# ATH-1105, a Small-Molecule Positive Modulator of Hepatocyte Growth Factor (HGF)/MET, Is Neuroprotective in a TDP-43 Mouse Model of Amyotrophic Lateral Sclerosis

Kayla Kleist,* Andrée-Anne Berthiaume,* Jewel Johnston, Sherif Reda, Hans J. Moebius, Kevin J. Church

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## CONCLUSIONS

Treatment with ATH-1105 in TDP-43$^{A315T}$ ALS mice resulted in

1. Improvement in balance, coordination, and muscle strength in motor function tests
2. Protection against body weight reduction
3. Preservation of nerve function and structure
4. Reduction of plasma biomarkers of systemic inflammation and neurodegeneration
5. Prolonged survival and delayed time to first mortality

## KEY TAKEAWAY

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



**Acknowledgments**

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**Disclosures**

Kayla Kleist, Andrée-Anne Berthiaume, Jewel Johnston, Sherif Reda, Hans J. Moebius, and Kevin J. Church are employees and stockholders of Athira Pharma, Inc.

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## INTRODUCTION

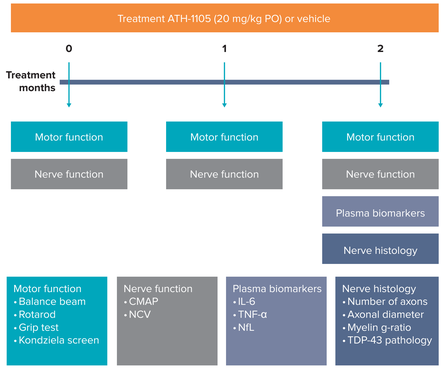
- ALS is characterized by progressive motor neuron degeneration, demyelination, and systemic inflammation[1,2]
  - Up to 97% of people with ALS exhibit TDP-43 proteinopathy[3]
- Beginning at 2 months of age, TDP-43$^{A315T}$ mice develop ALS-like deficits in motor and nerve function, motor neuron loss, and systemic inflammation, contributing to early mortality[4]
- Enhancing HGF/MET signaling may counteract the neurodegeneration observed in ALS via neuroprotective mechanisms that counteract neurotoxicity, inflammation and oxidative stress-induced damage[5-8]
  - See supplemental information for more details (**QR code**)

## OBJECTIVE

To evaluate the neuroprotective effects of ATH-1105, a small-molecule positive modulator of the HGF/MET system, on function and survival in a TDP-43 mouse model of ALS

## METHODS

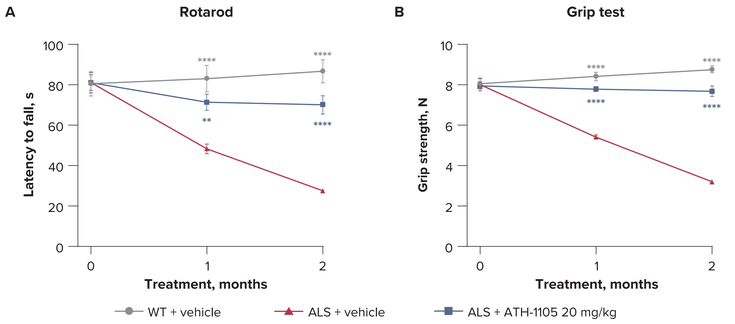
### Figure 1. Study design




- One-month-old male mice were sorted into 3 groups of 10 animals each and given daily treatment from 1-3 months of age (total of 2 treatment months)
  - WT + vehicle (healthy control): WT mice treated with vehicle (PO)
  - ALS + vehicle (disease control): TDP-43$^{A315T}$ mice treated with vehicle (PO)
  - ALS + ATH-1105 20 mg/kg: TDP-43$^{A315T}$ mice treated with ATH-1105 (PO)
  - Behavioral tests, sciatic nerve electrophysiology, histology, and plasma biomarker analyses were carried out as described in the supplemental information (**QR code**)
  - No mortality was observed at, or prior to, 3 months of age
- To evaluate effects on survival, a separate cohort of 1-month-old TDP-43$^{A315T}$ mice were sorted into groups of 20 animals each and given daily treatment from 1-5 months of age (total of 4 treatment months)
  - ALS + vehicle (PO)
  - ALS + ATH-1105 20 mg/kg (PO)
  - The governing ethics committee required humane killing at 5 months of age for this ALS model

## RESULTS

### Figure 2. ATH-1105 significantly improves balance, coordination, and muscle strength

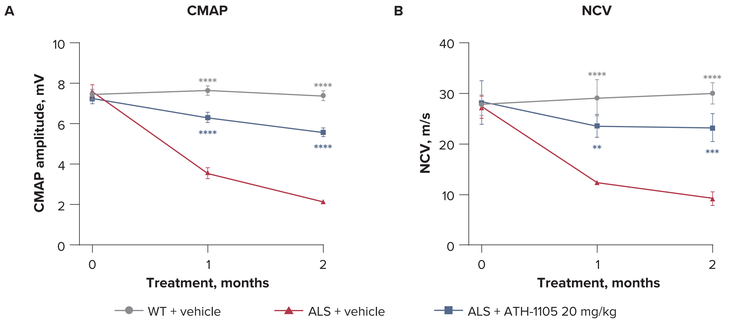



Graphical representation of (A) rotarod latency to fall and (B) grip strength at baseline and after 1 and 2 months of treatment. Significant effects in balance beam cross time and Kondziela screen latency to fall were also observed, as well as significant protection from body weight reduction; these results are presented in the supplemental information (**QR code**).

