CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

216660Orig1s000

NON-CLINICAL REVIEW(S)

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration
Center for Drug Evaluation and Research

Date: September 29, 2022

From: Lois M. Freed, Ph.D.
Director, Division of Pharmacology/Toxicology-Neuroscience
Office of Neuroscience

Subject: NDA 216660 (Relyvrio; AMX0035)

The sponsor (Amylyx Pharmaceuticals) submitted NDA 216660 on October 29, 2021, for AMX0035, a fixed-dose combination of sodium phenylbutyrate (3 gm) and tauroursodeoxycholic acid (1 gm) intended for the treatment of patients with amyotrophic lateral sclerosis (ALS).

The nonclinical studies have been reviewed in detail by Dr. David Carbone (Pharmacology/Toxicology NDA Review and Evaluation, NDA 216660, May 18, 2022) who has concluded that the nonclinical data are adequate and support approval of the NDA.

The nonclinical information is summarized briefly below.

Phenylbutyrate (PB) is a pan-histone deacetylase (HDAC) inhibitor, and tauroursodeoxycholic acid (also referred to as taurursodiol, ursodoxicoltaurine, TURSO, and TUDCA by the sponsor) is a taurine-conjugated bile acid. The sponsor acknowledges that the pathophysiology is "complex" and that "…the precise causes of neurodegenerative diseases are unknown" but proposes that AMX0035 exerts neuroprotective effects through HDAC inhibition (PB) and anti-apoptotic (TUDCA) mechanisms. A series of in vitro studies were conducted by the sponsor to characterize the pharmacological activity of PB and TUDCA, each alone and in combination, related to these mechanisms. However, the mechanisms by which PB or TUDCA might exert therapeutic effects in ALS are unknown.

The majority of the in vitro assays were conducted using embryonic or newborn rat mixed (neurons and glial) cortical cultures or rat primary spinal motor neurons exposed to hydrogen peroxide or glutamatergic stress. In general, the results of

these assays did not consistently demonstrate neuroprotective effects of PB or TUDCA alone or in combination on multiple endpoints (cell viability, neurite network, motor neuron survival, TDP-43 mis-localization). In several assays, riluzole or edaravone was tested as a comparator. Riluzole, but not PB, TUDCA, or the combination, consistently increased neuronal survival or the integrity of the neurite network, or corrected TDP-43 mis-localization.

The sponsor conducted only one in vivo primary pharmacology study in an animal model of ALS. In what was characterized as an exploratory study, AMX0035 (100 mg/kg PB + 200 mg/kg TUDCA) was orally administered BID to SOD-1 *G93A* transgenic mice from postnatal day (PND) 50 (presymptomatic) to end stage (approximately PND 150). Neither component was administered alone. AMX0035 had no significant effect on survival (129.8 ± 2.28 days vs 130.9 ± 2.01 days for control), improvement in rotarod performance, body weight, or clinical score.

The PK of sodium phenylbutyrate (NaPB) and TUDCA ((b) (4)) was evaluated in male Sprague Dawley rat. NaPB and TUDCA, each at doses of 50, 100, and 300 mg/kg), were administered in combination as a single dose. For PB, mean T_{max} and $t_{1/2}$ in plasma were 0.5 hr and 0.334-0.708 hrs, respectively. PK parameters for csf could either not be calculated (50 and 100 mg/kg) or were based on extrapolated values (300 mg/kg). For TUDCA, mean plasma T_{max} was 3.5-5.83 hrs; $t_{1/2}$ could not be calculated; csf levels were below the level of quantification at all doses. No estimates of absorption, V_d, or clearance were provided for either drug.

In tissue distribution studies in male Sprague Dawley (n = 3) and Long-Evans (n = 12) rats administered a single oral dose (60 mg/kg) of [^{14}C]NaBP + TUDCA or NaBP + [^{14}C]TUDCA (1 animal/sampling time), radioactivity was widely distributed in both strains. The highest concentrations were detected in kidney for [^{14}C]NaBP and GI contents and bile for [^{14}C]TUDCA. With [^{14}C]NaBP, concentrations in the CNS were low in both strains. With [^{14}C]TUDCA, radioactivity was not detectable in the CNS of Long-Evans rat and detectable only at very low levels at one time point (24 hrs post dose) in CNS of Sprague Dawley rat.

The metabolic profiles of [^{14}C]PB and [^{14}C]TUDCA were investigated in male Sprague Dawley rat. Following a single oral dose of 60 mg/kg [^{14}C]PB, parent compound accounted for 19.3% of total drug-related material in plasma. Four metabolites were detected; M135 (phenylacetate), M192 (phenylacetyl glycine), M161 (phenyl crotonate), and M179 (hydroxyl phenylbutyrate) accounted for 15.6%, 45.5%, 18.0%, and 1% of total drug-related material in plasma, respectively. Following a single oral dose of 60 mg/kg [^{14}C]TUDCA, parent compound accounted for 9.21% of total drug-related material. One metabolite, M516_2 (oxidation product), was detected, which accounted for the majority (90.8%) of drug-related material in plasma.

The in vivo metabolic profiles of NaBP and TUDCA were not assessed in human, so interspecies comparisons cannot be made.

The pivotal (GLP-compliant) oral toxicity studies were conducted in Sprague Dawley rat and minipig (Gottingen, Hanford).

In rat, NaBP/TUDCA were administered orally (gavage) at doses of 0/0, 250/83.32, 750/250, and 1000/333.28 mg/kg QD for 28 days, and AMX0035 was administered orally (gavage) at doses of 0, 250, 420, and 840 mg/kg QD for 26 weeks (+ 28-day recovery period). According to the sponsor, the high dose for the 26-week study was selected to "…show drug-specific effects but not produce an incidence of fatalities that would prevent a meaningful evaluation." However, there were no drug-related deaths in rat; only "Red discoloration of the pinnae" was observed at 1500 mg NaPB/500 mg/kg TUDCA in a dose-ranging study in rat.

In the 28-day study, the only finding was hypertrophy/hyperplasia of the nonglandular epithelium, observed at a similar incidence at all doses. The high dose was identified as a no-adverse-effect level (NOAEL). In the 26-week study, the primary findings were microscopic changes in female reproductive organs (ovarian follicular cysts at all doses, and increased mucification of the cervix and vagina and lobuloalveolar hyperplasia of the mammary gland at the mid and high doses). Of uncertain relationship to drug was "regionally extensive" bone marrow atrophy with angiectasia, observed in two males (main study, recovery) and one female (main study) at the high dose. Plasma exposures on Day 182 of the 26-week study are summarized in the following table:

DRUG (mg/kg)	**Cmax (ng/mL)**			**AUC (ng*hr/mL)**		
	250	**420**	**840**	**250**	**420**	**840**
MALES						
NaPB	10100	19500	22800	15300	43100	67000
TUDCA	282	327	244	2880	3470	3400
FEMALES						
NaPB	11900	16800	26300	16400	28700	57300
TUDCA	546	607	2460	2970	8890	25800

In the nonrodent studies, NaBP/TUDCA were administered to Gottingen minipigs at oral (gavage) doses of 0/0, 250/83.32, 750/250, and 1000/333.28 mg/kg QD for 28 days; AMX0025 was administered to Hanford minipigs at oral (gavage) doses of 0, 250, 423, and 845 mg/kg QD for 9 months. No drug-related effects were observed in either study. According to the sponsor, the doses tested in the 9-month study were "…similar to those used in a previous 28 day toxicity study…in which all dose levels were well tolerated and there were no adverse effects observed." However, the 9-month study was conducted in a different

strain of minipig than used in the dose-ranging studies, and the 1000/333.28 mg/kg doses in Gottingen minipig were identified by the sponsor as both maximum tolerated doses and no adverse effect levels. Doses above 1000/333.28 mg/kg were not tested in either strain of minipig.

Plasma exposures on Day 273 of the 9-month study are summarized in the following table:

DRUG (mg/kg)	Cmax (ng/mL)			AUC (ng*hr/mL)		
	250	420	840	250	420	840
MALES						
NaPB	9730	15400	64500	14000	24700	138000
TUDCA	110	58.9	589	1190	745	7930
FEMALES						
NaPB	9110	24600	89300	14300	46600	155000
TUDCA	188	173	458	2490	2160	5160

For comparison to the plasma exposure data in rat and minipig, plasma exposures in humans at the maximum recommended human dose (6 gm NaPB and 3 gm TUDCA per day) are as follows:

- Cmax: 188,000 ng/mL (PB) and 740 ng/mL (TUDCA)
- $AUC_{(0\text{-}24\ h)}$: 472,000 ng*hr/mL (PB) and 8720 ng*hr/mL (TUDCA).

A full battery of reproductive and developmental toxicology studies was conducted for AMX0035. In the pivotal studies in CD-1 mouse and Sprague Dawley rat, AMX0035 was administered by oral gavage at doses of 0, 187.5, 375, and 750 mg/kg BID (0, 375, 750, and 1500 mg/kg/day). Toxicokinetic analysis was not included in any of the studies.

In the fertility and early embryonic development (to implantation) study, AMX0035 was administered to rats at oral (gavage) prior to mating, throughout mating, and, in females, through gestation day (GD) 7. No maternal toxicity or adverse effects on fertility parameters were observed.

Effects on embryofetal development (EFD) were tested in mouse and rat. Rabbit was not used because of severe body weight loss and reduced food consumption at all doses (125-750 mg/kg BID; 250-1500 mg/kg/day) tested in a dose range-finding study. In the mouse and rat EFD studies, AMX0035 was administered during GDs 6-15 (mouse) or GDs 6-17 (rat). No adverse effects on embryofetal development were observed in either species.

In the pre- and postnatal development study in rat, AMX0035 was administered from GD 6 to lactation day 20. Findings consisted of an increase in the number of dams with stillborn pups at all doses (4.55, 15.00, 23.81, and 28.57% at 0, 375,

750, and 1500 mg/kg/day, respectively) and in postnatal pup deaths at the high dose, primarily during PNDs 0-4 (2, 1, 5, and 19 at 0, 375, 750, and 1500 mg/kg/day, respectively). No adverse drug-related effects were observed on developmental endpoints, including physical development, sexual maturation, neurological assessments, learning and memory (Morris water maze), and reproductive function.

