022). The results are summarised in Table 31, providing evidence that Albrioza treatment results in a significant lowering of YKL-40 levels compared with placebo.

Table 31: Plasma YKL-40 after 24 weeks in the CENTAUR study

	Week 24 Geometric LS Mean (SE)		Geometric LS Mean Ratio	
Plasma Biomarker	AMX0035 (n=81)	Placebo (n=45)	(95% CI)	p-value
YKL-40 (ng/mL)	**31.4** (1.1)	**38.8** (1.1)	**0.8** (0.7, 0.9)	**0.0078**

CI: confidence interval, LS: least squares, SE: standard error

YKL-40 in plasma was also found to be correlated both with ALSFRS-R score and disease progression in the CENTAUR study over 24 weeks as presented below:

- YKL-40 correlation to ALSFRS-R score (r = -0.21, p = 0.0001)
- YKL-40 correlation to ALSFRS-R progression rate (r = -0.19, p = 0.004)

At this time, biomarkers in ALS are under intense study but to date should be evaluated with caution in terms of their ability to support a treatment effect. However, the effect of Albrioza on YKL-40, which in both natural history studies and in CENTAUR correlated with ALS disease progression, is promising, and provides further evidence for pharmacologic engagement with Albrioza.

The applicant also wishes to note that Albrioza has demonstrated changes in biomarkers in Alzheimer's as well, including on markers that may be relevant to ALS. In the randomised, placebo-controlled PEGASUS study in 95 participants with Alzheimer's disease, CSF was drawn prior to first dosing and at the 24-week visit (or early discontinuation visit, as applicable) to evaluate the effect of treatment with Albrioza on biomarker concentrations in the CNS. The biomarker results from PEGASUS are shown in Table 32 with markers showing a significant benefit for Albrioza therapy highlighted in blue.

As previously noted, the observed lowering of YKL-40 has evidence supporting its importance in ALS.

CSF total tau levels are also markedly increased in both Alzheimer's and ALS (Wattmo et al., 2020, Scarafino et al., 2018). A 2018 study was conducted involving 85 participants with ALS, 30 participants with ALS-mimicking diseases, and 51 participants with other non-neurodegenerative diseases. In this study, a higher level of total tau was found in CSF in participants with ALS compared to ALS-mimicking

diseases (p=0.006) and other non-neurodegenerative diseases (p<0.001). Additionally, CSF levels of total tau correlated with historical rate of disease progression since first symptom (ΔFS score) at the time of spinal tap (r=0.257, p=0.02) and respiratory function loss as measured by SNIP (r=0.315, p=0.03). This suggests that total tau could be a relevant biomarker in ALS (Scarafino et al., 2018).

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

These findings support the clinical findings from the CENTAUR ALS study by providing mechanistic evidence that Albrioza is active against important neurodegenerative processes in the central nervous system.

Table 32: Biomarker Results from PEGASUS Clinical Study in Alzheimer's disease

		Change from Baseline at Week 24			
	Biomarker	**Albrioza**	**Placebo**	**Albrioza and Placebo LSMEAN Difference (95% CI)**	**p-value**
Neurodegeneration (pg/mL)	Total Tau	-64.9	8.82	-73.7 (-106.8, -40.7)	<0.0001
	Phosphorylated Tau (pTau 181)	-14.6	-0.27	-14.4 (-21.5, -7.2)	0.0002
	FABP3	-344.6	102.9	-447.5 (-684.6, -210.5)	0.0004
	Neurofilament Light Chain (NfL)	169.5	63.6	105.9 (-119.7, 331.5)	0.35
Synaptic Function	Neurogranin (pg/mL)	-81.2	-8.34	-72.9 (-110.8, -34.9)	0.0003
Inflammation (pg/mL)	YKL-40	-14635	1508	-16143 (-26996, -5291)	0.004
	IL-15	-0.02	0.25	-0.28 (-0.49, -0.06)	0.01
	IL-6	644.4	565.9	78.5 (-1042.5, 1199.4)	0.89
	IL-8	1.54	1.17	0.37 (-4.37, 5.11)	0.88
	GFAP	821.7	488.1	333.5 (-2080.2, 2747.2)	0.78
	MCP-1	-1.97	-0.79	-1.2 (-21.2, 18.8)	0.91
Core AD pathology	AB_{42} / AB_{40} ratio	0.0039	-0.0051	0.0090 (0.0029, 0.0151)	0.005
	AB_{42} (pg/mL)	-8.09	-41.46	33.37 (-38.37, 105.11)	0.36
	AB_{40} (pg/mL)	-752.70	-754.81	2.11 (-1007.67, 1011.88)	1.0
Metabolism / Oxidative Stress (pg/mL)	8-OHdG	0.31	-0.13	0.44 (0.13, 0.74)	0.006
	24-OHC	-0.20	-0.07	-0.13 (-0.67, 0.41)	0.63
	Leptin	0.45	4.53	-4.09 (-25.71, 17.54)	0.71
	sIR	-0.04	-0.19	0.15 (-0.25, 0.55)	0.47
Neurovascular	MMP-10 (pg/mL)	-3.13	-0.92	-2.21 (-8.48, 4.05)	0.48

CI: confidence interval, LSMEAN: least squares mean.

Conclusion on ground for re-examination 2

The applicant believes that the secondary clinical endpoints, while generally not nominally significant, provide additional support for the efficacy claim of the primary outcome.

All clinical secondary endpoints for muscle strength (ATLIS) and respiratory function (SVC) favour Albrioza and show a similar effect size (Figure 15).

While there is still much to learn about biomarkers in neurodegenerative diseases including ALS and they should be interpreted with caution, the demonstrated biological changes in plasma the CENTAUR study, including a significant reduction in YKL-40, may support the efficacy findings (Table 31).

The significant biomarker results in CSF in the Pegasus study of Alzheimer's disease on markers of YKL-40, tau, and phosphorylated-tau that have been implicated in ALS provide additional data on the biological activity of Albrioza (Table 32).

CHMP position on the second ground for re-examination

The second ground for the refusal states that there is no convincing support of any of the secondary efficacy endpoints, including the biomarker pNF-H (biomarker indicating neuronal injury), which would have been expected to support the proposed mechanism of action, further increasing the uncertainty.

The applicant argues that the trends of the secondary outcomes are supportive of the primary analysis in favour of treatment effect related to Albrioza on slowing the rate of disease progression and that there is biomarker support other than pNF-H available for the treatment benefit.

Secondary efficacy outcomes in the 24-week main phase of CENTAUR included (in hierarchical order) the rate of decline in isometric muscle strength as measured by the ATLIS device, the decline in plasma levels of the pNF-H, the rate of decline in the SVC, and survival (including death or death equivalent at 24 weeks).

The muscle strength was measured by the ATLIS scale which according to the applicant was used first time in the CENTAUR clinical trial and, thus, the capability of this scale to detect treatment effects on muscle strength is in not very well known. Some numerical changes in favour of the AMX0035 arm vs placebo were observed both for muscle strength as well as respiratory function but did not reach statistical significance (Figure 15). The interpretation of the effect on muscle strength and SVC results was even further hampered by the fact that 36.5% of study participants were not able to complete ATLIS assessment and 29% of study participants did not complete SCV test. Results provided by the applicant assume missing at random.

Neurofilament is considered to be an interesting biomarker in ALS to assess the disease progression and potential response to treatment, as it reflects neuronal degeneration. The mean rate of change 3.58 pg/mL per month with AMX0035 and -2.34 pg/mL per month with placebo (difference, 5.93 pg/mL per month; 95% CI, -4.41 to 16.26, p=0.2601), are unexpected findings as the increase in the AMX0035-group would imply increased neuronal degeneration and the decrease in the Placebo group a decrease in neuronal degeneration. However, the finding is not significant and neurofilament is still not accepted as a valid surrogate biomarker and needs further validation. Thus, this secondary endpoint also does not support the efficacy of Albrioza treatment. Neither does it support the suggested mechanism of action.

Survival (death and death equivalent [defined as death, tracheostomy or PAV]) was no different in participants treated with Albrioza or placebo after the double-blind randomized phase of 24 weeks. As anticipated survival of 6 months was an inclusion criterion, this is an expected result and survival after 24 weeks cannot support efficacy of Albrioza.

The applicant presented data on treatment effects on another biomarker, YKL-40, measured in available plasma samples from patients from the CENTAUR study. This is a *post hoc* analysis and the changes of levels of this biomarker can be considered only as exploratory at present. The validation of it as a biomarker measuring specific treatment effect of Albrioza or a biomarker of neurodegeneration responding to any treatment used for ALS would require additional efforts.

In conclusion, although several of the secondary endpoints directionally support an effect of the treatment, the results are based on a very small data set with a high amount of missing data, adding to the uncertainty of data interpretation. Besides, none of them shows statistically significant results in a type I error controlled analysis. This may be expected and acceptable in a *proof of concept* phase II study, but in a pivotal study, with no other studies supporting the results, it is however a fact that such results do not lend a warranted support to the primary efficacy endpoint.