Data presented as mean ± SEM. Statistical significance was determined by 2-way ANOVA with Dunnett's test versus ALS + vehicle.

$^{**}p < 0.01$; $^{****}p < 0.0001$.

### Figure 3. ATH-1105 significantly improves muscle and nerve function

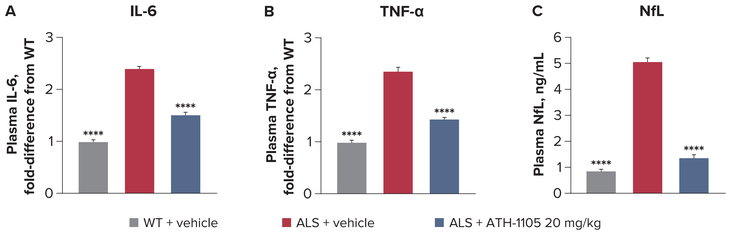



Graphical representation of (A) CMAP amplitude and (B) NCV at baseline and after 1 and 2 months of treatment.

Data presented as mean ± SEM. Statistical significance was determined by 2-way ANOVA with Dunnett's test versus ALS + vehicle.

$^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$.

### Figure 4. ATH-1105 reduces plasma biomarkers of inflammation and neurodegeneration

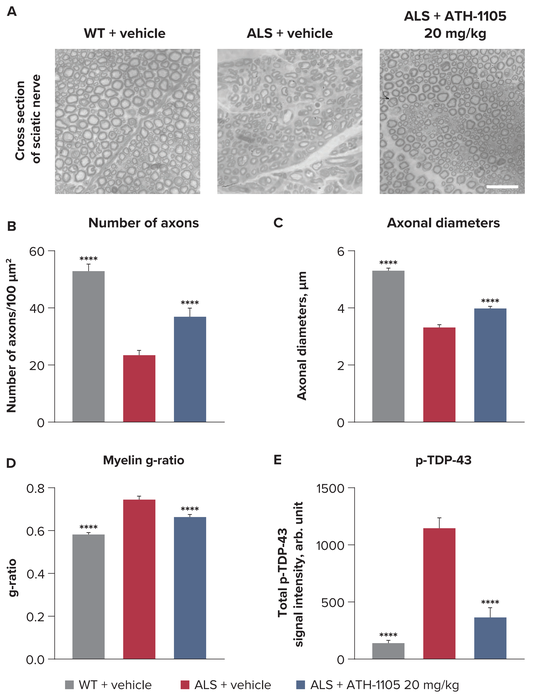



Graphical representation of plasma (A) IL-6 and (B) TNF-α, in fold-difference over the WT + vehicle group at 2 months of treatment. (C) Plasma NfL concentration at 2 months of treatment.

Data presented as mean + SEM. Statistical significance was determined by 1-way ANOVA with Dunnett's test versus ALS + vehicle.

$^{****}p < 0.0001$.

### Figure 5. ATH-1105 protects against axon degeneration and demyelination, and reduces p-TDP-43 accumulation

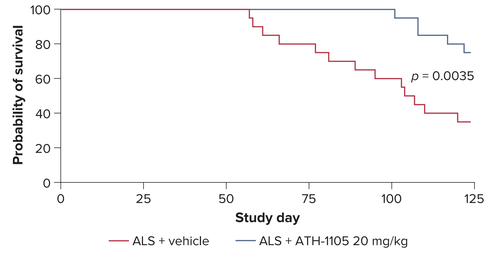



(A) Histology images of sciatic nerve cross sections stained with toluidine blue to label myelin. Scale is 10 µm (all panels). Graphical representation of (B) the number of axons, (C) axonal diameters, and (D) myelin g-ratio (defined as the ratio of the inner axonal diameter to the total axonal diameter) after 2 months of treatment. (E) Quantification of p-TDP-43 in sciatic nerve sections dual labeled for p-TDP-43 (Ser409/410) and the axonal marker Tuj1 by IHC; representative images are shown in the supplemental information (**QR code**).

Data presented as mean + SEM. Statistical significance was determined by 1-way ANOVA with Dunnett's test versus ALS + vehicle.

$^{***}p < 0.001$, $^{****}p < 0.0001$.

### Figure 6. ATH-1105 prolongs survival and delays time to first mortality




Survival curves up to 4 months of treatment (study day 124; 5 months of age). Time to first mortality with ALS + vehicle was 59 days versus 101 days with ALS + ATH-1105 20 mg/kg.

Data presented as Kaplan-Meier survival curves. Statistical significance was determined by the log-rank (Mantel-Cox) test.

