Small-Molecule Hepatocyte Growth Factor/MET Positive Modulator ATH-1105 Is Neuroprotective in the TDP-43 Mouse Model of Amyotrophic Lateral Sclerosis

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CONCLUSIONS

Treatment with ATH-1105 in TDP-43 ALS mice resulted in

1. Improvement in balance, coordination, and muscle strength in motor behavior tests
2. Protection of nerve function and structure
3. Normalization of plasma biomarkers of systemic inflammation and neurodegeneration

KEY TAKEAWAY

This study highlights the therapeutic potential of ATH-1105 in a representative mouse model of ALS and supports further investigation of ATH-1105 in this disease indication

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https://bit.ly/3z2jdre

Acknowledgments

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Disclosures

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Presented at the Virtual 33rd International Symposium on ALS/MND; December 6-9, 2022

INTRODUCTION

- Activation of the MET receptor by its ligand hepatocyte growth factor (HGF) promotes neuroprotective, neurotrophic, and anti-inflammatory mechanisms[1-3]
- ALS is characterized by progressive motor neuron degeneration, demyelination, and systemic inflammation, with up to 97% of patients exhibiting TDP-43 proteinopathy[4]
- Promotion of HGF/MET signaling may counteract the neurodegeneration observed in ALS via protective mechanisms against neurotoxicity, neuroinflammation, and oxidative stress–induced damage[5-7]
- A series of small-molecule positive modulators of HGF/MET was developed, including ATH-1105, a brain-penetrant, orally bioavailable compound with favorable pharmacokinetic properties
- The effects of daily oral treatment with ATH-1105 for 2 months were evaluated in the TDP-43 mouse model of ALS[8]

OBJECTIVE

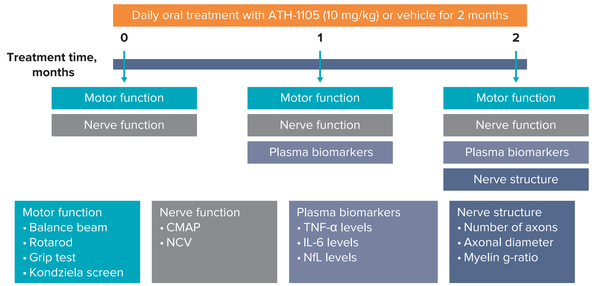
To determine the neuroprotective effects of ATH-1105, a positive modulator of the HGF/MET pathway, in the TDP-43 mouse model of ALS

METHODS

Study Design

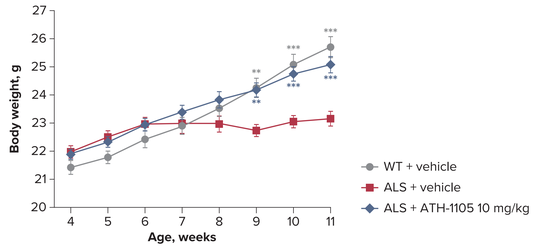
- 1-month-old mice were sorted into 3 groups of 10 animals each
  - Group 1 (healthy control) included WT mice treated once daily with oral vehicle
  - Group 2 (disease control) included TDP43^{A315T} mice treated once daily with oral vehicle
  - Group 3 (ALS + ATH-1105 10 mg/kg) included TDP43^{A315T} mice treated once daily with oral ATH-1105
- Animals were treated for a duration of 2 months from 1-3 months of age
- Behavioral tests, sciatic nerve electrophysiology and histology, and plasma biomarker analyses were carried out as described in the Supplemental Information (**QR code**)

Figure 1. Study design



RESULTS

Figure 2. ATH-1105 significantly protects against loss of body weight

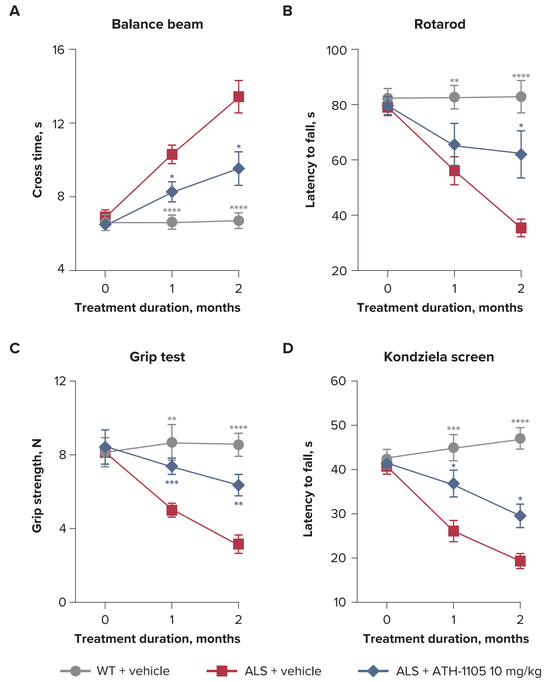


Graphical representation of animal body weight over time.