Point not resolved.

5.1.3. Ground #3

Finally, the survival data are not considered robust, the plausibility is questioned and hampered by several methodological issues, e.g., inherent limitations of a single pivotal trial, post-hoc data-collection and analyses without control for multiplicity.

Applicant's position on the third ground for re-examination

The applicant does not accept the CHMP's characterisation that the survival data were not robust. Mortality as a vital status represents an objective assessment and it is especially clinically meaningful in ALS as a rapidly fatal disease. If anything, the survival analysis performed by the applicant may be considered conservative, which is confirmed through alternative approaches. As such, the survival benefit provides confirmatory evidence for establishing the clinical efficacy of Albrioza, particularly in view of the fact that ALS is a universally fatal disease with a relatively short median overall survival from the initial diagnosis.

The submitted ITT overall survival analysis was a randomised, placebo-controlled comparison. All participants were tracked over time and assessed for mortality status (alive or dead) with nearly full follow-up (only 1/137 was censored early). Two groups were compared – the 89 participants that were randomised to Albrioza versus the 48 participants that were randomised to placebo. Both investigators and participants remained blinded to original treatment assignment through the duration of follow-up, which was up to 42 months (3.5 years) after randomisation.

While the placebo group was able to cross over to receive Albrioza after 24 weeks, this submitted analysis did not make any adjustment for placebo crossover. Placebo crossover would, if anything, work against a potential treatment effect (as will be demonstrated below), but ITT analysis was presented because it is the most straightforward and conservative survival analysis. A schematic outline of the survival analysis is provided below in Figure 16.

Figure 16 : CENTAUR Overall survival analysis comparing originally randomised Groups

The result was that the participants randomised to Albrioza had an overall survival (OS) benefit versus those randomised to placebo. Median difference in overall survival was:

- **4.8 months, hazard ratio = 0.64, p = 0.048** (Table 33).

This is the first time in the field of ALS that a treatment has demonstrated, in a randomised controlled trial, a benefit on both function and survival. A survival benefit of 4-5 months was considered by the SAG-N a "huge effect size."

Table 33: ITT Overall survival favours group initially randomised to Albrioza

	Albrioza (N = 89)	Placebo (N = 48)
Median OS, months	**23.5**	**18.7**
Difference	**4.8 months**	
HR (95% CI)[1]	**0.64** (0.4, 1.0)	
p-value	**0.048**	
# of events	**94**	

CI: Confidence interval, HR: Hazard ratio, ITT: Intent-to-treat

However, the Ground for Refusal 3 cites several concerns about the survival result, including plausibility of the survival outcome and methodological issues e.g., inherent limitations of a single pivotal trial, which are discussed below.

Single pivotal study

The applicant acknowledges that the clinical experience with Albrioza is currently based on a single efficacy-safety study. Comprehensive evidence will be able to be provided post authorisation as is appropriate for a conditional MA. Nevertheless, the currently available data consistently point in favour of Albrioza across primary outcome of functional benefit in the 24-week main study, secondary outcomes for ATLIS and SVC, and survival across the entire study follow-up for up to 42 months. This remains the only study in ALS to demonstrate differences on function and survival.

Post hoc data-collection and analyses without control for multiplicity

Survival was a pre-specified endpoint in the randomised phase and the open label extension phase and the hierarchy for each was described in a statistical plan submitted to US FDA prior to unblinding. Results should be considered nominal since endpoints in the hierarchy prior to survival failed.

However, it should be noted there is no possibility for selection bias in the survival analysis and all efforts were made for this to be as robust and conservative an analysis as possible. All study participants were included and there was effectively no missing data through the final date of follow-up. Mortality was determined as all-cause mortality, without any 'judgement' – simply if participants were alive or dead. No placebo crossover was imputed, groups were analysed as randomised. So, while there can be inherent

challenges in such an analysis, the applicant believes the submitted overall survival analysis should be viewed as free from selection bias, complete, robust and conservative.

Plausibility of survival outcome

Plausibility of the survival outcome is questioned in the CHMP AR as follows:

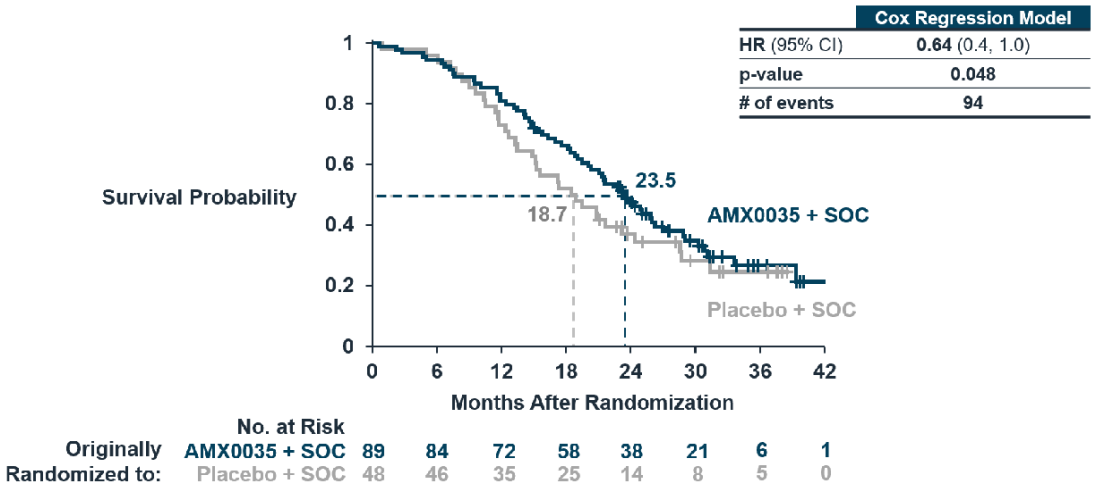
'The observed effect on survival is not considered plausible, as functional loss precedes mortality, and slowing of functional loss cannot be concluded through the methodological issues observed.'

As noted in the Grounds for Re-examination 1, the applicant disagrees with the conclusion that a difference on function has not been shown. Additionally, as a matter of consistency in regulatory decision-making, reference is made to the published EPAR for centrally authorised riluzole, a product indicated for ALS treatment, which states:

> EPAR Conclusion: *"Riluzole has been demonstrated to extend survival in two studies conducted in patients with ALS, but not in a third trial. Survival was the main efficacy criteria and was considered as a strong outcome measure. The failure to find any effect on functional end-points does not affect the reliability of the survival results."*

Furthermore, CHMP has questioned the shape of the Kaplan-Meier survival curve and that there should be clear change in the curve in participants on placebo and switching to active treatment (crossover) in the open-label extension phase (**Figure 17**).

Figure 17: Overall survival ITT observed in AMX0035 and placebo groups in CENTAUR



CI: confidence interval, HR: hazard ratio, ITT: intent-to-treat, SOC: standard of care.

However, the submitted analysis is a comparison between two groups only – randomised to placebo and randomised to active – so this is a pure group-to-group comparison. This was submitted as the most conservative analysis.

Placebo crossover analyses

Three independent analyses including two external datasets were additionally used to characterise the observed crossover survival benefit. All three analyses yielded similar results, showing a large and significant survival benefit of Albrioza.

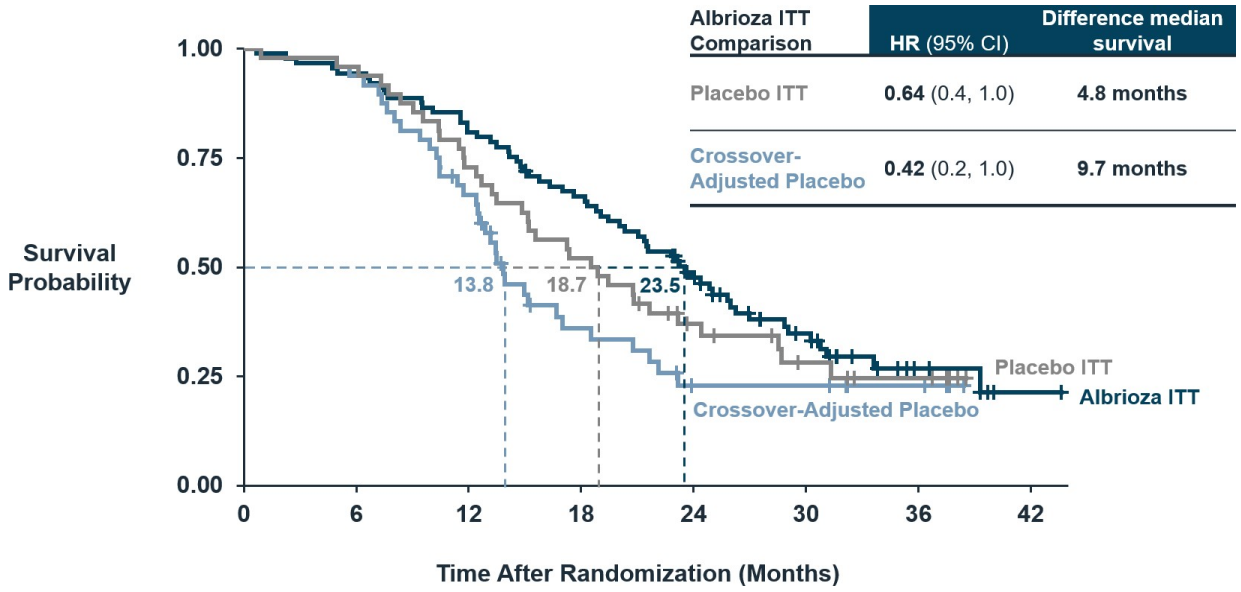
Rank-preserving structural failure time (RPSFT)

The applicant used the method known as Rank-preserving structural failure time (RPSFT) to estimate placebo survival in absence of crossover. RPSFT is a method frequently employed in oncology clinical

trials to account for treatment crossover since oncology trials often include an option for placebo crossover in the case of disease progression. The RPSFT model provides an estimate of the overall survival estimate for the placebo group had treatment switching (crossover) not occurred (Robins et al., 1991, Latimer et al., 2014, Jonsson et. al., 2014).