The genotoxic potential of AMX0035 was tested in a standard battery of in vitro (Ames, chromosomal aberration in human lymphocytes) and in vivo (micronucleus in CD-1 mouse) assays, which were adequate and negative.

Carcinogenicity studies have not been conducted. Carcinogenicity studies in mouse and rat may be conducted post-approval, as agreed to by the Division (Meeting Minutes, April 7, 2020, and July 22, 2021) because of the seriousness of the indication.

Conclusions and Recommendations

The sponsor has submitted a battery of nonclinical studies to support the NDA for AMX0035 for the proposed indication. A number of in vitro assays and one in vivo study in an ALS-relevant animal model of disease were conducted to characterize the pharmacological activity and potential efficacy of AMX0035 for the treatment of ALS. Overall, the results of these studies were inconsistent or failed to demonstrate beneficial effects of AMX0035. Tissue distribution studies in rat indicated transport of phenylbutyrate into the CNS, whereas CNS levels of TUDCA were near or below the limit of quantification.

The general toxicology, genetic toxicology, and reproductive and developmental toxicology studies of AMX0035 were, in general, adequately conducted and did not identify serious general or developmental toxicity or genotoxic potential. It is, however, of note that the data available from the general toxicology studies indicate that the plasma exposures for PB and TUDCA achieved in animals at the highest doses tested in the nonclinical studies were lower than those in humans at the maximum recommended human doses (6 gm NaPB and 3 mg TUDCA).

From a safety standpoint, taking into consideration the available clinical safety data, the nonclinical studies support approval of the NDA. If the NDA is approved, carcinogenicity studies in two species (mouse and rat) should be conducted as post-marketing requirements, as previously agreed to by the Division and communicated to the sponsor.

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

LOIS M FREED
09/29/2022 10:39:31 AM

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	216660
Supporting documents:	1, 23
Applicant's letter date:	October 29, 2021
CDER stamp date:	October 29, 2021
Product:	AMX0035
Indication:	Amyotrophic Lateral Sclerosis
Applicant:	Amylyx Pharmaceuticals
Clinical Review Division:	DN1
Reviewer:	David L. Carbone, Ph.D.
Supervisor:	Lois M. Freed, Ph.D.
Division Director (DN1):	Teresa Buracchio, M.D.
Project Manager:	Michelle Mathers

TABLE OF CONTENTS

1 Executive Summary

1.1 Introduction

AMX0035 has been developed by Amylyx Pharmaceuticals for the treatment of amyotrophic lateral sclerosis.

1.2 Brief Discussion of Nonclinical Findings

AMX0035 is an orally administered fixed dose combination of 3 g sodium phenylbutyrate (PB) and 1 g tauroursodeoxycholic acid (TUDCA; TURSO). Dosing is to be once daily for 3 weeks and twice daily thereafter. There are no safety concerns regarding impurities or excipients.

PB and TUDCA are thought by the sponsor to suppress mitochondrial and endoplasmic reticulum dysfunction, respectively, which may play roles in the pathogenesis of ALS. The sponsor conducted in vitro primary pharmacology studies in cyclid models of mitochondrial disease, and cell culture models of hydrogen peroxide and glutamate toxicity. The sponsor claims that these in vitro studies demonstrate (b) (4) however, such claims are not supported by the data which are inconsistent (i.e., PB/TUDCA combinations in models of glutamatergic stress were comparable to TUDCA alone). Additionally, a study was submitted by the sponsor in which twice-daily oral administration of 100 mg/kg PB/200 mg/kg TUDCA for approximately 100 days in the SOD1G93A transgenic mouse model of ALS had no effects on lifespan or markers of disease progression.

Standard PK parameters for plasma and CSF were assessed in rats administered PB (mg/kg)/TUDCA (mg/kg) combinations of 50/50, 100/100, and 300/300. In CSF, PB was only detected in CSF at quantifiable levels following administration of the high dose, at which CSF C_{max} and AUC were approximately 28 and 19%, respectively, those in plasma. CSF TUDCA levels were below the limits of quantification at all doses. ADME data were collected in a single distribution and excretion mass balance study of orally-administered PB/TUDCA in SD rat in which PB, TUDCA, or both components were radiolabeled. Based on radioactivity, PB-related material accumulated in kidney while CNS levels were minimal (i.e., 10-fold lower than kidney); radiolabeled TUDCA-related material was below the limits of quantification in CNS tissue. According to the Clinical Pharmacology team, a mass balance study is not needed in humans to support AMX0035; it is, therefore, unknown whether there are any major circulating human metabolites that would warrant testing in nonclinical studies.

Pivotal general toxicology studies with AMX0035 included daily oral dosing in SD rat and Gottingen minipig for 26 or 39 weeks, respectively. There was no notable toxicity in either species. PB and TUDCA were individually assessed in Ames and in vitro chromosomal aberration assays and were found to be negative, and AMX0035 was found to be negative in an in vivo micronucleus assay in CD1 mice at up to 2000 mg/kg. There were no adverse findings following oral administration of AMX0035 in a fertility study in SD rat, embryofetal development studies in CD1 mice and SD rats, or a pre-

and postnatal development study in SD rats at doses up to 1500 mg/kg (750 mg/kg BID). Carcinogenicity studies are to be conducted as post-marketing requirements.

1.3 Recommendations

1.3.1 Approvability

The nonclinical data support approval of AMX0035.

1.3.2 Additional Nonclinical Recommendations

None

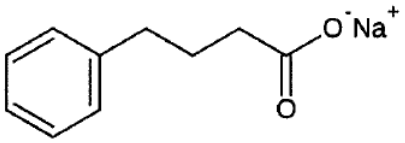
1.3.3 Labeling

(b) (4)

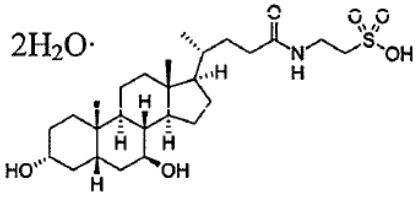
1 Page of Draft Labeling has been Withheld in Full as b4 (CCI/TS) immediately following this page

2 Drug Information

2.1 Drug



(Sponsor's Figure)



(Sponsor's Figure)

Phenylbutyrate (PB)
EPC: pan-HDAC inhibitor
CAS: 1716-12-7
IUPAC: Sodium 4-phenylbutanoate

Formula: $C_{10}H_{11}NaO_2$
MW: 186.2 g/mol

Tauroursodeoxycholic acid (TUDCA)
EPC: Bile acid
CAS: 14605-22-2
IUPAC: N-3a, 7b-dihydroxy-24-oxo-5b-cholan-24-yl dihydrate

Formula: $C_{26}H_{45}NO_6S2H_2O$
MW: 499.7 g/mol

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 129563 (AMX0035 for ALS; Amylyx Pharmaceuticals)

(b) (4)

2.3 Drug Formulation

AMX0035 is formulated as a fixed-dose combination of 3 g PB and 1 g TUDCA in a sachet, using conventional excipients.

2.4 Comments on Novel Excipients

There are no concerns regarding excipients.

2.5 Comments on Impurities/Degradants of Concern

Based on discussion with the CMC team, there are no concerns regarding impurities.

2.6 Proposed Clinical Population and Dosing Regimen

AMX0035 is intended for use in ALS patients. The proposed dosing is 3 g PB/1 g TUDCA daily for 3 weeks and twice daily thereafter.

2.7 Regulatory Background

IND 129563
pIND meeting (March 21, 2016)
pNDA meeting (February 23, 2021)

3 Studies Submitted

3.1 Studies Reviewed

Pharmacology
Effects of PB and/or TUDCA in cybrid models of mitochondrial disease, cell culture models of H2O2 or glutamate-induced toxicity, and disease progression in the SOD1G93A model of ALS. Respiratory and CV safety pharmacology.

PK/ADME
Standard plasma and CSF PK in rat. Tissue distribution and metabolite profiling in rat.

General Toxicology
Single dose studies in SD rat, beagle dog, and Gottingen minipig; 28-day repeat oral dose studies in SD rat and Gottingen minipig; 26-week daily oral dosing in SD rat; 9-month daily oral dosing in Gottingen minipig.

Genetic Toxicity
Ames and chromosomal aberration for PB and TUDCA (alone); in vivo mouse micronucleus study with AMX0035.

Reproductive and Developmental Toxicity
Fertility study in rat; embryofetal development in mouse and rat; pre- and postnatal development in rat.

Impurities
13-day repeat daily dosing in rat with stressed an unstressed AMX0035 batches.

3.2 Studies Not Reviewed

Primary pharmacology studies for indications other than ALS.

3.3 Previous Reviews Referenced

IND 129563: Nonclinical review by David L. Carbone, Ph.D. (June 20, 2019)

4 Pharmacology

4.1 Primary Pharmacology

AMX0035 is intended to treat mitochondrial and endoplasmic reticulum (ER) dysfunction, which may be involved in the pathogenesis of ALS.

