**INTRODUCTION**

- Mitochondrial dysfunction is believed to play a prominent role in the pathophysiology ofAmyotrophic Lateral Sclerosis (ALS).
- SBT-272 is an investigative molecule currently in preclinical development for the treatment of patients with rare neurodegenerative disease through the promotion of mitochondrial health.
  - SBT-272 preserves the inner mitochondrial membrane structure by interacting with the phospholipid cardiolipin, a critical mediator of the mitochondrial cristae network, and key structural and functional element of mitochondrial respiratory super-complexes.
- Through its protective effect on cardiolipin, SBT-272 has been shown to reduce the generation of harmful reactive oxygen species (ROS) while also increasing the efficiency of electron transport under conditions of stress, leading to a net gain in bioenergetic output and a decrease in oxidative damage.
- Importantly, both mechanisms - damage from ROS and bioenergetic deficit - have been described in the mitochondria obtained from subjects with ALS.

**OBJECTIVE**

- To investigate the potential efficacy of SBT-272 in treating ALS, mice expressing the mutant human SOD1 G93A transgene were treated with daily intraperitoneal injections of SBT-272 starting at 8 weeks of age.
- Consistent with the onset of neuromuscular dysfunction in mice as measured by electromyography, the circulating neurofilament biomarker, were not affected by drug despite comparable levels of

**METHODS**

- A total of 108 mutant human transgenic SOD1 mice (B6SJL-Tg(SOD1*G93A)1Gur/J) were used for the study.
- Animals were assigned to one of two groups, one dedicated to histology and one dedicated to behavior analyses and lifespan.
- Prevented animals began when reached 8 weeks of age.
- Animals in the dedicated histology group were treated with daily intraperitoneal injections of drug or vehicle for six weeks at age; vehicle (6 male/6 female); SBT-272 at 0.5mg/kg (6 male/6 female); SBT-272 at 5.0mg/kg (6 male/6 female).
- Histological analyses are pending and will include sciatic nerve axon counts, spinal cord Iba-1 and GFAP-1 staining, spinal cord neuromuscular junction occupancy, and electron microscopy of spinal cord sections to investigate mitochondrial morphology.
- Animals in the dedicated behavioral/lifespan group were treated with daily intraperitoneal injections of drug or vehicle until humane end of life as follows: vehicle (10 male/10 female); SBT-272 at 0.5mg/kg (10 male/10 female); SBT-272 at 5.0mg/kg (10 male/10 female).
- In observations included daily weight gain, weekly forelimb grip strength, and weekly neurological assessments, which was assessed using a four-point qualitative ratings scale.
- Bi-weekly retro-orbital blood draws were performed to verify drug exposure and for biomarker analyses exposure.

**RESULTS**

- Treatment of symptomatic ALS mice resulted in therapeutic benefit that was sex dependent.
- Male mice treated with high dose of drug experienced a dose-dependent delay in the onset of neurological symptoms, as assessed by a qualitative ratings scale, and a dose-dependent survival benefit that was highly correlative with plasma neurofilament levels (extended lifespan) (Figure 1).
- Both results were statistically significant.
- Decline in grip strength was attenuated in male mice treated with high dose of drug, although these results were not statistically significant (Figure 2).
- Circulating plasma levels of the neuroinflammatory biomarker neurofilament light chain, an emerging biomarker of axonal damage, were significantly decreased at high dose of drug – these results were statistically significant (Figure 3).
- Notably, survival and biomarker levels were highly correlative in male mice (Figure 4).
- Female SOD1 G93A mice, which present with a milder phenotype and displayed lower levels of the circulating neuroinflammatory biomarker, were not affected by drug despite comparable levels of exposure.
- Confirmatory histology studies are ongoing and are expected to corroborate the in-life and biomarker observations.

**CONCLUSIONS**

- SBT-272-treated ALS mice experienced a dose dependent delay in the onset of neurological disease, a reduction in systemic markers of neurodegeneration and prolonged lifespan.
- Notably, these effects were sex dependent, despite no overt differences in drug exposure between sexes (data not shown).
- The reason for this observed sex difference is currently unknown, although biomarker analyses corroborate a milder disease course in affected female mice which is consistent with survival data.
- Pending histological analyses should shed further insight into the mechanism of SBT-272 neuroprotection in male ALS mice.
- The data presented here establish SBT-272 as a potential therapeutic agent, with mitochondria as a tractable target for the treatment of patients with ALS.
- Additional studies in TDP-43 model systems are currently planned to corroborate findings seen in the SOD1 model and to gain additional mechanistic insights.