The results of the RPSFT analysis to estimate the effect of placebo crossover is shown below (Figure 18).

Figure 18: Overall survival ITT observed in AMX0035 and placebo groups in CENTAUR compared with Crossover-adjusted placebo using RPSFT model



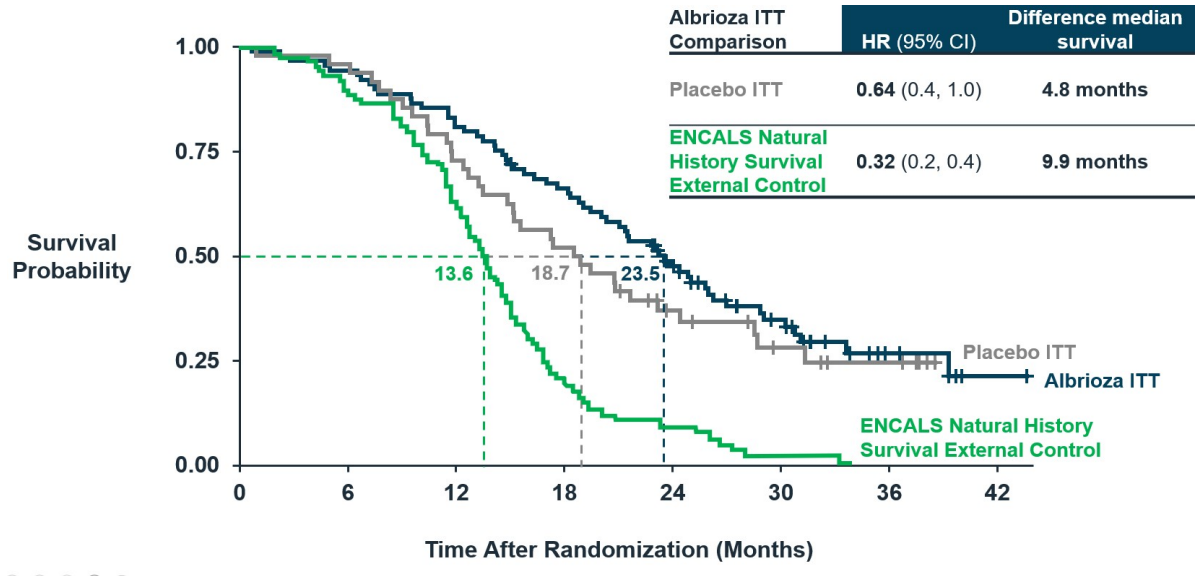
CI: confidence interval, HR: hazard ratio, ITT: intent-to-treat, RPSFT: Rank-preserving structural failure time.

European Network to Cure ALS (ENCALS) simulation

The European Network to Cure ALS (ENCALS) has collected data on more than 10,000 people living with ALS and used this data to create a prognostic model based on baseline factors to predict patient survival time (Westeneng et al, 2018). Amylyx collaborated with the originators of this model to predict treatment naïve overall survival time for each individual participant in CENTAUR. The originators of the model were blinded to treatment assignments when creating their survival estimates.

The predicted (treatment naïve) survival data generated using this model were compared against the actual observed survival data in the CENTUR study and are shown below (Figure 19).

Figure 19: Overall survival ITT observed in AMX0035 and placebo groups in CENTAUR compared with Crossover-adjusted placebo using ENCALS simulation



CI: confidence interval, HR: hazard ratio, ITT: intent-to-treat.

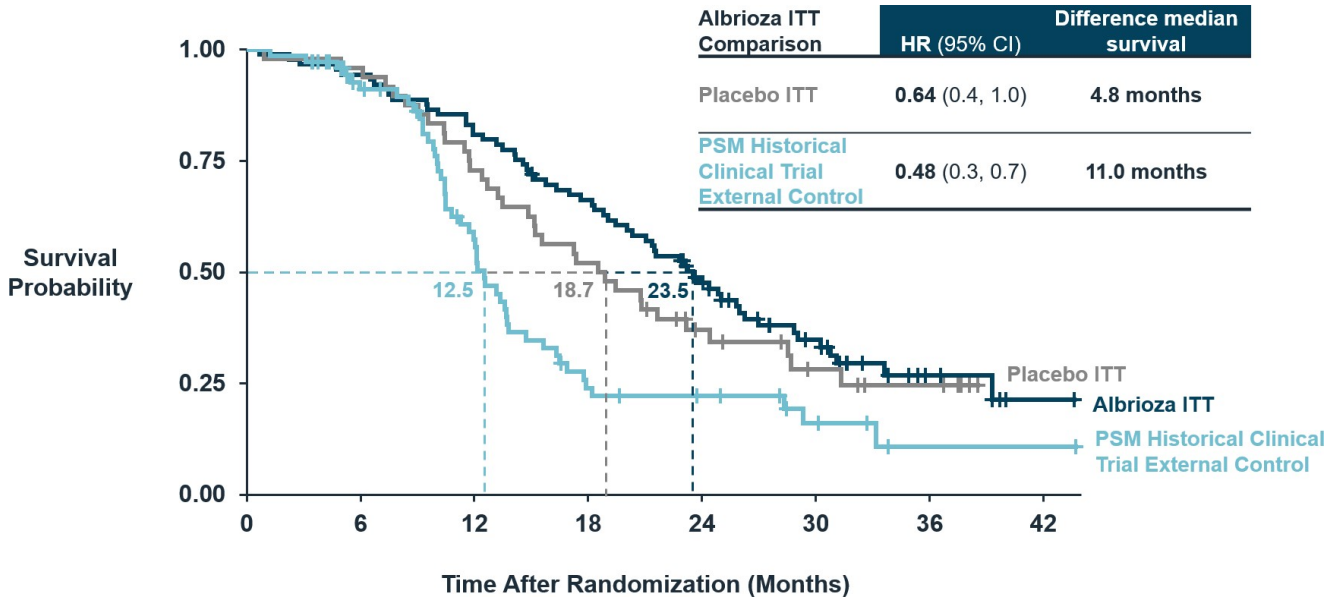
PRO-ACT natural history comparison

The United States PRO-ACT database is a database which collects clinical trial data from completed trials. PRO-ACT contains over 11,000 de-identified patient records from past clinical trials. A clinical trial natural history group was chosen from the PRO-ACT based on the key inclusion/exclusion criteria from CENTAUR to ensure a good comparison group.

Additionally, propensity score matching (PSM) was conducted for the AMX0035 treatment group from CENTAUR and the PRO-ACT historical control group (1:1 ratio) using a caliper width of 0.4 of the standard deviation (SD) of the logit of the propensity score on key prognostic variables including time since ALS symptom onset, pre-baseline ALSFRS-R slope, slow vital capacity and age.

The results of the survival comparison between the groups are shown below (Figure 20).

Figure 20: Overall survival ITT observed in AMX0035 and placebo groups in CENTAUR compared with Crossover-adjusted placebo using PRO-ACT database



CI: confidence interval, HR: hazard ratio, ITT: intent-to-treat, PSM: propensity score matched.

All three analyses support the overall survival finding from CENTAUR

Taken together these external cohort comparisons provide clear evidence for a survival benefit in the placebo cohort that had the opportunity to crossover to active therapy in the open-label extension. That is participants in the ITT placebo arm (who were allowed to cross over to active treatment) survived longer than would have been expected had they remained treatment naïve, suggesting a benefit from crossover to active drug. It is additionally encouraging that three separate methods of estimating treatment-naïve survival in the CENTAUR population had similar findings estimating a treatment naïve cohort to survive between 12.5 and 13.8 months at median.

Most importantly, these analyses further support the robustness and importance of the ITT, overall survival analysis from the CENTAUR trial.

Overview of ground 3 regarding ITT survival analysis

The applicant believes the survival data submitted are robust and clinically meaningful in ALS.