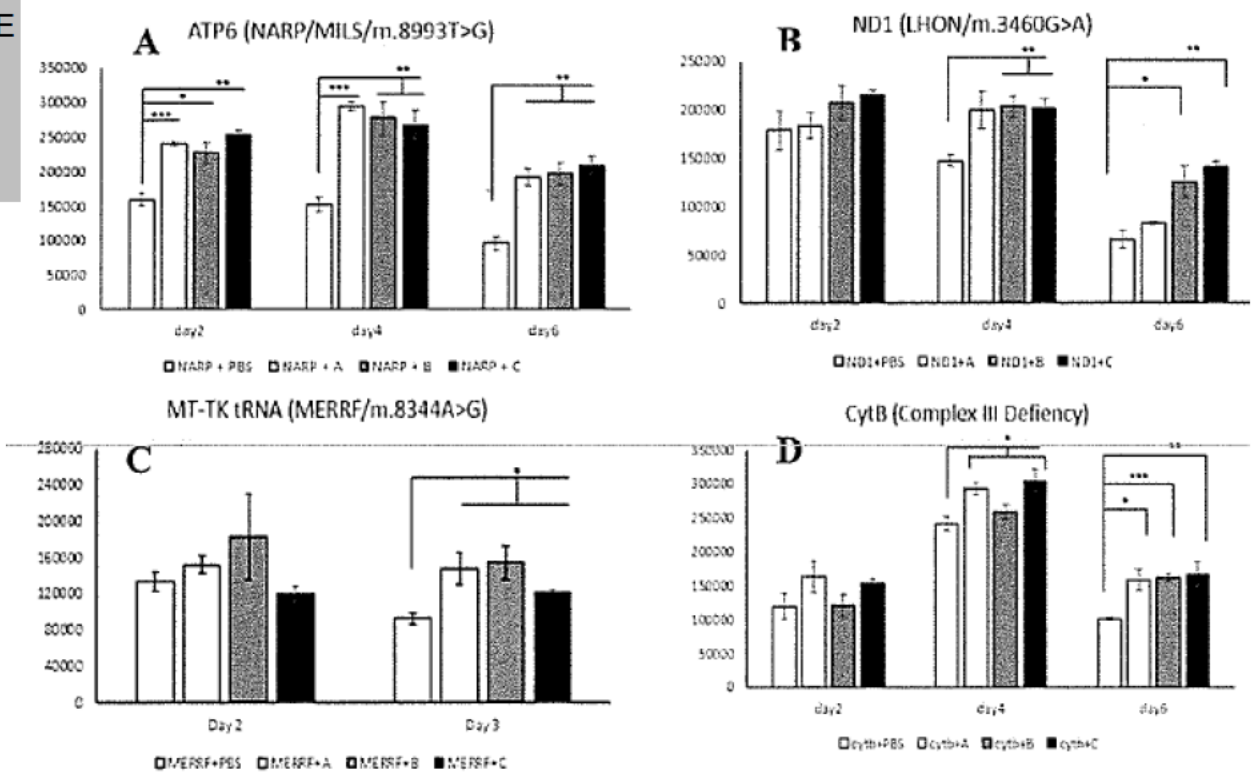
Mitochondrial Disease

According to the sponsor, TUDCA is thought to incorporate into mitochondrial membranes where it reduces mitochondrial permeability and increases the apoptotic threshold; PB is thought to ameliorate ER stress by upregulation of the chaperone regulator DJ-1. The sponsor tested PB and TUDCA alone and in combination in 6 cybrid models of primary mitochondrial disease (TCM) in which mtDNA is depleted from 143B-osteosarcoma cells and replaced, by cytoplasmic transfer, with mtDNA selected to model a specific disease. Endpoints included growth rate of the TCMs, mitochondrial membrane potential, and mitochondrial mass.

- neuropathy, ataxia, and retinitis pigmentosa **ATP6** (NARP/MILS/m.8993T>G),
- Leber's hereditary optic neuropathy **ND1** (LHON/m.3460G>A),
- **CytB** (Complex III Defiency),
- myoclonic epilepsy with ragged red fiber **MT-TK tRNA** (MERRF/m.8344A>G),
- Cyclooxygenase 1(**COX-1**); mtDNA common deletion,
- NADH dehydrogenase (ubiquinone) iron-sulfur protein 4 (**NDUFS4**) KO MEFs (Leigh Syndrome/LHON)

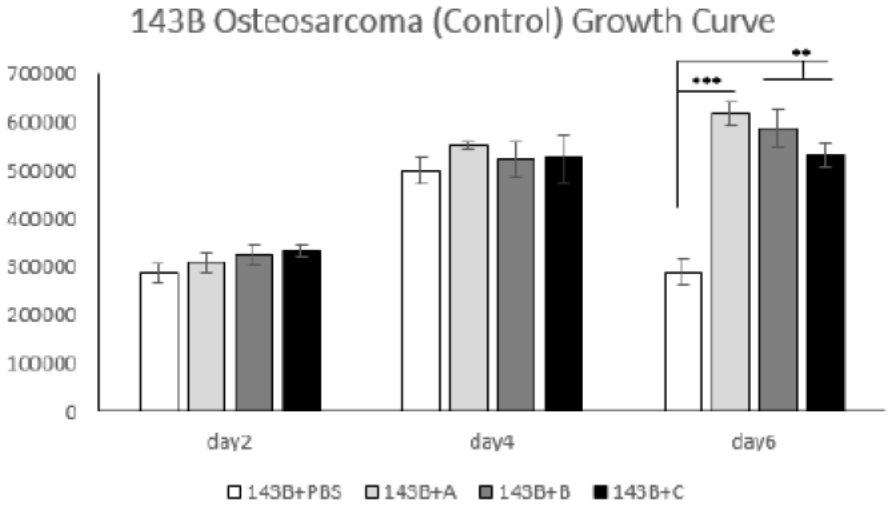
(Sponsor's Table: TCM disease models)

Individual and combination effects were assessed in the ATP6 model, indicating improvements in cell growth with 500 µM PB/50 µM TUDCA relative to 500 µM PB or 50 µM TUDCA alone; however, similar data assessing a combination effect were not provided for the remaining models. Additional experiments assessed µM PB/TUDCA ratios of 500/25, 500/50, or 500/100 (designated Group A, B, or C, respectively) on cell growth, indicating improvements over control in ATP6, ND1, and CytB models. However, it is unclear whether any observations on growth curves were due to an effect on the induced pathology or were an artifact of underlying PB/TUDCA effects on cell growth in the line itself (Figure 3A; cell line with no induced mitochondrial disease).



(Sponsor's Figure: Cell Growth)

Figure 3A

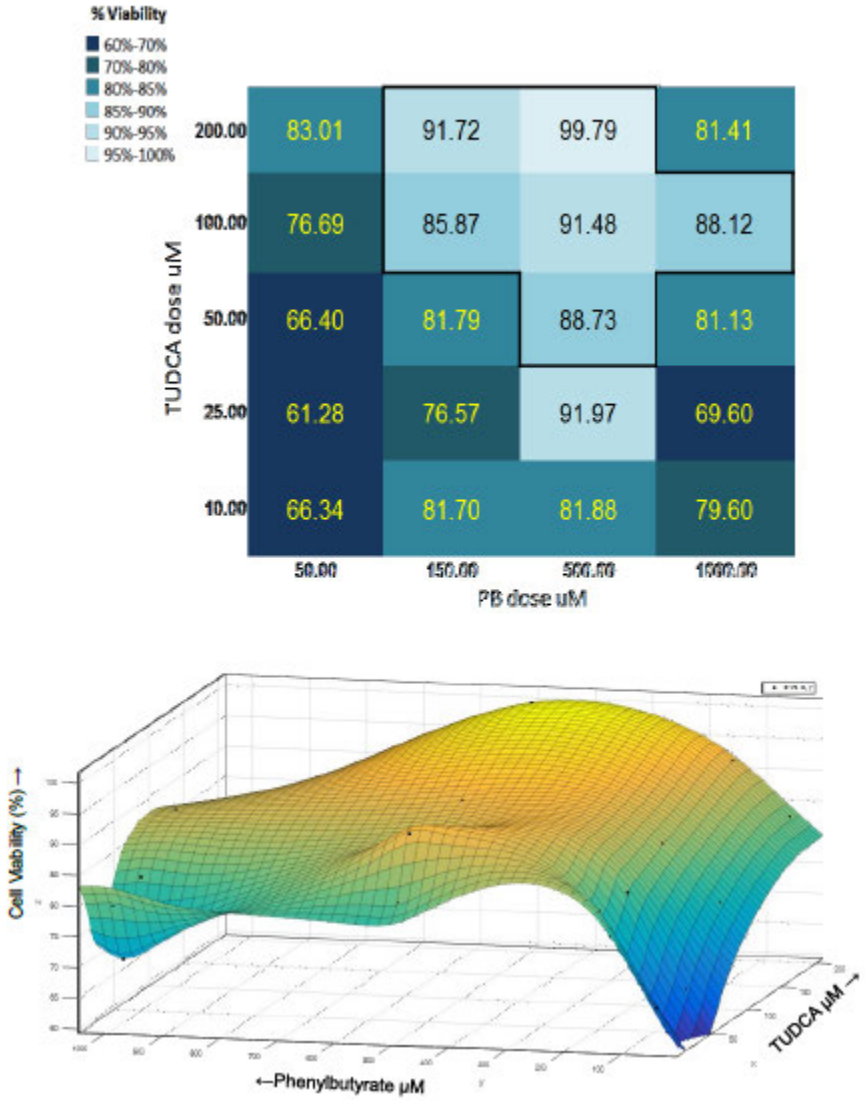


(Sponsor's Figure: Effects on control cells)

Oxidative Stress (H_2O_2):

Percent improvements in average cell viability (per LDH release) relative to control were assessed in primary mixed rat cortical cultures exposed to 40 µM H_2O_2 alone (48.56%) or in the presence of 100 µM TUDCA (58.59%), 1 mM PB (72.20%), or both (90.36%).

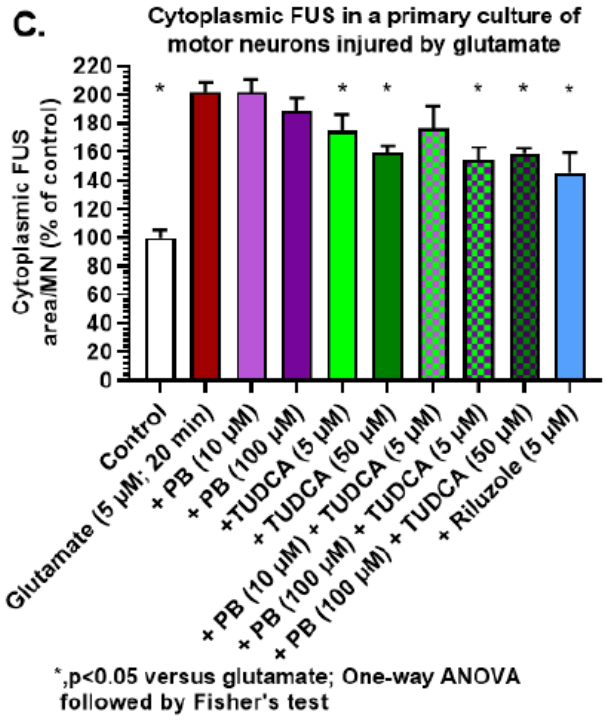
In a separate assay, cell viability was assessed following exposure to H_2O_2 alone (8.73%) or in combination with 500 µM PB and 100 µM TUDCA (5.21%). Using the same rat cortical culture system, the sponsor assessed several ratios of PB/TUDCA to determine an optimal ratio for AMX0035, indicating maximal protection from H_2O_2-induced toxicity by coadministration of 500 µM PB/200 µM TUDCA.