**Abbreviations** **ALS**, amyotrophic lateral sclerosis; **ANOVA**, analysis of variance; **CMAP**, compound muscle action potential; **HGF**, hepatocyte growth factor; **IHC**, immunohistochemistry; **IL-6**, interleukin 6; **NCV**, nerve conduction velocity; **NfL**, neurofilament light chain; **PO**, oral gavage; **p-TDP-43**, phosphorylated TDP-43; **SEM**, standard error of the mean; **TDP-43**, TAR DNA-binding protein 43; **TNF-α**, tumor necrosis factor α; **Tuj1**, class III beta-tubulin; **WT**, wild type.

**References** **1.** Hulisz D. *Am J Manag Care*. 2018;24(15):S320-S326. **2.** Tortelli R et al. *Front Neurol*. 2020;11:552295. **3.** Scotter et al. *Neurotherapeutics*. 2015;12(2):352-363. **4.** Bargsted et al. *Sci Rep*. 2017;7(1):14266. **5.** Nicoleau C et al. *Stem Cells*. 2009;27:408-419. **6.** Ko KR et al. *Sci Rep*. 2018;8:8316. **7.** Johnston JL et al. *Neurotherapeutics*. Published online December 20, 2022. doi: 10.1007/s13311-022-01325-5. **8.** Desole C et al. *Front Cell Dev Biol*. 2021;9:683609.

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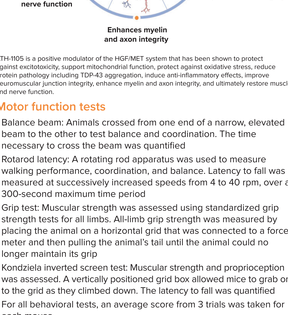
## SUPPLEMENTAL INFORMATION

### Supplemental Figure S1. Positive modulation of HGF/MET signaling has therapeutic potential in ALS




ATH-1105 is a positive modulator of the HGF/MET system that has been shown to protect against excitotoxicity, support mitochondrial function, protect against oxidative stress, reduce protein pathology including TDP-43 aggregation, induce anti-inflammatory effects, improve neuromuscular junction integrity, enhance myelin and axon integrity, and ultimately restore muscle and nerve function.

#### Motor function 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 the animal could no longer maintain its grip
- 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

- CMAP was 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 NCV was 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 µm², and the myelin g-ratio were quantified using the ImageJ g-ratio plug-in (http://gratio.efil.de/)

### Supplemental Table S1. Study groups

| Group | Genotype | Treatment | Dose | Administration route | Treatment timing | No. of mice (baseline) | No. of nerves for toluidine blue histology | No. of nerves for p-TDP-43 IHC |
|---|---|---|---|---|---|---|---|---|
| Group 1 healthy control | WT | Vehicle | – | PO | 1-3 months old | 10 | 10 | 7 |
| Group 2 ALS control | TDP-43$^{WT/Q}$ | Vehicle | – | PO | 1-3 months old | 10 | 10 | 7 |
| Group 3 ALS + ATH-1105 | TDP-43$^{WT/Q}$ | ATH-1105 | 20 mg/kg | PO | 1-3 months old | 10 | 10 | 7 |
| Group 4 survival ALS control | TDP-43$^{WT/Q}$ | Vehicle | – | PO | 1-5 months old | 20 | – | – |
| Group 5 survival ALS + ATH-1105 | TDP-43$^{WT/Q}$ | ATH-1105 | 20 mg/kg | PO | 1-5 months old | 20 | – | – |

### Supplemental Figure S2. ATH-1105 significantly improves balance, coordination, and muscle strength in several behavioral assays




Graphical representation of (A) Kondziela screen latency to fall and (B) balance beam cross time 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 Dunnett's test versus ALS + vehicle.
****$p < 0.0001$.

### Supplemental Figure S3. 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 Dunnett's test versus ALS + vehicle.
*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

### Supplemental Figure S4. ATH-1105 reduces p-TDP-43 levels in the sciatic nerve




10-µm thick cross sections of fixed sciatic nerves of the left side (n = 7 mice/group) were cut and labeled with anti-Tuj1, an axonal marker, and anti–p-TDP-43 (Ser409/410), a marker of TDP-43 aggregates. Intensity of p-TDP-43 labeling was quantified. Representative images are shown. Scale is 50 µm (all panels).

**Abbreviations** **ALS**, amyotrophic lateral sclerosis; **ANOVA**, analysis of variance; **CMAP**, compound muscle action potential; **ELISA**, enzyme-linked immunosorbent assay; **IHC**, immunohistochemistry; **IL-6**, interleukin 6; **NCV**, nerve conduction velocity; **NfL** neurofilament light chain; **NMJ**, neuromuscular junction; **PO**, oral gavage; **p-TDP-43**, phosphorylated TDP-43; **SEM**, standard error of the mean; **TDP-43**, TAR DNA-binding protein 43; **TNF-α**, tumor necrosis factor α; **Tuj1**, class III beta-tubulin; **WT**, wild type.

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