The primary, ITT overall survival analysis showed a statistically significant, 4.8 month difference in overall survival for participants originally randomised to Albrioza (Figure 16). This analysis was nearly complete (136/137 participants through last date of follow-up) and as free from judgement or bias as possible.

A 4-5 month difference in overall survival was considered "a huge effect size" by the experts at the SAG-N.

The methodological choices of the main ITT analysis were, if anything, conservative, as there was no adjustment for placebo crossover. Methods to account for placebo crossover, including RPSFT (Figure 17), the ENCALS Survival prediction algorithm (Figure 19), and propensity score matching against a clinical trial natural history database (Figure 20) all showed that participants who crossed over from placebo to active treatment lived longer than those who did not or than would have been expected.

CHMP position on the third ground for re-examination

The third ground for refusal questions the robustness of survival data, the plausibility is questioned and hampered by several methodological issues, e.g., inherent limitations of a single pivotal trial, *post hoc* data-collection and analyses without control for multiplicity.

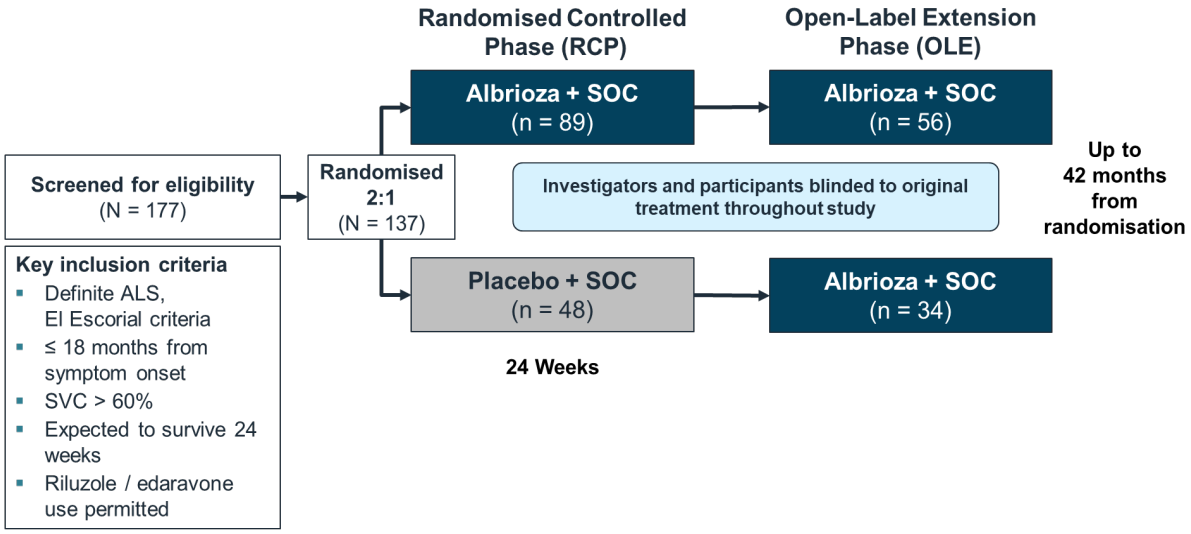
The applicant does not accept the CHMP's characterisation that the survival data were not robust. The applicant claims that the submitted ITT overall survival analysis with a maximum follow up time of 42 months (3.5 years) was a randomised, placebo-controlled comparison. All participants were tracked over time and assessed for mortality status (alive or dead, death equivalent not included) with nearly full follow-up (only 1/137 was censored early). The *post hoc* OL survival analysis was based on the initial randomisation at baseline of the double-blind part of the study. Nevertheless, there was no placebo control after 6 months whereafter all participants were given Albrioza, and the median exposure to Albrioza was considerably shorter than the maximum follow-up time of 42 months. The applicant claims that Median difference in overall survival was 4.8 months, hazard ratio = 0.64, p = 0.048. It should be noted that this is n nominal p-value without type-I error control.

The applicant's postulated statement that a survival benefit of 4-5 months observed during the OL phase is clinically significant for patients could be in principle agreed to by the CHMP. However, the aforementioned methodological limitations preclude this.

It is also noted that the applicant acknowledges that the clinical experience with Albrioza is currently based on a single phase II efficacy-safety study. Since clear benefit of the treatment with Albrioza cannot be established based on presented data (see assessment of grounds 1 and 2 above), these data on survival needs further confirmation.

The applicant also believes the submitted overall survival analysis should be viewed as free from selection bias, complete, robust and conservative. The applicant states that almost all randomized patients (136/137) were included in the survival analysis. Even if all 89 patients treated with Albrioza were included into the survival analysis only 56 patients entered the OLE phase of the study and were actually treated for a longer period than 6 months (Figure 21). Thus, it is unclear which patient group contributed mostly to the survival numbers – the one which continued treatment with Albrioza or the one which stopped treatment. Similar observation might be valid also for the patients from the placebo group since only 34 patients continued in the OLE phase of the study and switched to treatment with Albrioza while the remaining 12 patients were never exposed to the active treatment.

Figure 21: Patient disposition during the randomised controlled phase and the open label extension phase in CENTAUR study



Definite ALS, El Escorial criteria: meets criteria for ALS diagnosis and shows ALS upper and lower motor neuron symptoms in ≥ 3 body regions

The applicant also presented three additional placebo crossover survival analysis: 1) The RPSFT to estimate placebo survival in absence of crossover, 2) comparison based on the data collected by the European Network to Cure ALS (ENCALS) to create a prognostic model based on baseline factors to predict patient survival time and 3) the propensity score matching (PSM) was conducted for the AMX0035 treatment group from CENTAUR and the PRO-ACT historical control group (1:1 ratio) using a caliper width of 0.4 of the standard deviation (SD) of the logit of the propensity score on key prognostic variables including time since ALS symptom onset, pre-baseline ALSFRS-R slope, slow vital capacity and age. These are *post hoc* comparisons with essentially no details how these comparisons were performed. Thus, their value is of limited importance. The use of external data is not considered to add any valuable information to this randomised trial.

With regard to the applicant's comment on riluzole, it should be noted that riluzole was first authorised in 1996. The statement by the CHMP regarding survival and preserved function should be interpreted in its context. Survival at 12 months (the end of the placebo-controlled double-blind phase) was the primary efficacy endpoint and a statistically significant difference in favour of riluzole over placebo was shown.

In conclusion, with regard to Albrioza, the survival analysis is a post-hoc analysis, without type I error protected results, from a small sample without replication, with a borderline nominal p-value. The biological rational is still not clear and "death equivalents" were not included in the analysis.

Point not resolved.

5.1.4. Ground #4

Since efficacy of Albrioza has not been sufficiently shown, a favourable benefit/risk ratio cannot be concluded and, consequently, a conditional marketing authorisation cannot be granted.

Taken together, the available evidence does not support the conclusion of a positive benefit/risk of Albrioza for the treatment of ALS.

Applicants position on the fourth ground for re-examination

The applicant does not accept the CHMP's position that the clinical efficacy has not been sufficiently shown to support a positive benefit/risk balance. The CENTAUR study showed a clear and clinically

important treatment benefit and favourable safety profile for people with ALS, a critically unmet medical need. There are compelling grounds for Albrioza to be considered for grant of a CMA, given that all the prerequisite conditions are satisfied according to the established regulatory framework.

CENTAUR was a randomised placebo-controlled study which met its pre-specified primary outcome showing a significant and clinically meaningful 25% slowing in ALS functional decline for Albrioza vs. placebo (2.32 point treatment difference on the ALSFRS-R score over 24 weeks). In an ITT analysis with essentially no missing data, a 4.8 month longer overall survival benefit was observed for participants randomised to Albrioza vs. placebo. These analyses used standard and typical statistical approaches for the ALS field and results remained robust when statistical hypotheses were tested in sensitivity analyses.

These key outcomes of CENTAUR were supported by additional independent outcomes, including consistent effect sizes (although non-significant) on secondary outcomes including tests of breathing (23% slowing of decline in SVC) and muscle strength (15% and 20% slowing of decline in Total and Upper Limb ATLIS, respectively), and pre-specified long-term analyses such as a delay in time to first hospitalisation (almost 1.5 years delay). Biomarker changes in YKL-40 supported the efficacy outcomes, and additional changes on biomarkers relevant to ALS were observed in a randomised placebo-controlled trial in people with Alzheimer's disease.

These observed treatment benefits were accompanied by a favourable safety profile. There was a lower incidence of SAEs in the Albrioza vs. placebo group during the 24-week double-blind phase of CENTAUR. Most SAEs were consistent with the manifestations and complications of underlying ALS, such as respiratory failure, and the lower incidence corroborates the treatment benefit of Albrioza in slowing disease progression. Gastrointestinal adverse events are the main consideration for the safety profile of Albrioza; these have a higher incidence during the start of treatment with Albrioza, but otherwise show no considerable difference between placebo and Albrioza in the intensity and duration of these events. Events were generally mild in severity.