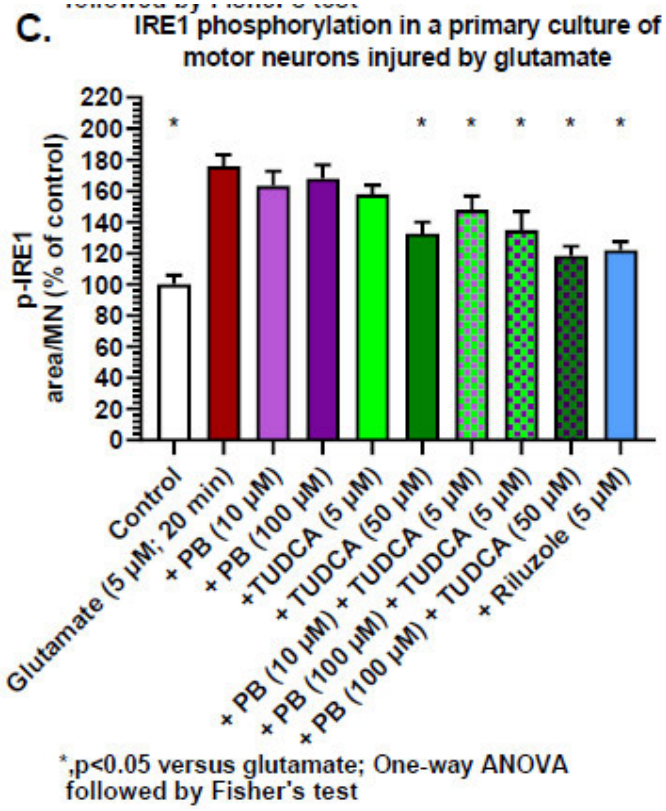


(Sponsor's Figure)

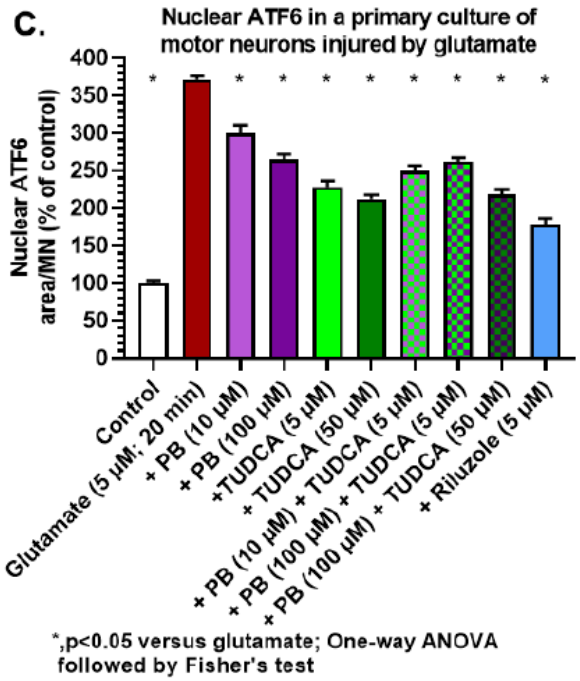
ER Stress

ER stress was induced by the addition of 5 µM glutamate to primary motor neuron cultures and the effects of PB and TUDCA alone or in combination on markers of ER stress (FUS, IRE1, and AATF6) were assessed; there were no clear improvements by PB/TUDCA combinations relative to TUDCA alone.





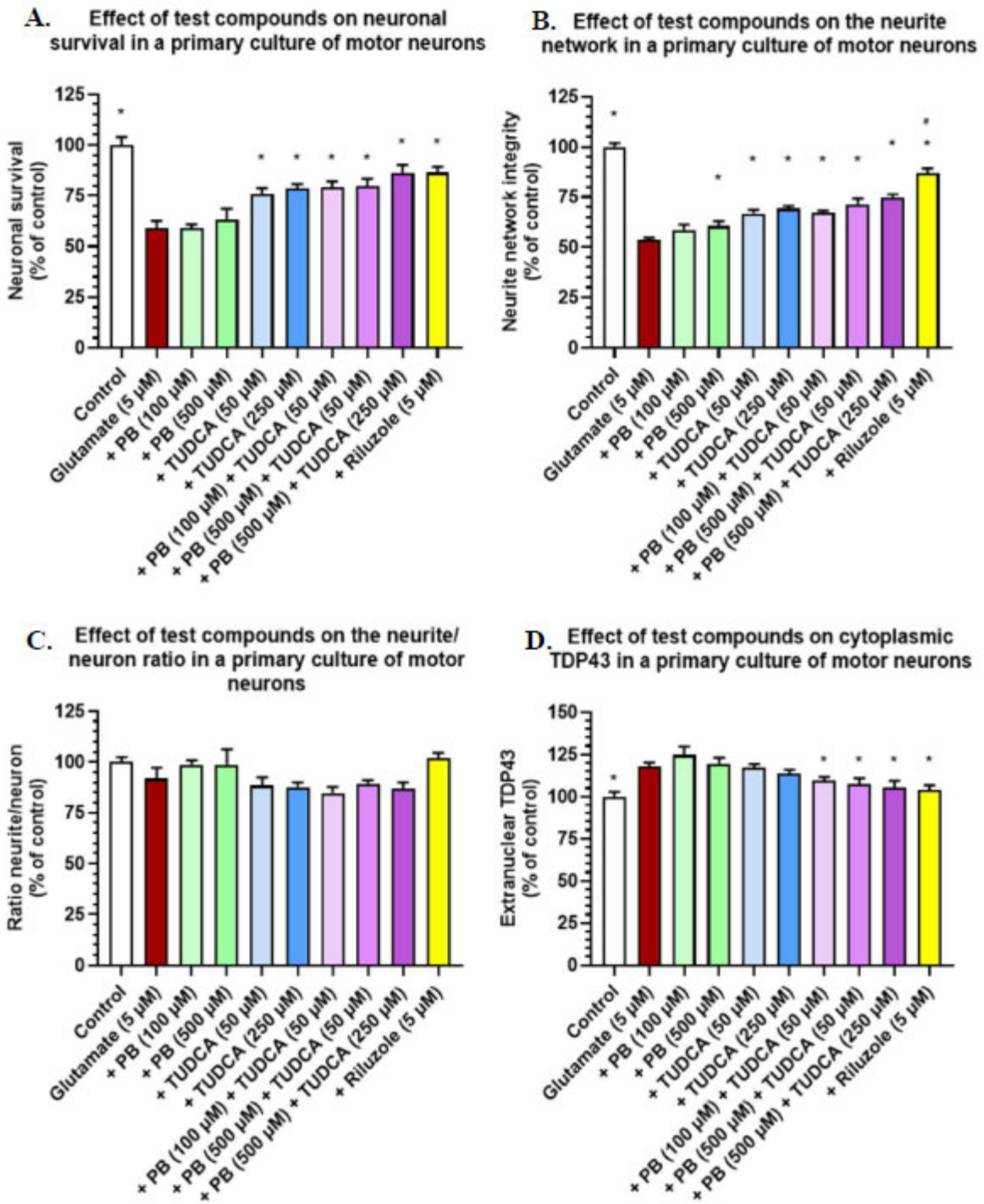
(Sponsor's Figures)



(Sponsor's Figure)

