Efficacy of Sodium Phenylbutyrate and Ursodoxicoltaurine Combination in Transgenic Mice Displaying Progressive Motor Neuron Degeneration Phenotype

Poster 51

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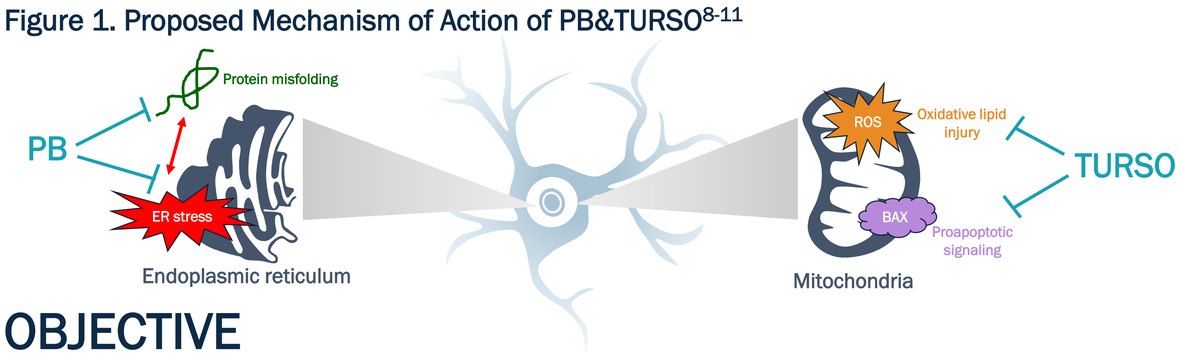
BACKGROUND

- Variants in the actin polymerization protein profilin 1 (PFN1) have been implicated as a genetic cause of amyotrophic lateral sclerosis (ALS)[1]
  - *In vitro/silico* and mouse models of ALS-associated variants in *PFN1* showed increased likelihood of protein misfolding/aggregation, resulting in neuronal death[1-4]
- A transgenic mouse model of ALS containing *PFN1* variants has been developed that displays a progressive motor neuron degeneration phenotype[3]
  - Loss of upper and lower motor neurons
  - Muscle atrophy
  - Weight loss
  - Decreased survival
- AMX0035, an oral, fixed-dose combination of sodium phenylbutyrate (PB) and ursodoxicoltaurine (TURSO, also known as taurursodiol) is hypothesized to reduce neuronal death by mitigating endoplasmic reticulum (ER) stress and mitochondrial dysfunction, which are 2 key pathways of ALS pathogenesis[5-9]
  - PB may function both as a chemical chaperone to stabilize protein folding and reduce ER stress and the unfolded protein response and as a transcriptional regulator of antiapoptotic and antioxidant proteins (**Figure 1**)[10,11]
  - TURSO may stabilize the mitochondrial membrane by reducing the translocation of BAX, a cell death regulator, leading to reduced reactive oxygen species (ROS) generation and increased apoptotic threshold of the cell (**Figure 1**)[8,9]

Figure 1. Proposed Mechanism of Action of PB&TURSO[8-11]



OBJECTIVE

- To determine if PB&TURSO combination conferred a greater therapeutic benefit compared with vehicle and PB or TURSO as individual components in a mouse model of motor neuron degeneration

METHODS

- Wild-type (WT) or *PFN1*^{C71G} mice were dosed once daily for 6 weeks (5 days/week) with vehicle or 400 mg/kg of PB, TURSO, or PB&TURSO combination (**Figure 2**)
  - Two age cohorts were used to ensure that the motor neuron degeneration phenotype was quantifiable, with dosing beginning at 12 or 16 weeks of age
  - Each treatment group had 8 mice per sex per genotype
- At the end of treatment, neuromuscular function was assessed with electromyography (EMG) recordings of peak compound muscle action potential (CMAP)
  - CMAP is a well-established method for measuring neuromuscular function that has been used in mouse models of ALS and in ALS clinical trials[12,13]
  - Stimulating electrodes were positioned on either side of the sciatic nerve at the proximal thigh, and motor response in the tibialis anterior muscle was recorded using an intramuscular needle electrode
- Two-way analysis of variance was used to test treatment and genotype effects and to identify statistically significant treatment effect differences

Figure 2. Summary of Study Design

Cohort 1

Mouse genotype	Treatment	Number of animals/sex
WT	Vehicle	8
PFN1^{C71G}	Vehicle	8
	PB	8
	TURSO	8
	PB&TURSO	8

Cohort 2

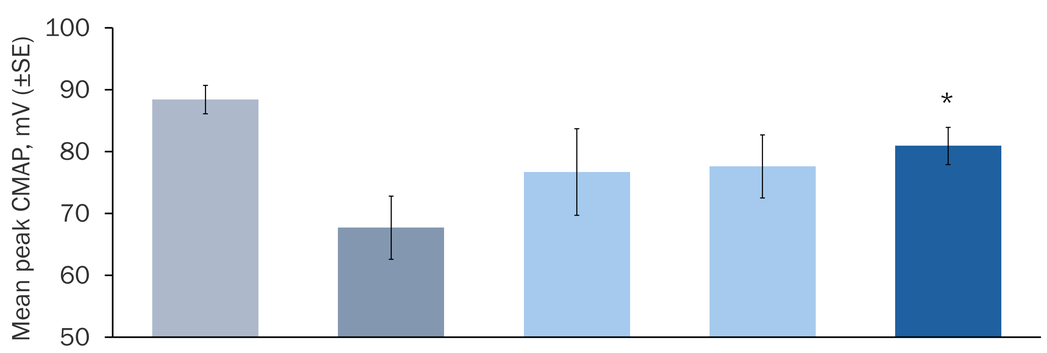
Mouse genotype	Treatment	Number of animals/sex
WT	Vehicle	8
PFN1^{C71G}	Vehicle	8
	PB	8
	TURSO	8
	PB&TURSO	8

CMAP, compound muscle action potential; PB, sodium phenylbutyrate; *PFN1*, profilin 1; TURSO, ursodoxicoltaurine; WT, wild type.