The consistent findings from the data support a true treatment effect based on a well-designed and conducted study, with concordant favourable outcomes across primary and key secondary endpoints, and with sensitivity analyses supporting the primary model. In addition, a structured analysis of treatment benefits and risks has been conducted and finds the benefit/risk balance of Albrioza to be positive even when taking account potential uncertainties.

The unmet medical need in ALS is undisputed. ALS is a rapidly progressive, fatal disease with an average life expectancy of about 2 years from diagnosis despite current standard of care. There is just one EMA-approved treatment for ALS (riluzole), and no treatments have been approved in the EU for over 25 years. It is an understatement that the ALS community is in desperate need of new treatments. Amylyx is applying for conditional MA for Albrioza, as the applicant believes that the benefit of immediate availability of Albrioza outweighs the risks inherent in the fact that the approval would rely on a single positive study and that comprehensive data will be provided post-approval.

Considering all the above, the CHMP is asked to reconsider its decision not to issue a positive opinion. The available data for Albrioza provide compelling support for a true treatment effect and favourable safety profile based on a well-designed and conducted study. The PHOENIX study, which has already been fully recruited with 664 participants, will provide additional evidence within a reasonable period of time - complementary to and extending on the existing dataset. The applicant contends that for people living with ALS the benefits of earlier availability substantially outweigh the risks and that there are compelling grounds for issuance of a CMA.

CHMP position on the fourth ground for re-examination

The fourth ground for refusal stated that since efficacy of Albrioza has not been sufficiently shown, a favourable benefit/risk ratio cannot be concluded and, consequently, a CMA cannot be granted.

The applicant does not accept the CHMP's position that the clinical efficacy has not been sufficiently shown to support a positive benefit/risk balance and essentially present the same arguments already lifted in the response to the first three grounds of refusal.

Furthermore, the applicant has performed a structured analysis of treatment benefits and risks. This analysis assumes benefits to have been established as true effects. Although the analysis allows size of effects to vary, the lowest effect used for functional decline is the point estimate of the CHMP preferred analysis, not considering the uncertainty of this estimate in form of the confidence interval.

The CHMP considers that the efficacy data provided in the single study are neither robust nor statistically compelling and it does not allow to conclude that the ALS patients will have clear benefit from treatment with Albrioza (see assessment of first and second grounds of refusal above). The safety profile includes gastro-intestinal tolerability issues, potentially affecting the patients' quality of life. Since efficacy of Albrioza has not been sufficiently shown, the benefit risk is currently negative. As positive benefit risk should be established for the CMA, the opinion of the CHMP remains that the CMA cannot be granted at present moment.

The presented data need further confirmation and the ongoing PHEONIX study could provide such data.

Point not resolved.

5.2. Report from the SAG-N

Following a request from the applicant at the time of the re-examination, the CHMP convened a Scientific Advisory Group (SAG) inviting the experts to provide their views on the CHMP grounds for refusal, taking into account the applicant's response.

1. Considering the results for the primary endpoint in the Centaur study as evaluated by the primary analysis as well as the sensitivity analysis recommended by the CHMP, do you consider that a statistically robust effect with respect to slowing of functional loss has been documented for Albrioza?

The SAG-N experts noted a positive difference in the amyotrophic lateral sclerosis functional rating scale revised (ALSFRS-R) score in favour of Albrioza in the phase II CENTAUR study. However, the SAG-N experts identified a number of limitations in connection with the same results for the primary endpoint in the study.

In particular, it was highlighted that the effect size was marginal insofar as the significance level of the observed efficacy was borderline. It was also noted that the study was based on a short study duration. Additionally, the study population enrolled in the CENTAUR study was considered to be poorly representative of the whole population of people with ALS. To illustrate this consideration, one of the experts noted that only 6% of the participants enrolled in the Swedish ALS registry could have met the eligibility criteria for participating in the CENTAUR trial. In turn, this was considered to undermine the external validity of the results.

Finally, some SAG-N experts noted past drug developments for which successful phase II studies were not confirmed in the subsequent phase III trials.

In light of the above considerations, the majority of the SAG-N experts considered that the results for the primary endpoint in the CENTAUR study were not capable of demonstrating a statistically robust effect with respect to the slowing of functional loss; and, therefore, could not be considered as sufficient to support a recommendation for the granting of a conditional marketing authorisation. In furtherance of that position, it was indicated that, based on the current limitations identified, it would be preferable

to await the results of the larger confirmatory PHOENIX phase III trial before potentially being able to conclude on the effectiveness of Albrioza in respect to the slowing of functional loss.

In addition, some SAG-N experts raised the possibility that the uneven distribution of adverse events could have impacted the blinded evaluation of the clinical efficacy assessment of the participants. However, one expert pointed out that the ALSFRS-R score is a robust enough outcome not to be affected even in the case whereby there are indications of adverse events by the evaluating physician. Overall, these observations did not alter the finding (based on the conclusion reached by a majority of the SAG-N) that, based on available evidence, a statistically robust effect with respect to the slowing of functional loss has not been documented for Albrioza.

One of the patient representatives expressed the (minority) view that the results are positive, Further, in the view of the patient representation, a positive recommendation concerning the granting of a marketing authorisation for Albrioza should not be delayed considering, in their view, the large unmet medical need at issue.

2. Do you consider that the results for the secondary efficacy endpoints, including the biomarker pNF-H (biomarker indicating neuronal injury) are supporting the results for the primary endpoint taking into account limitations such as missing data?

Some SAG-N experts considered that the results for the secondary efficacy endpoints demonstrated some indication of a (marginal) treatment effect. However, the results did not contribute or not add much information as regards the results on the primary end point (ALSFRS-R). The experts considered that clinical outcome measures other than ALSFRS-R could be more variable and less sensitive than ALSFRS-R. As a result, it was considered that the CENTAUR design setting and sample size were not considered adequate to capture a clinical effect on these outcomes. Other SAG-N experts considered that results for the secondary efficacy endpoints were not supportive, particularly considering that those endpoints were not controlled for multiplicity. It was noted that this should be considered when determining the role of these endpoints in the context of assessment of efficacy.

Some SAG-N experts highlighted that the plasma neurofilament heavy chain (pNF-H) is not a validated biomarker so the absence of positive results on pNF-H is irrelevant and did not add any supportive value on the evaluation of efficacy.

3. Do you consider the survival data to be robust, considering the plausibility of the results in relation to the effect on functional loss as well as methodological issues, e.g., inherent limitations of a single trial, post-hoc data-collection and analyses without control for multiplicity?

The SAG-N experts agreed by consensus that the survival data is not robust considering the methodological limitations that were identified, such as the *post hoc* data collection and analysis, small sample size and short time of follow-up. While it was observed that the results were informative, they were not considered robust enough in and by themselves to support the conclusion that Albrioza increases survival time in people with ALS.

The patient representative highlighted the *post hoc* nature of the survival data.

Observations from the applicant

The applicant has provided observations about the SAG Neurology responses relative to the primary and secondary efficacy endpoints including survival. In the view of the applicant, the SAG-N answers justify the CMA based on the agreement on CENTAUR as a positive study on the primary outcome, the agreement by some SAG-N experts that the secondary endpoints are supportive and that neurofilaments findings are not relevant and the agreement that data on survival is informative even if there is disagreement on the robustness of findings. Further, the applicant reiterated that all criteria required for

a CMA are met. The CHMP has taken into account and assessed these observations before adopting the final opinion.

5.3. Oral explanation

The applicant was invited to present its position during an oral explanation (OE). On 10 October 2023, a presentation to address the grounds for refusal was made in front of the CHMP plenary meeting.

However, after this OE, the Committee concluded that the grounds for refusal, with the uncertainties and concerns regarding the efficacy of Albrioza as expressed above, still remained.

5.4. Overall conclusion on grounds for re-examination

The CHMP assessed all the detailed grounds for re-examination and argumentations presented by the applicant and considered the views of the Scientific Advisory Group.

The CHMP concluded that none of the grounds for refusal have been solved by the applicant.

6. Benefit-risk balance following re-examination

6.1. Therapeutic Context

6.1.1. Disease or condition

With a median survival of approximately 2 years from diagnosis, ALS is a universally fatal neurodegenerative disease marked by rapid loss of motor function due to degeneration of motor neurons. Most affected patients eventually require assistance with activities of daily living, with subsequent progression leading to respiratory compromise, complete paralysis, and respiratory failure, the leading cause of death in ALS.

The applicant is seeking a CMA of Albrioza for the following indication: the treatment of ALS.

6.1.2. Available therapies and unmet medical need

There is currently one approved product for ALS treatment in Europe: riluzole (Rilutek®), which blocks glutamatergic neurotransmission in the CNS. This product showed a survival benefit (~3-6 months).

Despite the availability of riluzole, a high unmet medical need remains for patients with ALS who face rapid morbidity and mortality even with this therapy.