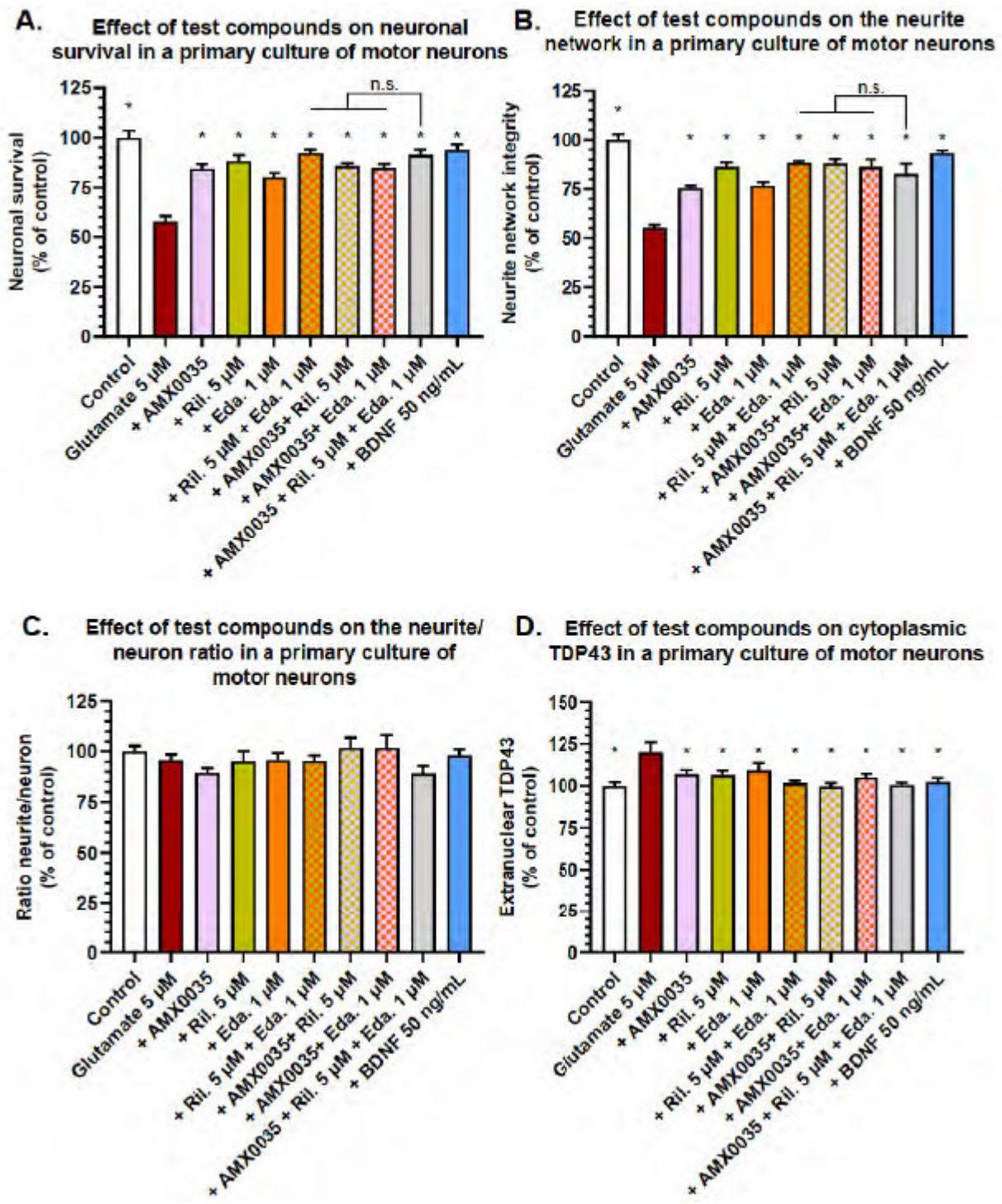
Glutamatergic Stress

Rat spinal motor neuron cultures were derived from GD14 embryos and subject to glutamatergic toxicity following the addition 5 µM glutamate for 20 min, resulting in loss of motor neurons, reductions in neurite network length, and abnormal TDP-43 translocation into the cytoplasm. PD effects by the combination at concentrations up to 500 µM PB/ 250 µM TUDCA were generally similar to those seen with 250 µM TUDCA alone, and were similar or less pronounced than those observed following administration of 5 µM riluzole.



(Sponsor's Figures)

An additional study using the same model indicated that there were no synergistic effects between PB/TUDCA in combination with either riluzole or edaravone and that PD effects with PB/TUDCA were generally equivalent to those seen with 1 µM edaravone and were less effective than that seen with 5 µM riluzole.



(Sponsor's Table)

A pretreatment-effect for suppression of glutamatergic toxicity in rat primary spinal cord neurons was assessed following 1, 8, or 24 h pretreatment with PB, TUDCA, or both prior to the addition of 5 µM glutamate. There were no protective effects of 10 or 100 µM PB, while both 5 and 50 µM TUDCA resulted in measurable PD effects following 1 h preincubation. There was no significant increase in effects by 100 µM PB/50 µM

TUDCA when compared to TUDCA alone, and effects did not exceed those observed with 5 µM riluzole or 1 µM edaravone.

In Vivo
Minimal improvements in locomotor function were observed following administration of 1 or 10 µM PB in a drosophila model of TDP43 pathology; however, there were no effects following similar administration of TUDCA, and the combination of PB/TUDCA was not assessed. There was no drug-related survival prolongation or improvements in clinical course in SOD1G93A transgenic mice administered a 100 mg/kg PB/200 mg/kg TUDCA combination BID for approximately 100 days.

4.2 Secondary Pharmacology

Not evaluated.

4.3 Safety Pharmacology

Summarized from nonclinical review of IND 129563:
Cardiovascular and respiratory safety pharmacology endpoints following single oral doses of PB/TUDCA up to 1000/333.3 mg/kg were assessed in male Gottingen minipigs and SD rats, respectively. Findings in minipigs included a 50 BPM increase at the high dose that resolved after 20 h, and transient decreases in systolic, diastolic, and mean arterial pressures. There were no drug effects on respiratory rate, tidal volume, or minute volume in rat.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Absorption
Standard PK parameters for plasma and CSF were evaluated in male SD rats administered single oral doses of 50/50, 100/100, or 300/300 mg/kg TUDCA/PB. An additional study evaluated plasma PK in male rats administered 45 mg/kg PB, 15 mg/kg TUDCA, or a 3:1 combination of PB/TUDCA (45/15 mg/kg).

Dose (mg/kg/mg/kg)	PB				TUDCA			
	C_{max} (ng/mL)	AUC_{0-x} (ng*h/mL)	T_{max} (h)	$t_{1/2}$ (h)	C_{max} (ng/mL)	AUC_{0-x} (ng*h/mL)	T_{max} (h)	$t_{1/2}$ (h)
Plasma								
50/50	2780	1820	0.5	0.334	70	305	4.83	N/C
100/100	21300	12500	0.5	0.381	160	768	3.5	N/C
300/300	55200	53800	0.5	0.708	202	767	5.83	N/C
CSF								
50/50	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
100/100	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
300/300	15700	9950	0.5	0.521	BQL	BQL	BQL	BQL

Dose	PB				TUDCA			
	C_{max} (ng/mL)	AUC_{last} (ng*h/mL)	T_{max} (h)	$t_{1/2}$ (h)	C_{max} (ng/mL)	AUC_{last} (ng*h/mL)	T_{max} (h)	$t_{1/2}$ (h)
Plasma								
45 mg/kg PB	6960	5470	0.25	0.8	N/A	N/A	N/A	N/A
15 mg/kg TUDCA	N/A	N/A	N/A	N/A	293	10800	4	N/C
45/15 mg/kg	168	5760	12	1.8	154	5100	12	N/C

(BQL=below quantification; N/C=not computed)

ADME

Tissue distribution, excretion mass balance, and metabolite profiling was assessed in male SD and LE rats following a single oral administration of 60 mg/kg AMX0035 in which PB, TUDCA, or PB and TUDCA were radiolabeled. The highest tissue distribution for radioactivity was in kidney; radioactivity in eye, bone, white adipose, brain, spinal cord, and seminal vesicles was at least 10-fold lower than that in kidney. Administration of AMX0035 in which PB was radiolabeled indicated that PB accounted for 19.3% of circulating drug-related material, while the metabolites M135 (phenyl acetate), M192 (phenylacetyl glycine), and M161 (phenyl crotonate) accounted for 15.6, 45.5, and 18.9% of total circulating PB-related material. Administration of AMX0035 in which TUDCA was radiolabeled indicated TUDCA accounted for 9.2% of circulating TUDCA-related material while the oxidation product referred to by the sponsor as metabolite M516_2 accounted for 90.8% of total circulating TUDCA-related material; however, the identity of M516_2 was not provided, and it is, therefore, unclear whether this metabolite is present in humans.

6 General Toxicology

6.1 Single-Dose Toxicity

Summarized from nonclinical review of IND 129563:
Single, oral doses of PB/TUDCA up to 1500/500 and 1000/333.28 mg/kg were evaluated in male SD rats and male and female Gottingen minipigs, respectively. The high dose in rat resulted in transient red discoloration of the pinnae; there were no adverse effects in minipig. A single-dose study was initiated in male and female beagle dogs with PB/TUDCA doses of 62.5/20.83 and 125/41.66 mg/kg but was discontinued due to emesis, retching, tremors, loose feces, and increases in respiratory rate at all doses.

6.2 Repeat-Dose Toxicity

28-Day Studies (summarized from nonclinical review of IND 129563):
28-day repeat-doses studies were conducted in male and female SD rats and Gottingen minipigs with oral PB/TUDCA doses up to 1000/333.3 mg/kg in both species. Drug-related effects in rats consisted of hypertrophy and hyperplasia of the nonglandular stomach epithelium, and approximate 7% reductions in RBC, hemoglobin, and hematocrit in HDM; these findings were not present after a 14-day recovery period. There were no adverse effects in minipigs.

Study title: A 26-Week Oral Gavage Toxicity Study of AMX0035 in Sprague Dawley Rats with a 4-Week Recovery Period

Study no.:	01217005
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 20, 2018
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AMX0035, Lot CBTDN, 99.7% (PB) and 98.3% (TUDCA)

Methods

Doses:	0, 250, 420, 840 mg/kg
Frequency of dosing:	Daily
Route of administration:	Oral Gavage
Dose volume:	5.91 to 9.94 mL/kg
Formulation/Vehicle:	Water
Species/Strain:	SD Rat
Number/Sex/Group:	15 main, 5 recovery (C and HD)
Age:	7 weeks at initiation of dosing
Weight:	155 to 275 g at initiation of dosing
Satellite groups:	TK (6/S/G)
Unique study design:	n/a
Deviation from study protocol:	No significant deviations

Observations and Results

Mortality and Clinical Signs

All animals were observed twice daily for mortality or signs of morbidity. Detailed examinations were conducted weekly. There were no drug-related mortalities or clinical signs.

Body Weights and Food Consumption

Body weights and food consumption were assessed weekly. There were no drug-effects on body weight or food consumption.

Ophthalmoscopy

Examinations by indirect ophthalmoscopy and slit lamp biomicroscopy were conducted prior to the initiation of dosing and on Day 178. There were no drug-related findings.

ECG

Not evaluated.

Hematology, Clinical Chemistry, and Urinalysis

Blood and urine samples were collected from fasted animals prior to necropsy. There were no drug effects on hematology, clinical chemistry, or urinalysis parameters.

Gross Pathology and Organ Weights

There were no drug-related gross findings or effects on organ weights.

Histopathology

Adequate Battery: Yes

Adrenal glands (2) Aorta Bone with marrow Sternum Femur (with joint) Bone marrow smear (from femur)[a] Brain Cervix Epididymides (2)[b] Eyes with optic nerves (2)[c] Gastrointestinal tract Esophagus Stomach Duodenum Jejunum Ileum Cecum Colon Rectum Harderian glands (2)[d] Heart Kidneys (2) Larynx Liver (sections of 2 lobes) Lungs (including bronchi, fixed by inflation with fixative) Lymph node Axillary (2) Mesenteric Mandibular (2)[d]	Ovaries (2) with oviducts[e] Pancreas Peripheral nerve (sciatic)[d] Peyer's patches Pharynx Pituitary Prostate Salivary glands (mandibular [2])[d] Seminal vesicles (2) Skeletal muscle (quadriceps) Skin with mammary gland[f] Spinal cord Cervical Lumbar Thoracic Spleen Testes (2)[b] Thymus Thyroid (with parathyroids [2])[e] Tongue Trachea Uterus Urinary bladder Vagina Gross lesions (per SOP)

[a] Not taken from animals found dead; not placed in formalin; examined only if scientifically warranted (based on hematology and histopathologic findings).
[b] Fixed in modified Davidson's solution.
[c] Fixed in Davidson's solution.
[d] One of the pair (or side) was examined.
[e] Parathyroids and oviducts were examined if in the plane of section and in all cases where a gross lesion of the organ was present.
[f] For females; a corresponding section of skin was be taken from the same anatomic area for males.