RESULTS

- The study included 160 mice; treatment groups were well-balanced based on age, sex, genotype, and average body weight for each sex
- In the cohort initiating treatment at 12 weeks of age, modest motor function decline were observed in the vehicle-treated *PFN1*^{C71G} mice (**Figure 3**)
  - Vehicle-treated *PFN1*^{C71G} mice showed a 23.4% peak CMAP reduction (67.7±5.1 mV) vs vehicle-treated WT mice (88.4±2.3 mV)
  - PB&TURSO-treated *PFN1*^{C71G} mice showed statistically significant partial rescue of CMAP decline relative to vehicle-treated WT mice (80.9±3.0 mV; *P*<.05)
- In the cohort initiating treatment at 16 weeks of age, major motor function decline were observed in the vehicle-treated *PFN1*^{C71G} mice (**Figure 4**)
  - A 52.2% reduction was observed in vehicle-treated *PFN1*^{C71G} mice (36.9±5.1 mV) versus vehicle-treated WT mice (77.4±2.5 mV)
  - PB&TURSO-treated *PFN1*^{C71G} mice showed statistically significant partial rescue of this decline (54.6±5.7 mV; *P*<.05)
- Only cohorts treated with PB&TURSO demonstrated significantly different peak CMAP compared with vehicle-treated controls; treatment with PB or TURSO alone did not

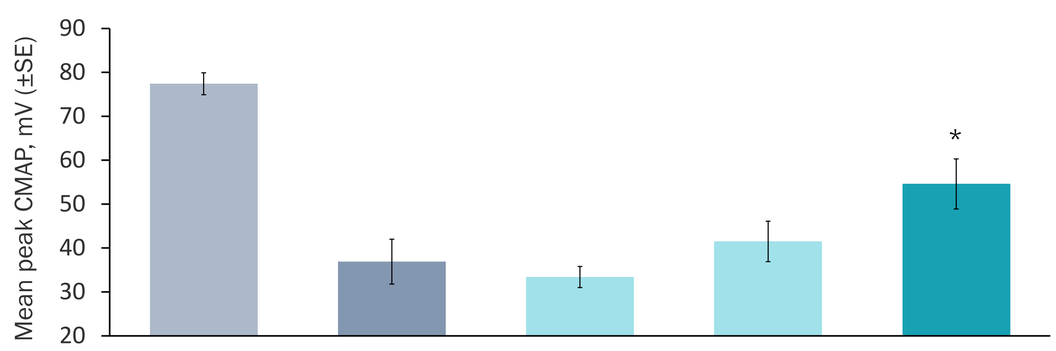
Figure 3. Peak CMAP in *PFN1*^{C71G} Mutant Mice Dosed for 6 Weeks Beginning at 12 Weeks of Age (Cohort 1)



	Vehicle	Vehicle	PB only	TURSO only	PB & TURSO
Mean peak CMAP (SE)	88.4 mV (2.3)	67.7 mV (5.1)	76.7 mV (7.0)	77.6 mV (5.1)	80.9 mV (3.0)

*P<.05 compared with *PFN1*^{C71G} vehicle.
CMAP, compound muscle action potential; PB, sodium phenylbutyrate; *PFN1*, profilin 1; TURSO, ursodoxicoltaurine; WT, wild type.

Figure 4. Peak CMAP in *PFN1*^{C71G} Mutant Mice Dosed for 6 Weeks Beginning at 16 Weeks of Age (Cohort 2)



	Vehicle	Vehicle	PB only	TURSO only	PB & TURSO
Mean peak CMAP (SE)	77.4 mV (2.5)	36.9 mV (5.1)	33.4 mV (2.4)	41.5 mV (4.6)	54.6 mV (5.7)

*P<.05 compared with *PFN1*^{C71G} vehicle.
CMAP, compound muscle action potential; PB, sodium phenylbutyrate; *PFN1*, profilin 1; TURSO, ursodoxicoltaurine; WT, wild type.

CONCLUSIONS

- The mouse model was representative of both mild impairment (cohort treated at 12 weeks of age) and severe impairment (cohort treated at 16 weeks of age)
- The combination of PB&TURSO significantly decreased motor function decline in a mouse model of ALS
- This benefit was only seen when combining PB&TURSO and not with individual PB or TURSO treatment

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Disclosures

JT is an employee of and has stock option ownership of Amylyx Pharmaceuticals, Inc. JC and JK are co-CEOs of and own stock in Amylyx Pharmaceuticals, Inc.

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AMX0035 is an investigational drug in the EU and UK and not approved for use in ALS.