6.1.3. Main clinical studies

The application is based on one study. Study AMX3500 was a phase II randomized, double-blind, placebo-controlled study which evaluated the fixed-dose combination AMX0035 (3g sodium PB / 1g TUDCA) in patients with ALS in the United States. The study consisted of a 24-week double-blind treatment period. Upon completion, subjects could enter the OLE phase of the study, where subjects on placebo switched to active treatment.

Subjects aged 18 to 80 years of age with a definite diagnosis of sporadic or familial ALS as defined by the El Escorial criteria with less than or equal to 18 months since ALS symptom onset were eligible to enter into the study. In addition, subjects had to have a SVC of >60% of the predicted value for gender, height and age. Subjects were permitted to use riluzole and/or edaravone.

There were 137 subjects randomized into the study: 89 to AMX0035 and 48 to placebo. During the initial 3 weeks of treatment, subjects were instructed to take 1 sachet of the study drug daily. If well-tolerated, subjects could increase their dose to the recommended dosing of 2 sachets daily, corresponding to a total daily dose of 6g PB/2g TUDCA in the AMX0035 group.

The primary endpoint was the rate of decline in the total ALSFRS-R score at week 24.

Secondary endpoints included the rate of decline in ATLIS; the impact on plasma concentration of pNF-H; the rate of decline in SVC, and the impact on survival (defined as death, tracheostomy or permanent artificial ventilation).

6.2. Favourable effects

The estimated total ALSFRS-R scores at week 24 were 29.06 for the AMX0035 group and 26.71 for the placebo group. The estimated difference between the groups was 2.32, which was statistically significant in favour of AMX0035 (p= 0.0340).

Numerical improvements with AMX0035, were observed, for

- the estimated total ATLIS scores; 39.08 for the AMX0035 group and 36.26 for the placebo group, with an estimated difference of 2.82 at week 24,
- the SVC % predicted; 66.17 for AMX0035 and 61.06 for placebo, with an estimated difference of 5.11 at week 24,
- the estimated percentage of the event of death or death equivalent; 2.8 for the AMX0035 group and 4.4 for the placebo group (hazard ratio 0.632) at week 24. For death events, this was 2.6 for subjects treated with AMX0035 and 2.6 for subjects treated with placebo (hazard ratio 1.016).

Survival was also captured retrospectively at two-time points in the OLE phase of the study. At the most recent cut-off (March 1, 2021), the median survival estimate for death or death equivalent was 23.5 months for subjects who received AMX0035 throughout the study and 17.9 months for subjects initially randomized to placebo (hazard ratio 0.597). For time to death, the median survival estimates were 23.5 and 18.7 (hazard ratio 0.619) for subjects treated continuously with AMX0035 and those who switched to AMX0035 in the OLE, respectively.

6.3. Uncertainties and limitations about favourable effects

Based on the applicant's original analysis, the CENTAUR study, the only study in the development program performed including ALS-patients, met its primary efficacy objective by showing a statistically significant difference in favour of AMX0035 in the rate of decline in the ALSFRS-R.

The applicants pre-specified primary analysis was questioned by the CHMP from four aspects.

- First, the use of mITT population instead of the ITT population was questioned. The mITT population excluded two subjects, both from the experimental arm and both died. Excluding subjects based on events occurring after randomisation can introduce bias in the analysis, especially if the reason for exclusion is death.

- Secondly, the primary analysis of the rate of decline (slope) used a shared-baseline, mixed-effects analysis, including age, the estimated rate of disease progression (i.e., ΔFS), and baseline of the efficacy outcome of interest (other than ALSFRS-R) as covariates and interacting with time. This is not considered an appropriate method. The method strongly relies on linearity of disease progression, and although progression has been shown to decrease in a linear fashion over the course of a typical clinical trial, the linearity is disputed over longer periods (Karanevich 2018), as is the case here since the model includes the progression rate from symptom onset to baseline as covariate.
- Furthermore, the primary analysis model also assumed data to be MAR. This is not considered a reliable assumption as it is unlikely that patients that discontinue or die will still experience a treatment effect.
- Lastly, handling death in the same way as other missing data was not agreed.

Therefore, the applicant was requested to perform and present an analysis of the primary endpoint using the change from baseline for the ITT population, with time as a fixed categorical factor instead of continuous in the model (i.e. not relying on linearity) and using a placebo based imputation for discontinuations and worst-case imputation (ALSFRS-R score of "0") in case of death

In the most optimistic case, i.e. the applicant's original analysis, the effect was 2.32 (95% CI 0.18, 4.47) and the difference between treatment arms was statistically significant (p=0.034). In the most conservative case, i.e. the CHMP's requested analysis as described above, the effect size is smaller, and the primary endpoint is no longer statistically significant (1.52, 95% CI -0.80, 3.84, p=0.20). Additional sensitivity analyses fall somewhere in between these estimates (e.g., placebo-based imputation for death: 1.96, 95% CI -0.02, 3.94, p=0.052). This indicates that the effect estimate and clinical significance for the primary endpoint vary according to the applied method for analysis. Thus, the true treatment effect of AMX0035 cannot be reliably estimated due to the uncertainties highlighted above. The results, therefore, are neither robust nor statistically compelling.

The SAG-N experts noted a positive difference in the ALSFRS-R score in favour of Albrioza in the phase II CENTAUR study. However, they also noted some of the above limitations and consequently, the majority of the SAG-N experts considered that the result for the primary endpoint in the CENTAUR study was not capable of demonstrating a statistically robust effect with respect to the slowing of functional loss.

Moreover, there is no support from any of the secondary efficacy endpoints, e.g. ATLIS, biomarker pNF-H and SVC neither in terms of statistical significance or effect size. Some SAG-N experts considered that secondary endpoints did not add much information as regards the results on the primary endpoint and the remaining considered that they were not supportive. Further, the key secondary endpoint ATLIS is currently not fully validated and the contribution of the findings on this endpoint to the overall conclusions of the study is considered very limited.

Post hoc analyses performed over the placebo-controlled + OLE period suggest that AMX0035 had a beneficial effect on survival. The requested re-analysis (Cox regression model) appears to be consistent with the initial findings and the reported HR and p-value are more positive compared to the original analysis. However, this needs to be considered in the context of a study failing to provide robust evidence of efficacy for the primary endpoint based on an appropriate analysis. The SAG-N experts also noted methodological limitations such as *post hoc* data collection and analysis, small sample size and short time of follow-up and concluded that while informative, results were not considered robust enough in and by themselves to support the conclusion that Albrioza increases survival time in people with ALS.

Furthermore, the plausibility of the presented survival effect observed remains questionable. It would be expected that the survival curve should change in subjects initially randomised to placebo after having

switched to active treatment after similar exposure time. However, no favourable effect in this regard could be observed.

Finally, the lack of support from the analysis of the main biomarker pNF-Hs lowers the evidentiary value of the downstream results. ALS symptom progression results from motor neuron degeneration, causing the loss of motor function. It is hypothesized that AMX0035 reduces neuronal cell death by the PB/TURSO combination via mitigating both ER- and oxidative stress. Notable, the mean rate of change in the AMX group was 3.58 pg/mL per month (consistent with increased neuronal death), and the mean rate of change in the placebo group was -2.34 pg/mL per month (consistent with decreased neuronal cell death). However, the difference (5.93 pg/mL per month; 95% CI, -4.41 to 16.26) is not significant, p=0.26. In total, neither the results on the biomarker pNF H (biomarker indicating neuronal injury), nor those on functional decline provide convincing support for the proposed mechanism of action. In any case, the CHMP noted that some SAG-N experts highlighted that results of the plasma neurofilament heavy chain (pNF-H) – suggesting and increased neuronal death compared to placebo- are irrelevant as in their view pNF-H is not a validated biomarker.

6.4. Unfavourable effects

A total of 137 subjects were available for safety analysis during the main phase of study AMX3500 of which 48 were treated with placebo and 89 with AMX0035. During this phase, the mean exposure was 21.5 weeks (Q1-Q3: 22.8 ;24.1) in the placebo group and 19.7 weeks (16.3; 24.4) in the AMX0035 group.

A total of 34/48 subjects from the former placebo group switched to AMX0035 in the OLE phase. For subjects initially randomized to AMX0035, 56/89 subjects rolled over into the OLE phase. The median exposure in the OLE phase was 15.4 weeks in placebo to AMX0035 (RP) and 31 weeks in AMX0035 to AMX0035 (RA). Expressed in patient-years, the exposure to AMX in the main phase of the study was 33.7 patient-years, in OLE 68.4 patient-years and cumulatively 103 patient-years.

The most common AEs, i.e. AEs reported with a frequency of >10% main phase are diarrhoea (8 (16.7%) vs. 19 (21.3%)), nausea (6 (12.5%) vs. 16 (18.0%)), constipation (12 (25.0%) vs. 12 (13.5%)), salivary hypersecretion (1 (2.1%) vs. 10(11.2%)), muscular weakness (9 (18.8%) vs. 18 (20.2%)), neck pain (5 (10.4%) vs. 2 (2.2%), fall (18 (37.5%) vs. 25 (28.1%)), headache (11 (22.9%) vs. 12 (14.6), dizziness (2 (4.2%) vs. 9 (10.1%)), viral upper respiratory tract infection (2 (4.2%) vs. 10 (11.2%), dyspnoea (4 (8.3%) vs. 9 (10.1%)), for placebo and AMX0035 respectively.