(Sponsor's Table)

Signed Pathology Report: Yes

Peer Review: No

Histological Findings: Drug-related findings occurred in the ovary, vagina, cervix, and mammary gland. None of the findings were considered adverse; however, according to the sponsor the findings in the vagina, cervix, and mammary glands indicate potential disruption of estrous cycling.

Text Table 23
Incidence of Selected Histopathologic Findings, Day 183/184 Terminal Euthanasia

	Females			
Dosage (mg/kg/day):	**0**	**250**	**420**	**840**
Ovary [a]	**15**	**15**	**14**	**15**
Cyst; follicle (present)	0	**3**	**3**	**4**
Vagina [a]	**15**	**15**	**14**	**15**
Mucification, increased	0	0	**3**	**4**
Minimal	-	-	**2**	**2**
Mild	-	-	0	**1**
Moderate	-	-	**1**	**1**
Cervix [a]	**15**	**15**	**14**	**15**
Mucification, increased	0	0	**2**	**2**
Minimal	-	-	**2**	0
Mild	-	-	0	**1**
Moderate	-	-	0	**1**
Mammary gland [a]	**15**	**13**	**13**	**13**
Hyperplasia; lobuloalveolar	0	0	**4**[b]	**4**[b]
Minimal	-	-	**3**	**3**
Mild	-	-	**1**[b]	**1**[b]
Dilatation	0	0	**2**	**2**
Minimal	-	-	**2**	**0**
Mild	-	-	0	**2**

[a] Number of tissues examined from each group.
[b] Includes 1 case of focal atypical (lobuloalveolar) hyperplasia.
- = No noteworthy findings.
BOLDED incidences represent effects of AMX0035 administration.

(Sponsor's Table)

Special Evaluation

Not evaluated.

Toxicokinetics

TK parameters for PB and TUDCA were assessed on Days 1 and 182. Plasma C_{max} and AUC for PB were similar between males and females, but TUDCA exposures were higher in females. C_{max} and AUC increased with repeat dosing. TK data were not provided for major metabolites.

Text Table 21
Summary of Toxicokinetic Parameters - Sodium phenylbutyrate (PB)

Dosage	AUC(0-t) (ng•hr/mL)		Cmax (ng/mL)		Tmax (hr)	
	Day 1	Day 182	Day 1	Day 182	Day 1	Day 182
Males						
250 mg/kg/day	7260	15,300	3440	10,100	1	1
420 mg/kg/day	23,000	43,100	12,300	19,500	1	1
840 mg/kg/day	54,100	67,000	20,400	22,800	1	1
Females						
250 mg/kg/day	12,200	16,400	6460	11,900	1	1
420 mg/kg/day	16,200	28,700	6830	16,800	1	1
840 mg/kg/day	50,600	57,300	15,300	26,300	1	1

Text Table 22
Summary of Toxicokinetic Parameters - Tauroursodeoxycholic acid (TUDCA)

Dosage	AUC(0-t) (ng•hr/mL)		Cmax (ng/mL)		Tmax (hr)	
	Day 1	Day 182	Day 1	Day 182	Day 1	Day 182
Males						
250 mg/kg/day	55.6	2880	11.0	282	2	1
420 mg/kg/day	1400	3470	408	327	2	2
840 mg/kg/day	981	3400	61.6	244	4	2
Females						
250 mg/kg/day	657	2970	50.9	246	8	8
420 mg/kg/day	906	8890	181	607	1	2
840 mg/kg/day	1420	25,800	100	2460	1	1

(Sponsor's Table)

Dosing Solution Analysis

Dosing solutions ranged from 91.4 to 104% of their respective target concentrations.

Study title: AMX0035: 9-Month Toxicity Study in Miniature Swine

Study no.: S15821
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: November 20, 2018
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: AMX0035, Lot CBTDN, 99.7% (PB) and 98.3% (TUDCA)

Methods

Doses:	0, 250, 423, 845 mg/kg
Frequency of dosing:	Daily
Route of administration:	Oral Gavage
Dose volume:	5.91 to 10 mL/kg
Formulation/Vehicle:	Water
Species/Strain:	Minipig
Number/Sex/Group:	4 main, 2 recovery (C and HD only)
Age:	3 to 4 months at initiation of dosing
Weight:	10.7 to 17.5 at initiation of dosing
Satellite groups:	None
Unique study design:	No comments
Deviation from study protocol:	1 MDM, 1 HDM, 1 LDF, and 1 HDF were euthanized following gavage error.

Observations and Results

Mortality and Clinical Signs

Animals were observed twice daily for mortality or signs of morbidity. Detailed examinations were conducted weekly. There were no drug-related mortalities or clinical signs.

Body Weights and Food Consumption

Body weight and qualitative assessments of food consumption were conducted weekly; there were no drug effects.

Ophthalmoscopy

Examinations by indirect ophthalmoscopy and slit lamp biomicroscopy were conducted prior to the initiation of dosing, on Dosing Day 269, and Recovery Day 24. There were no drug-related findings.

ECG

ECG was evaluated prior to the initiation of dosing, Dosing Day 269, and on Recovery Day 23. There were no drug-related findings.

Hematology, Clinical Chemistry, and Urinalysis

Blood samples were collected prior to the initiation of dosing, On Days 94 and 266 of the dosing phase and on Day 22 of the recovery phase. Urine samples were collected prior to the initiation of dosing, on Days 97 and 266 of the dosing phase and Day 22 of the recovery phase. There were no drug effects on hematology, clinical chemistry, or urinalysis parameters.

Gross Pathology and Organ Weights

There were no drug-related gross findings or effects on organ weights.

Histopathology

Adequate Battery: Yes

Artery, Aorta	Kidneys	Spinal Cord, Cervical
Bone, Femur	Large Intestine, Cecum	Spinal Cord, Lumbar
Bone, Sternum	Large Intestine, Colon	Spinal Cord, Thoracic
Bone Marrow, Femur	Large Intestine, Rectum	Spleen
Bone Marrow, Sternum	Gross Lesions**	Stomach, cardiac glandular
Brain	Liver/Gallbladder	Stomach, fundic
Cervix	Lung/Bronchus	Stomach, esophageal
Epididymis	Lymph Node, Mandibular	Stomach, pyloric glandular
Esophagus	Lymph Node, Mesenteric	Testes*
Eyes with optic nerve*	Muscle, Skeletal (quadriceps femoris)	Thymus
Gland, Adrenal	Nerve, Sciatic	Tongue
Gland, Lacrimal	Ovaries/Oviducts	Trachea
Gland, Mammary	Pancreas	Urinary Bladder
Gland, Pituitary	Skin – abdominal	Uterus
Gland, Prostate	Small Intestine, Duodenum	Vagina
Gland, Salivary, Mandibular	Small Intestine, Ileum	
Gland, Thyroid	Small Intestine, Jejunum	
Heart		

* Eyes and testes were fixed in Davidson's and Modified Davidson's, respectively for 1 to 3 days and stored in 70% alcohol (eyes) and 10% NBF (testes).

**Gross lesions were collected at the discretion of the Pathologist conducting the necropsy.

(Sponsor's Table)

Signed Pathology Report: Yes

Peer Review: No

Histological Findings: there were no drug-related findings.

Special Evaluation

No evaluated

Toxicokinetics

TK parameters for PB and TUDCA were assessed on Days 1 and 273. TK data for major metabolites were not provided.

AMX0035 (mg/kg)	Day	Males				Females			
		C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)	AUC_{last} (ng*h/mL)	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)	AUC_{last} (ng*h/mL)
PB Parameters									
250	1	7680	1	3.3	16200	7080	1.3	N/D	13000
	273	9730	1	2.2	14000	9110	1.0	1.7	14300
423	1	19100	1	3.9	30400	13900	1.0	1.7	24900
	273	15400	1	2.8	24700	24600	1.0	1.4	46600
845	1	36000	1	0.9	77800	32000	1.0	3.1	76100
	273	64500	1	4.6	138000	89300	1.0	1.2	155000
TUDCA Parameters									
250	1	113	12	N/D	2030	53.9	12	N/D	279
	273	110	10	N/D	1190	188	24	N/D	2490
423	1	235	12	N/D	1210	89.3	12	N/D	372
	273	58.9	12	N/D	745	173	12	N/D	2160
845	1	615	12	N/D	5540	274	12	N/D	2590
	273	589	12	N/D	7930	458	12	N/D	5160

Dosing Solution Analysis

Dosing solutions were withing 10% of their respective target concentrations.

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: AMX0035: Bacterial Reverse Mutation Test

Study no.: HUD0476
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: July 23, 2015
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: PB, Lot SPB 013/14-15, 99.7%; TUDCA, Lot 2015010026, 100.2%

Methods

Strains:	TA1535, TA1537, TA98, TA100, WP2 *uvr*A
Concentrations in definitive study:	-/+S9: 0, 5, 15, 50, 150, 500, 1500, 5000 µg/plate
Basis of concentration selection:	OECD Guidelines
Negative control:	PBS
Positive control:	-S9: SA (TA100 and TA1535), 9-AA (TA1537), 2-NF (TA98), 4-NQO (WP2 *uvr*A). + phenobarbital/5, 6-benzoflavone induced SD rat S9 fraction: 2-AA (TA100, TA1535, WP2 *uvr*A), BAP (TA98, TA1537)
Formulation/Vehicle:	PBS
Incubation & sampling time:	72 h

Study Validity

This study was consistent with OECD guidelines.

Results

AMX0035 was negative for mutagenicity.