Data presented as mean ± SEM.

Statistical significance was determined by 2-way ANOVA with the Dunnett test versus ALS + vehicle. $^{**}P < 0.01$; $^{***}P < 0.001$.

Figure 3. ATH-1105 significantly improves balance, coordination, and muscle strength

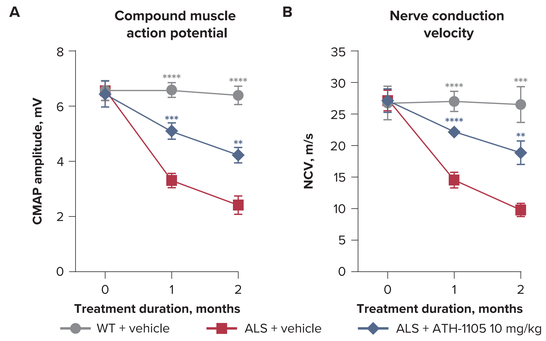


Graphical representation of (A) balance beam cross time, (B) rotarod latency to fall, (C) grip strength, and (D) Kondziela screen latency to fall at baseline and after 1 and 2 months of ATH-1105 treatment.

Data presented as mean ± SEM.

Statistical significance was determined by 2-way ANOVA with the Dunnett test versus ALS + vehicle. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$.

Figure 4. ATH-1105 significantly improves compound muscle action potential amplitude and nerve conduction velocity

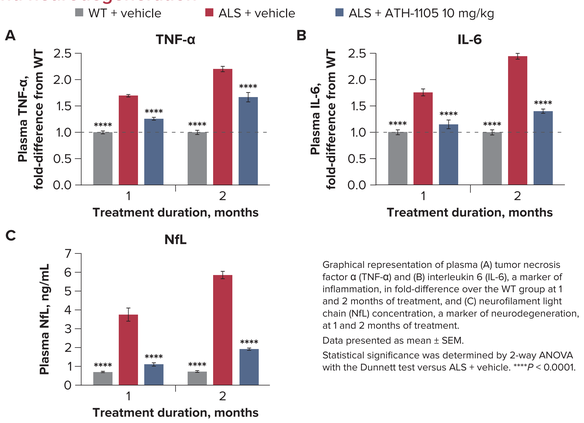


Graphical representation of (A) CMAP amplitude and (B) NCV at baseline and after 1 and 2 months of ATH-1105 treatment.

Data presented as mean ± SEM.

Statistical significance was determined by 2-way ANOVA with the Dunnett test versus ALS + vehicle. $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$.

Figure 5. ATH-1105 reduces plasma markers of inflammation and neurodegeneration

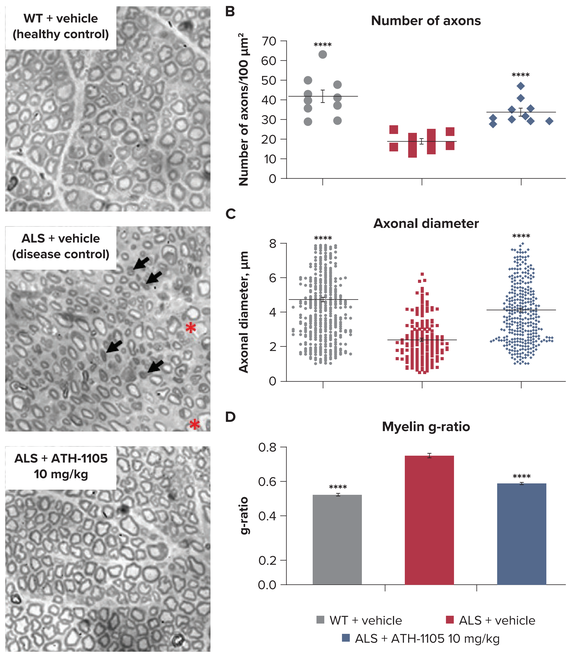


Graphical representation of plasma (A) tumor necrosis factor α (TNF-α) and (B) interleukin 6 (IL-6), a marker of inflammation, in fold-difference over the WT group at 1 and 2 months of treatment, and (C) neurofilament light chain (NfL) concentration, a marker of neurodegeneration, at 1 and 2 months of treatment.

Data presented as mean ± SEM.

Statistical significance was determined by 2-way ANOVA with the Dunnett test versus ALS + vehicle. $^{****}P < 0.0001$.