During the main phase, AEs leading to treatment interruption were reported in 5 (10.4%) vs. 12 (13.5%) subjects, dose reduction in 0 vs. 4 (4.5%) subjects or drug withdrawal in 5 (10.4%) vs. 18 (20.2%) of the subjects in the placebo and AMX0035 group, respectively. Reasons for discontinuations were primarily gastrointestinal disorders (9 (6.6%) subjects of which (1 (2.1%) in placebo and 8 (15.7%) in AMX0035) group respectively.

The provided data on AEs causally related to AMX0035 suggests a dose-response relationship in most AEs. The majority of patients were able to follow the 2 sachet dose throughout the study, albeit some with temporary interruptions or dose reductions due to AEs. Data regarding the used dose on re-start of treatment and treatment continuation thereafter shows a variable pattern. Altogether based on the provided data regarding temporary drug interruption, re-start of treatment and recurrence of AEs, and taking into account the unknown efficacy of reduced dose, no specific advice on risk mitigation through these measures can be recommended.

At least one SAE was reported in 8 (16.7%) placebo subjects and 11 (12.4) AMX0035 subjects during the main study part, and in 18 (37.5%) RP and 27 (30.3 %) RA subjects during the overall study,

respectively, Apart from one SAE of nephrolithiasis considered related by the investigator in each, the AMX0035 and the placebo group, no further SAE or death was considered treatment-related by the investigator during the main and OLE study part. This can be agreed.

SAE or death was considered treatment-related by the investigator during the main and OLE study part. This can be agreed. Seven subjects died during the main phase of the study with an incidence of 5 subjects [5.6%] AMX0035 group and 2 subjects [4.2%] placebo group. The cause of death was respiratory failure in 3 subjects in the AMX0035 and 2 subjects in the placebo group. Other causes of death in the AMX0035 group included subdural hematoma, and diverticular perforation. Cumulatively (main and OLE phase) a total of 22 (16.1%) of the subjects died. Fourteen (10.2%) due to respiratory failure.

Three PB-related impurities have not been structurally identified as requested and, therefore, not qualified for genotoxicity. During the procedure, the applicant identified (preliminary data) the likely root cause for the formation of certain unidentified impurities. However, the actual analytical data have not been provided nor have the impurities been structurally characterised. It is agreed that the applicant could have addressed these uncertainties during the post-authorisation phase.

6.5. Uncertainties and limitations about unfavourable effects

The use of AMX0035 in elderly subjects is considered relevant given the underlying disease. While the comparison of data in subjects aged ≤65 and > 65 years of age suggests a similar safety profile of AMX0035 in these subgroups, a limited number of subjects above the age of 65 years was included in the study.

No clinical data in patients with moderate to severe renal impairment or hepatic impairment (all severity) is available. The need for dosing recommendation in these patient populations from a safety perspective is unclear, taking into account that the dose-response relationship in AEs was not addressed by the time of opinion.

6.6. Effects Table

Table 34: Effects Table for Albrioza

Effect	Short Description	Unit	AMX0035	Placebo	Uncertainties/ Strength of evidence	References
Favourable Effects						
ALSFRS-R	the rate (slope) of decline in the total ALSFRS-R score at week 24	Estimate (SE)	29.06 (0.781)	26.73 (0.975)	**SoE**: Estimated difference: 2.32 (1.094) vs. placebo p < 0.0340 **Unc:** (I) Effect is not robust. In sensitivity analyses using different assumptions the treatment effect becomes smaller and stat. significance is lost. (smallest estimated difference 1.52, p=0.20) (II) Clinical relevance questioned and effect not supported by any of the secondary endpoints. (III) The sequential testing procedure already stopped at the first secondary endpoint (ATLIS, p= 0.1129)	Study AMX3500 (double-blind phase)
ATLIS	The rate (slope) of decline in the total ATLIS score at week 24	Estimate (SE)	39.08 (1.990)	36.26 (2.224)	**Unc:** Estimated difference: 2.82 (1.774) vs. placebo p = 0.1129 The endpoint is not validated in ALS	Study AMX3500 (double-blind phase)

Effect	Short Description	Unit	AMX0035	Placebo	Uncertainties/ Strength of evidence	References
SVC	The rate (slope) of SVC decline at week 24	Estimate (SE)	66.17 (2.327)	61.06 (2.812)	**Unc:** Estimated difference: 5.11 (2.872) Nominal p = 0.0763	Study AMX3500 (double-blind phase)
Survival	Time to death or death equivalent	Median survival estimate (months)	23.5	17.9	**SoE:** Hazard ratio: 0.597 (95% CI 0.387, 0.923) Nominal p = 0.0203 **Unc**: (I) No difference between placebo/AMX0035 observed in double-blind treatment period (nominal p > 0.5960). (II) Not formally tested;	Study AMX3500 (double-blind phase; open-label phase) [March 1, 2021 cut-off]
	Time to death	Median survival estimate (months)	23.5	18.7	**SoE** Hazard ratio: 0.619 (95% CI 0.399, 0.960 Nominal p =0.0324 **Unc**: (I) No difference between placebo/AMX0035 observed in double-blind treatment period (nominal p > 0.9873). (II) Not formally tested. (III) Plausibility of survival benefit questioned. Delay in separation of curves late in the study and no benefit for subjects who switched from placebo to active treatment in OLE. (III) A slowing of functional loss is not observed, which is expected to precede survival.	Study AMX3500 (double-blind phase; open-label phase) [March 1, 2021 cut-off]
Unfavourable Effects						
Gastrointestinal disorders	Adverse event	N (%)	59 (66.3)	30 (62.5)	**Unc**: (I) SOC common reason for study drug discontinuation, temporary suspension or dose reduction. (II) Unclear at what dose (i.e. 1 or 2 sachets) event occurred and whether could be effectively mitigated with dose reduction or temporary discontinuation.	Study AMX3500 (double-blind phase)

Abbreviations: ALSFRS-R= Amyotrophic Lateral Sclerosis Functional Rating Scale – Revised, AMX0035= 3g sodium phenylbutyrate / 1g ursodoxicoltaurine, ATLIS= Accurate Test of Limb Isometric Strength, OLE= open-label extension PB= phenylbutyrate SE= standard error, SoE= Strength of Evidence, SVC= Slow Vital Capacity, Unc= Uncertainty

6.7. Benefit-risk assessment and discussion

6.7.1. Importance of favourable and unfavourable effects

The applicant has performed a phase II proof of concept study (CENTAUR) with Albrioza (TUDCA and PB) in the treatment of ALS. This is the first, and the only, study performed with Albrioza in the treatment of ALS with available data.

Mechanism of action

The mechanism by which Albrioza exerts its therapeutic effects in patients with ALS is not very well-understood. According to the applicant, PB and TUDCA, in combination, demonstrate synergistic activity in reducing neuronal death, hypothesised to occur through simultaneous inhibition of ER and mitochondrial stress-related cell death. PB is a HDAC inhibitor that decreases ER stress through the upregulation of chaperone proteins and acts as a small molecular chaperone. TUDCA decreases mitochondrial stress by reducing mitochondrial permeability and increasing the apoptotic threshold of

the cell through inhibition of the BAX protein. The combination of the two components is meant to mitigate ER stress and mitochondrial dysfunction simultaneously, aiming at reducing neurotoxic inflammation and ultimately neuronal death.

Primary efficacy endpoint

The analysis of the primary endpoint showed a reduction in ALSFRS-R of 2.32 points in the AMX0035 treated group compared to the Placebo group after 24 weeks (p=0.034). A verified result of this magnitude would usually be considered as clinically relevant, even if a larger difference (3.2 Points in ALSFRS-R per 6 months) has been suggested to ensure a minimal important difference according to a patient-defined approach (Fournier *et al* 2022).

The model used when deriving the effect of 2.32 points per 24 weeks used assumptions not considered adequate by the CHMP requesting a sensitivity analysis simultaneously addressing four aspects of the primary model: the ITT population should be used instead of mITT, categorical time should be used (i.e., not relying on assumptions on linearity), using a placebo-based imputation for discontinuations instead of missing at random, and using a worst-case imputation (ALSFRS-R score of "0") in case of death, was requested. This model showed a point estimate of the difference between the two groups in ALSFRS-changes from baseline at 24 weeks of 1.52 points, p=0.20. Thus, the primary analysis of the efficacy endpoint is not considered as robust since the CHMP preferred analysis with a reasonably conservative approach for missing data and less dependent of model assumptions results in a different conclusion than the primary analysis.