Study title: Bacterial Reverse Mutation Assay

Study no.:	(b) (4)
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 29, 2020
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TUDCA, Lot 1086840, 100.1%

Methods

Strains: TA98, TA100, TA1535, TA1537, WP2 uvrA
Concentrations in definitive study: 50, 150, 500, 1500, 5000 µg/plate (+/-S9)
Basis of concentration selection: Limit dose
Negative control: PBS
Positive control:

Strain	-S9	+S9
TA98	2-NF	2-AA
TA100	NaN_3	2-AA
TA1535	NaN_3	2-AA
TA1537	9-aminoacridine	2-AA
WP2uvrA	methyl methanesulfonate	2-AA

Formulation/Vehicle: PBS
Incubation & sampling time: 48 to 72 hours

Study Validity

Assay was conducted according to OECD guidelines. S9 fraction was prepared from male SD rats administered Aroclor 1254. Preparations were assessed for the ability to metabolize benzo(a)pyrene and 2-AA to forms mutagenic to TA100.

Results

TUDCA was negative for mutagenicity.

Study title: Bacterial Reverse Mutation Assay

Study no.: (b) (4)
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: June 29, 2020
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: Sodium Phenylbutyrate, Lot 1086013, 99.9%

Methods

Strains: TA98, TA100, TA1535, TA1537, WP2 uvrA
Concentrations in definitive study: 50, 150, 500, 1500, 5000 µg/plate (+/-S9)
Basis of concentration selection: Limit dose
Negative control: PBS
Positive control:

Strain	-S9	+S9
TA98	2-NF	2-AA
TA100	NaN_3	2-AA
TA1535	NaN_3	2-AA
TA1537	9-aminoacridine	2-AA
WP2uvrA	methyl methanesulfonate	2-AA

Formulation/Vehicle: PBS
Incubation & sampling time: 48 to 72 hours

Study Validity

Assay was conducted according to OECD guidelines. S9 fraction was prepared from male SD rats administered Aroclor 1254. Preparations were assessed for the ability to metabolize benzo(a)pyrene and 2-AA to forms mutagenic to TA100.

Results

PB was negative for mutagenicity.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: In Vitro Mammalian Chromosomal Aberration Test in Human Lymphocytes

Study no.: XQ29VJ
Study report location: AMX0035: In Vitro Mammalian Aberration Test in human Lymphocytes
Conducting laboratory and location: (b) (4)
Date of study initiation: October 28, 2015
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: PB, Lot SPB 013/14-15, 99.7%; TUDCA, Lot 2015010026, 100.2%

Methods

Cell line:	Human lymphocytes
Concentrations in definitive study:	0, 180, 300, 500 μg/mL
Basis of concentration selection:	Preliminary toxicity study
Negative control:	PBS
Positive control:	Mitomycin c (-S9), cyclophosphamide (+S9)
Formulation/Vehicle:	PBS
Incubation & sampling time:	3 h (+/- phenobarbital/5, 6-benzoflavone induced SD rat S9 fraction), 21 h (-S9)

Study Validity

This study was consistent with OECD guidelines and recommendations included in S2(R1).

Results

AMX0035 was negative for clastogenicity.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: In Vitro Chromosomal Aberration Assay in Human Peripheral Blood Lymphocytes (HPBL)

Study no.:	(b) (4)
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 14, 2020
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TUDCA, Lot 1086841, 100.1%

Methods

Cell line:	HPBL
Concentrations in definitive study:	240, 350, 500 µg/mL
Basis of concentration selection:	Limit dose
Negative control:	PBS
Positive control:	Mitomycin C (-S9) Cyclophosphamide (+S9)
Formulation/Vehicle:	PBS
Incubation & sampling time:	4 h (+/-S9), 20 h (-S9)

Study Validity

Assay was consistent with OECD guidelines. HPBL were obtained from a healthy, non-smoking individual with no recent history of radiotherapy, viral infection, or

administration of drugs. The metabolic activation system consisted of Aroclor 1254-induced rat liver S9 fraction that was assayed for the ability to convert benzo(a)pyrene and 2-AA to forms mutagenic to *S. typhimurium* strain TA100.

Results

TUDCA was negative for clastogenicity.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: In Vitro Mammalian Chromosomal Aberration Assay in Human Peripheral Blood Lymphocytes (HBPL)

Study no.:	(b) (4)
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 14, 2020
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Sodium Phenylbutyrate, Lot 1086013, 99.9%

Methods

Cell line:	HBPL
Concentrations in definitive study:	50, 100, 200 µg/mL
Basis of concentration selection:	Limit dose
Negative control:	PBS
Positive control:	MMC (-S9), CP (+S9)
Formulation/Vehicle:	PBS
Incubation & sampling time:	4 (+/-S9), 20 h (-S9)

Study Validity

Assay was consistent with OECD guidelines. HPBL were obtained from a healthy, non-smoking individual with no recent history of radiotherapy, viral infection, or administration of drugs. The metabolic activation system consisted of Aroclor 1254-induced rat liver S9 fraction that was assayed for the ability to convert benzo(a)pyrene and 2-AA to forms mutagenic to *S. typhimurium* strain TA100.

Results

TUDCA was negative for clastogenicity.

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: AMX0035: CD1 Mouse In Vivo Micronucleus Test

Study no:	Mouse Micronucleus
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Not provided
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PB, Lot SPB 013/14-15, 99.7%; TUDCA, Lot 2015010026, 100.2%

Methods

Doses in definitive study:	0, 500, 1000, 2000 mg/kg
Frequency of dosing:	Once daily × 2 days
Route of administration:	Oral gavage
Dose volume:	1.88 to 7.5 mL/kg
Formulation/Vehicle:	3:1 PB/TDUCA in PBS
Species/Strain:	Male CD-1 mice
Number/Sex/Group:	6M/group
Satellite groups:	None
Basis of dose selection:	Preliminary toxicity test
Negative control:	PBS
Positive control:	Mitomycin c

Study Validity

This study was consistent with OECD guidelines.

Results

AMX0035 was negative for clastogenicity.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: Twice Daily Oral Gavage and Fertility and Early Embryonic Development to Implantation Study with AMX0035 in Rats

Study no.:	8453763
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 30, 2020
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AMX0035, Lot 1697227, 98.7% (PB), 98.6% (TUDCA)

Methods

Doses:	0, 375, 750, 1500 mg/kg/day
Frequency of dosing:	BID
Dose volume:	5 mL/kg
Route of administration:	Oral Gavage
Formulation/Vehicle:	Water
Species/Strain:	SD rat
Number/Sex/Group:	22
Satellite groups:	None
Study design:	Males were dosed for 4 weeks prior to pairing, and throughout the 2-week mating period. Females were dosed for 14 days prior to pairing, throughout the mating period, and through GD 7. Animals from respective groups were paired 1:1. Males were necropsied within 2 days after the mating period. Cesarean sections were performed on GD 13.
Deviation from study protocol:	No significant deviations

Observations and Results

Mortality and Clinical Signs

Animals were observed twice daily for mortality or signs of morbidity. Detailed examinations were conducted weekly. There were no drug-related mortalities or clinical signs.

Body Weight and Food Consumption

Males were weighed twice weekly. Females were weighed twice weekly during the premating and mating periods, and weekly from GDs 0 to 13. Food consumption was

assessed weekly for males. Food consumption in females was assessed weekly during the premating phase and from GDs 0 to 10 but was not assessed during the mating period. There were no drug effects on body weight in males or females.

Toxicokinetics

Not evaluated.

Dosing Solution Analysis

Dosing solutions were within 15% of their respective target concentrations.

Estrous Cycle

Estrous cycle was evaluated by daily vaginal lavage; there were no drug effects.

Necropsy

There were no drug effects on male or female fertility indices; however, although female fertility indices were within the historical range for the testing laboratory, historical data were not provided for males. There were no effects on sperm parameters in males. There were no effects on corpora lutea, implantation sites, preimplantation loss, or early resorptions.

9.2 Embryofetal Development

Dose-ranging studies were conducted in CD1 mouse, SD rat, and NZW rabbit. There were no adverse effects on maternal body weight or intrauterine fetal growth, survival, or external morphology in mice administered daily oral doses of 1500 mg/kg AMX0035 in mouse from GDs 6 to 15, or rat from GDs 6 to 17. In rabbits, daily oral administration of AMX0035 from GDs 7 to 21 resulted in maternal toxicity and reduced fetal size at 250 mg/kg; doses above 250 mg/kg were not tolerated. Pivotal studies were conducted in mice and rats.

Study title: A Twice Daily (BID) Oral (Gavage) Study of the Effects of AMX-0035 on Embryo/Fetal Development in Mice

Study no.:	01217009
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 22, 2019
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AMX0035, Lot CBTDN, 100.5% (PB) and 98.9% (TUDCA)

Methods

Doses:	0, 375, 750, 1500 mg/kg/day
Frequency of dosing:	BID
Dose volume:	5 mL/kg
Route of administration:	Oral Gavage
Formulation/Vehicle:	Water
Species/Strain:	CD1 mice
Number/Sex/Group:	22 females/group
Satellite groups:	None
Study design:	Pregnant mice were administered daily, oral doses of AMX0035 from GDs 6 to 15. Scheduled necropsy was on GD 18.
Deviation from study protocol:	No significant deviations

Observations and Results

Mortality and Clinical Signs

Animals were observed twice daily for mortality or signs of morbidity. Detailed examinations were conducted daily. There were no drug-related mortalities or clinical signs.

Body Weight and Food Consumption

Body weights and food consumption were assessed daily from GD 5 to 18; there were no drug effects.

Toxicokinetics

Not evaluated

Dosing Solution Analysis

TUDCA concentrations ranged from 98.0 to 104% of their respective target concentrations. PB concentrations ranged from 103 to 109% or their respective target concentrations.

Necropsy

There were no drug-related gross findings.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

There were no effects on intrauterine growth or survival, male/female ratio, or number of corpora lutea, implantations, implantation loss, early or late resorptions, or total number of fetuses.

Offspring (Malformations, Variations, etc.)

There were no drug-related external, visceral, or skeletal malformations.