Figure 6. ATH-1105 protects against axon degeneration and demyelination



Histology images of sciatic nerve semi-thin cross sections (A) stained with toluidine blue; arrows point to degenerated axons; stars indicate regions with loss/thinning of myelin. Graphical representation of (B) the number of axons (per 100 µm²), (C) axonal diameter (in micrometers), and (D) mean of myelin g-ratio, defined as the ratio of the inner axonal diameter to the total axonal diameter, after 2 months of treatment.

Data presented as mean ± SEM.

Statistical significance was determined by 1-way ANOVA with the Dunnett test versus ALS + vehicle. $^{****}P < 0.0001$.

Abbreviations **ALS**, amyotrophic lateral sclerosis; **ANOVA**, analysis of variance; **CMAP**, compound muscle action potential; **HGF**, hepatocyte growth factor; **IL-6**, interleukin 6; **N**, Newtons; **NCV**, nerve conduction velocity; **NfL**, neurofilament light chain; **SEM**, standard error of the mean; **TDP-43**, TAR DNA-binding protein 43; **TNF-α**, tumor necrosis factor alpha; **WT**, wild type.

References **1.** Vallarola A et al. *Int J Mol Sci*. 2020;21:8542. **2.** Gong Z et al. *J Biomed Res*. 2022;36(5):336-342. **3.** Tortelli R et al. *Front Neurol*. 2020;11:552295. **4.** Hulisz D. *Am J Manag Care*. 2018;24(15):S320-S326. **5.** Desole C et al. *Front Cell Dev Biol*. 2021;9:683609. **6.** Nicoleau C et al. *Stem Cells*. 2009;27:408-419. **7.** Ko KR et al. *Sci Rep*. 2018;8:8316. **8.** Wegorzewska I et al. *Proc Nat Acad Sci U S A*. 2009;106(44):18809-18814.

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SUPPLEMENTAL INFORMATION

Behavioral Tests

- Balance beam: Animals crossed from one end of a narrow elevated beam to the other to test balance and coordination. The time necessary to cross the beam was quantified
- Rotarod latency: A rotating rod apparatus was used to measure walking performance, coordination, and balance. Latency to fall was measured at successively increased speeds from 4 to 40 rpm, over a 300-second maximum time period
- Grip test: Muscular strength was assessed using standardized grip strength tests for all limbs. All-limb grip strength was measured by placing the animal on a horizontal grid that was connected to a force meter and then pulling the animal's tail until it could no longer maintain its grip on the grid
- Kondziela inverted screen test: Muscular strength and proprioception was assessed. A vertically positioned grid box allowed mice to grab on to the grid as they climbed down. The latency to fall was quantified
- For all behavioral tests, an average score from 3 trials was taken for each mouse

Sciatic Nerve Electrophysiology

- CMAPs were recorded from the intrinsic foot muscles of anesthetized mice using steel-needle electrodes (MLA1302; AD Instruments)
- Amplitude and latency of CMAP were determined
- The distance between the 2 sites of stimulation was measured alongside the skin surface with the animal's legs fully extended, and NCVs were calculated from latency measurements

Plasma Biomarkers

- Quantification of IL-6, TNF-α, and NfL was performed in duplicate for each animal in 96-well plates by ELISA (RAB0308 and RAB0477; Sigma Aldrich and NBP2-80299; Novus Biologica)

Sciatic Nerve Histology (toluidine blue staining)

- Semi-thin cross sections of fixed sciatic nerves of the left side were cut and stained with toluidine blue, 0.5 %, + borax, 1 % , + MilliQ water 100 mL
- The axonal diameter, number of myelinated motor axons per 100 μm^2, and the myelin g-ratio were quantified using the ImageJ g-ratio plug-in (http://gratio.efil.de/)

Supplemental Table S1. Composition of the Study Groups

Group	Genotype	Treatment	Dose	Administration route	Treatment timing	No. of mice (baseline)	No. of nerves for histology
Group 1–Healthy control	Wild type	Vehicle	–	Oral gavage	Once a day from 1 to 3 months old	10	10
Group 2–Disease control	$TDP43^{A315T}$	Vehicle	–	Oral gavage	Once a day from 1 to 3 months old	10	10
Group 3–ALS + ATH-1105 10 mg/kg	$TDP43^{A315T}$	ATH-1105	10 mg/kg	Oral gavage	Once a day from 1 to 3 months old	10	10

Abbreviations **ALS,** amyotrophic lateral sclerosis; **CMAP,** compound muscle action potential; **ELISA**, enzyme-linked immunosorbent assay; **IL-6,** interleukin 6; **NCV,** nerve conduction velocity; **NfL,** neurofilament light chain; **TNF-α**, tumor necrosis factor alpha.

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