It may be argued that patients may die before reaching a score of ALSFRS-R 0 and that imputing death with 0 is not appropriate since it does not reflect actual disease trajectory and it inappropriately biases the ALSFRS-R. It is agreed that participants may die before reaching ALSFRS-R 0, but patients with a score of 0 may also be alive. A strictly conservative analyses would impute a negative value for ALSFRS-R. However, the scale floor is 0 and negative imputation has not been requested. A less conservative analysis with imputation of the mean ALSFRAS-R value of Placebo-treated participants is possible, however such an analysis clearly risks overestimating the ALSFRS-R of participants who pass away since the average placebo score is achieved among living participants, not even reflecting the lower range of the score in these participants.

By using continuous time in the model, the method strongly relies on linearity of disease progression, and although progression has been shown to decrease in a linear fashion over the course of a typical clinical trial, the linearity is disputed over longer periods (Karanevich 2018), as is the case here since the model includes the progression rate from symptom onset to baseline as covariate. Thus, using time as a categorial variable is more adequate.

Furthermore, excluding subjects based on events occurring after randomisation can introduce bias in the analysis, especially if the reason for exclusion is death. Hence the ITT population is preferred with conservative imputation of missing data.

Secondary efficacy endpoints

Secondary efficacy outcomes in the 24-week main phase of CENTAUR included the rate of decline in isometric muscle strength as measured by the ATLIS device, the decline in plasma levels of the pNF-H, the rate of decline in the SVC, and survival (including death and death equivalent).

The muscle strength was measured by the ATLIS scale which according to the applicant was used first time in the CENTAUR clinical trial and, thus, the capability of this scale to detect treatment effects on muscle strength is in not very well known. Some numerical changes in favour of the AMX0035 arm vs placebo were observed both for muscle strength as well as respiratory function but did not reach statistical significance (Figure 15). The interpretation of the effect on muscle strength and SVC results

was even further hampered by the fact that 36.5% of study participants did not complete ATLIS assessment and 29% of study participants did not complete SCV test. Results provided by the applicant assume missing at random. Thus, the secondary clinical endpoints do not support the primary endpoint.

Neurofilament is considered to be an interesting biomarker in ALS to assess the disease progression and potential response to treatment. However, no effect was seen in this secondary endpoint, and it is still not accepted as a valid surrogate biomarker and needs further validation.

Survival analyses, as expected, did not show any benefit of AMX0035 over Placebo after 24 weeks.

In conclusion, although several of the secondary endpoints directionally support an effect of the treatment, none of them shows statistically significant results in a type I error-controlled analysis. Furthermore, the results are based on a very small data set with a high amount of missing data, adding to the uncertainty of conclusions. This may be acceptable in a *proof of concept* phase II study, but in a single pivotal study, it is however a fact that such results do not lend a warranted support to the primary efficacy endpoint.

Post hoc analyses

Survival analyses

A survival analyses was performed based on the initial randomization groups. Of the 137 randomised, 136 patients could be followed up regarding death; death equivalents such as tracheostomy and PAV could not be assessed reliably and were not included in this analysis, i.e. some patients may be alive but in need of continuous ventilatory support. Even if all 89 patients treated with Albrioza were included into the survival analysis only 56 patients entered the OLE phase of the study and were actually treated for a longer period than 6 months. Thus, it is unclear which patient group contributed mostly to the survival numbers – the one which continued treatment with Albrioza or the one which stopped treatment. Similar observation might be valid also for the patients from the placebo group since only 34 patients continued in the OLE phase of the study and switched to treatment with Albrioza while remaining 12 patients were never exposed to the active treatment.

No survival benefit was observed during the double-blind treatment period. This could be understandable since the six months period is relatively short for detection of changes of survival. When the OL phase included the maximum follow-up time, 42 months (3.5 years) after randomisation, median duration of AMX treatment in the OLE was 4 months in former Placebo participants and 8 months in the former AMX participants. A median difference in overall survival was 4.8 months, hazard ratio = 0.64, p = 0.048. This is a *post hoc* analysis, without type I error control.

In the re-examination procedure, three additional placebo crossover survival analysis were submitted: 1) The RPSFT to estimate placebo survival in absence of crossover, 2) comparison based on the data collected by the ENCALS to create a prognostic model based on baseline factors to predict patient survival time and 3) the PSM was conducted for the AMX0035 treatment group from CENTAUR and the PRO-ACT historical control group (1:1 ratio) using a caliper width of 0.4 of the standard deviation (SD) of the logit of the propensity score on key prognostic variables including time since ALS symptom onset, pre-baseline ALSFRS-R slope, slow vital capacity and age. These are *post hoc* comparisons with essentially no details how these comparisons were performed. Thus, their value is of limited importance. The use of external data is not considered to add any valuable information to this randomised trial.

In conclusion, the survival analyses are *post hoc* analyses, without type I error protected results, from a small sample without replication. The biological rational is still not clear and "death equivalents" were not included in the analysis.

Safety

SAEs and deaths occurred at a similar frequency in the AMX0035 and Placebo groups and were not considered treatment related. Treatment emergent AEs were dominated by gastrointestinal disorders such as diarrhoea, nausea an abdominal pain. A number of patients discontinued due to such events, interrupted treatment or had dose reductions. Even if mostly mild or moderate in intensity, such AEs may be troublesome in patients with impaired nutrition and ability to perform their activities of daily living without the help of others. Other treatment related AEs were dizziness, hot flush, and fatigue. The safety profile of AMX0035 is overall acceptable. Nonetheless, this is given that there is an established effect. Survival is short in ALS patients and quality of life and hence tolerability of given medications is important. Patients should not be subjected to AEs potentially lowering their quality of life without earning an effect.

Benefit-risk balance

The applicant has performed *a structured analysis of treatment benefits and risks* as presented in addendum 1 to the detailed grounds for re-examination. This analysis assumes benefits to have been established as true effects. Although the analysis allows size of effects to vary, the lowest effect used for functional decline is the point estimate of the CHMP recommended analysis, not considering the uncertainty of this estimate in form of the confidence interval.

The CHMP considers that the efficacy data provided in the single study are neither robust nor statistically compelling and it does not allow to conclude that the ALS patients will have clear benefit from treatment with Albrioza (see assessment of first and second grounds of refusal above).

6.7.2. Balance of benefits and risks

The effect of Albrioza on function as evaluated by the primary endpoint is not considered robust since the CHMP preferred analysis representing a reasonably conservative approach for missing data and less dependent of model assumptions, results in an effect estimate of a small magnitude with the lower limit of the CI below zero. The support from secondary endpoints is limited, if any. The survival analysis is a *post hoc* analysis, without type I error protected results, from a small sample without replication. Considering the lack of demonstrated effect on functional endpoints, the plausibility of the *post hoc* survival analysis may be questioned. Thus, efficacy has not been established in a robust and compelling way and thus the benefit-risk balance is negative.

6.7.3. Additional considerations on the benefit-risk balance

Conditional marketing authorisation

As comprehensive data on the product are not available, a conditional marketing authorisation was requested by the applicant in the initial submission.

The CHMP considers that the product cannot be recommended for a conditional marketing authorisation as the benefit-risk balance is negative (as discussed).

Third party interventions

The CHMP received, during the assessment of the re-examination of this application, 14 correspondences from ALS associations or individuals (hereinafter referred to as "third parties") expressing the third parties' views about the efficacy and safety profile of Albrioza and the unmet medical need of ALS patients. The permission to share was granted for all except one (whereas the content of the latter was the same as some of the correspondences for which permission to share was granted). The applicant provided observations to all the correspondences that were shared.

The CHMP considered those interventions in the context of its assessment and concluded that the observations put forward by the ALS associations and Applicant's comments to them were already known by CHMP, and as such had no impact on the CHMP assessment or its conclusions.

6.8. Conclusions

The overall benefit/risk balance of Albrioza is negative.

7. Recommendations following re-examination

Based on the arguments of the applicant and all the supporting data on quality, safety and efficacy, the CHMP re-examined its initial opinion and in its final opinion concluded by consensus that the efficacy of the above-mentioned medicinal product is not sufficiently demonstrated and, therefore recommends the refusal of the granting of the conditional marketing authorisation for the above-mentioned medicinal product. The CHMP considers that:

- Efficacy has not been shown, as results were neither robust nor statistically compelling following a number of sensitivity analyses using different assumptions examining the robustness of the observed treatment effects and statistical inferences. Consequently, a slowing of functional loss has not been conclusively demonstrated.
- In addition, there is no convincing support of any of the secondary efficacy endpoints, including the biomarker pNF-H (biomarker indicating neuronal injury), which would have been expected to support the proposed mechanism of action, further increasing the uncertainty.
- Finally, the survival data are not considered robust, the plausibility is questioned and hampered by several methodological issues, e.g., inherent limitations of a single trial, post-hoc data-collection and analyses without control for multiplicity.

Since efficacy of Albrioza has not been sufficiently shown, a favourable benefit/risk ratio cannot be concluded and, consequently, a conditional marketing authorization cannot be granted.

Due to the aforementioned concerns a satisfactory summary of product characteristics, labelling, package leaflet, pharmacovigilance system, risk management plan and post-authorisation measures to address other concerns as outlined in the list of outstanding issues cannot be agreed at this stage.