Study title: A Twice Daily (BID) Oral (Gavage) Study of the Effects of AMX-0035 on Embryo/Fetal Development in Sprague Dawley Rats

Study no.:	01217003
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 22, 2019
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AMX0035, Lot CBTDN, 100.5% (PB) and 98.9% (TUDCA)

Methods

Doses:	0, 375, 750, 1500 mg/kg/day
Frequency of dosing:	BID
Dose volume:	5 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Water
Species/Strain:	SD rat
Number/Sex/Group:	25 females/group
Satellite groups:	None
Study design:	Pregnant females were administered AMX0035 from GDs 6 to 17. Scheduled necropsy was GD 21.
Deviation from study protocol:	No significant deviations

Observations and Results

Mortality and Clinical Signs

Animals were observed twice daily for mortality or signs of morbidity. Detailed examinations were conducted daily over the dosing period. There were no drug-related mortalities or clinical signs.

Body Weight and Food Consumption

Body weight and food consumption were assessed daily from GDs 5 to 8, and on GD 21; there were no drug effects.

Toxicokinetics

Not evaluated

Dosing Solution Analysis

PB and TUDCA concentrations ranged within 102 to 109% and 104 to 113%, respectively, of their intended concentrations.

Necropsy

There were no drug-related gross findings.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

There were no drug effects on intrauterine growth and survival, litter size, post implantation loss, viable fetuses, fetal weights, male/female ratio, or numbers of corpora lutea, implantations, or early or late resorptions.

Offspring (Malformations, Variations, etc.)

There were no drug-related external, visceral, or skeletal malformations, or effects on fetus weight.

9.3 Prenatal and Postnatal Development

Study title: Twice Daily Oral Gavage Prenatal and Postnatal Development Study, including Maternal Function, with AMX0035 in Rats

Study no.:	8465099
Study report location:	EDR
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	March 30, 2021
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AMX0035, Lot 1697227, 100.2% (PB), 99.5% (TUDCA)

Methods

Doses:	0, 375, 750, 1500 mg/kg/day
Frequency of dosing:	BID
Dose volume:	5 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Water
Species/Strain:	SD rat
Number/Sex/Group:	22 females/group
Satellite groups:	None
Study design:	AMX0035 was administered from GDs 6 to LD 20.
Deviation from study protocol:	No significant deviations

Observations and Results

F_0 Dams

Survival:	All animals survived until scheduled necropsy
Clinical signs:	There were no drug-related clinical signs or effects on lactation.
Body weight:	There were no drug effects on body weight
Food consumption:	There were no drug effects on food consumption
Uterine content:	The % females with stillborn pups were 4.55, 15.00, 23.81, and 28.57 in C, LD, MD, and HD, respectively.
Necropsy observation:	There were no drug-related gross findings.
Toxicokinetics:	Not evaluated
Dosing Solution Analysis	Dosing solutions were within 15% of nominal

F_1 Generation

Survival:	Pup deaths between PND 0 and 4 were 2, 1, 5, and 19 in C, LD, MD, and HD, respectively.
Clinical signs:	Litters were observed twice daily for mortality or signs of morbidity. There were no drug-related clinical signs.
Body weight:	Body weight and food consumption were assessed weekly; there were no drug effects.
Physical development:	There were no drug effects on pinna unfolding, righting reflex, incisor eruption, or eye opening.
Neurological assessment:	Locomotor activity and auditory startle were assessed on PNDs 56 and 62, respectively; there were no drug effects. Morris Water Maze testing began on Day 63 and included a spatial learning test over 4 days and a memory test on the 5th day; there were no drug-related findings.
Reproduction:	Estrous cycle was assessed daily, beginning 2 weeks prior to pairing at 12 weeks of age. There were no drug effects on estrous cycle or male or female reproductive indices.

F_2 Generation

Other:	Cesarean sections were conducted on GD 13. There were no effects on numbers of corpora lutea, implantation sites, preimplantation loss, or early resorptions.

10 Special Toxicology Studies

Impurities

A GLP-compliant study was conducted in male and female SD rats (5/sex/group) administered daily oral doses of vehicle (PBS), 1000 mg/kg non-stressed (2 months storage at 25 deg C, 60% humidity) AMX0035, and 1000 and 2000 mg/kg heat-stressed (2-months storage at 40 deg C, 75% humidity) AMX0035 for 14 days. There was no drug-related mortality or signs of morbidity, or effects on hematology, clinical chemistry, urinalysis, ophthalmology parameters. There were no drug-related gross or microscopic findings.

11 Integrated Summary and Safety Evaluation

Introduction

AMX0035 is an orally administered fixed dose combination of 3 g sodium phenylbutyrate (PB) and 1 g tauroursodeoxycholic acid (TUDCA) developed by Amylyx Pharmaceuticals for the treatment of amyotrophic lateral sclerosis (ALS). PB is approved for the treatment of urea cycle disorders; however, the sponsor did not develop AMX0035 using the 505(b)(2) pathway. TUDCA is not an FDA-approved product. According to the sponsor, PB and TUDCA are thought to suppress mitochondrial and endoplasmic reticulum dysfunction which may be involved in the pathogenesis of ALS. Dosing is to be once daily for 3 weeks, and twice daily thereafter.

Pharmacology

Primary Pharmacology

The sponsor assessed in vitro PD endpoints for AMX0035 using cyclid models of mitochondrial disease, cell culture models of H_20_2-induced stress, and glutamate-induced cell/ER stress. However, evidence that PB and TUDCA had a greater effect on the endpoints assessed in these studies when used in combination relative to each component alone was inconsistent. Specifically, although a combination effect appeared in cells stressed with H_2O_2, studies in which cells were subject to glutamatergic stress typically indicated a comparable effect between TUDCA and PB/TUDCA combinations. Collectively, the in vitro primary pharmacology studies do not provide convincing evidence that a PB/TUDCA combination would be potentially more effective than PB or TUDCA alone.

An in vivo primary pharmacology study was conducted in the SOD1 *G93A* transgenic mouse model of ALS in which oral BID doses of 100/200 mg/kg PB/TUDCA were administered for 50 days. There was no effect on lifespan or markers of disease progression that would suggest any therapeutic potential for AMX0035.

Secondary and Safety Pharmacology

Secondary pharmacology studies were not submitted. CNS safety pharmacology was not assessed; however, cardiovascular and respiratory safety pharmacology endpoints were assessed in standalone studies in Gottingen minipig and SD rat, respectively, following administration of 1000 mg/kg PB/333.3 mg/kg TUDCA and did not indicate any adverse effects.

PK/ADME

PK Parameters

Standard PK parameters for PB and TUDCA in plasma and CSF were assessed in rat. PB was only detected in CSF at quantifiable levels following administration of the high dose of 300 mg/kg PB/300 mg/kg TUDCA; CSF C_{max} and AUC for PB were approximately 28 and 19% that of plasma exposure, respectively. CSF TUDCA levels were below the limits of quantification at all doses.

ADME
ADME data were acquired from a single distribution and excretion mass balance study in SD rat following the administration of PB/TUDCA in which PB, TUDCA, or both components were radiolabeled. Based on radioactivity, PB-related material accumulated in kidney while CNS levels were minimal (i.e., 10-fold lower than kidney); radiolabeled TUDCA-related material was below the limits of quantification in CNS tissue. According to the Clinical Pharmacology team, a mass balance study in humans was not conducted for AMX0035. In the absence of data on the in vivo metabolic profile in humans, it is not known whether any major human metabolites are qualified by the nonclinical data.

Toxicology
In vivo toxicology studies were conducted using AMX0035.

General Toxicity
Single dose studies were conducted in male SD rats and male and female Gottingen minipigs at doses up to 1000 mg/kg PB/333.3 mg/kg TUDCA with no adverse effects. An additional study was initiated in beagle dog but was discontinued due to emesis, retching, tremors, loose feces, and increases in respiratory rate at all doses, including the low dose of 62.5 mg/kg PB/20.83 mg/kg TUDCA.

Repeat daily administration was assessed at oral doses up to 1000 mg/kg PB/333.3 mg/kg TUDCA in male and female SD rats and Gottingen minipigs for 28 days. Primary toxicity in rats consisted of hypertrophy and hyperplasia of the nonglandular stomach epithelium and approximate 7% reductions in RBC, hemoglobin, and hematocrit in HDM; these findings were not present after a 14-day recovery period. There were no adverse effects observed in minipigs. Pivotal toxicity studies consisted of daily oral dosing in SD rat (840 mg/kg AMX0035) and Gottingen minipig (845 mg/kg AMX0035) for 26 or 39 weeks, respectively. There was no notable toxicity in either species; however, it was unclear why the high dose in the 6- and 9-month studies was reduced to 845 mg/kg. The 845 mg/kg dose is not a limit, maximum feasible, or maximum tolerated dose, and it resulted in plasma C_{max} and AUC below those in humans administered the clinical dose. Additional nonclinical data may be necessary should the sponsor decide to evaluate a higher clinical dose.

Genetic Toxicity
PB and TUDCA were individually assessed in Ames and in vitro chromosomal aberration assays and were found to be negative. AMX0035 was tested at oral doses up to 2000 mg/kg and found to be negative in an in vivo micronucleus assay in CD mice. Collectively, these findings indicate a minimal potential for mutagenicity or clastogenicity.

Reproductive and Developmental Toxicity
Fertility was assessed following administered of AMX0035 at doses up to 1500 mg/kg/day (BID) for 4 weeks prior to pairing and through a 2-week mating period in males, and for 14 days prior to pairing until gestation day (GD) 7 in females.

Embryofetal development (EFD) effects were assessed in female SD rats and CD1 mice administered up to 1500 mg/kg/day (BID) AMX0035 between GDs 6 and 15 and GDs 6 and 17, respectively. Mice were selected for a second species based on maternal toxicity in New Zealand White rabbits following oral administration of 250 mg/kg AMX0035. Drug effects on pre- and postnatal development (PPND) were assessed in SD rats administered up to 1500 mg/kg/day (BID) AMX0035 from GD 6 to postnatal day (PND) 20. There were no adverse effects on maternal health or offspring health and development in fertility, EFD, or PPND studies. TK data were not collected so exposure multiples relative to humans at the recommended clinical dose cannot be determined; however, based on a mg/m^2 comparison, the fertility, EFD, and PPND studies in rat provide for an approximate 2-fold margin relative to the maximum recommended human dose of 6 mg PB/2 mg TUDCA per day.

Carcinogenicity
Carcinogenicity studies are to be conducted as post-marketing requirements.

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

DAVID L CARBONE
05/18/2022 12:15:19 PM

LOIS M FREED
05/18/2022 12:56:53 PM