ATH-1105, a Small-Molecule Positive Modulator of the Hepatocyte Growth Factor System, Is Neuroprotective in a Mouse Model of ALS When Administered Pre– or Post–Symptom Onset, or in Combination With Riluzole

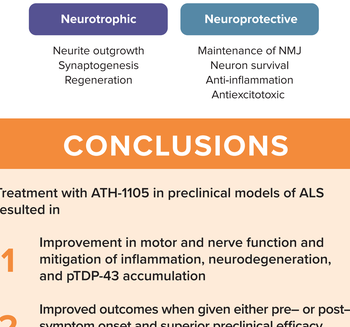
Andrée-Anne Berthiaume, Kayla N. Kleist, Sherif M. Reda, Sharay E. Setti, Robert W. Taylor, Jewel L. Johnston, Kevin J. Church

Athira Pharma, Bothell, WA, USA

INTRODUCTION

- ALS pathology is associated with progressive motor neuron degeneration, demyelination, systemic inflammation, glutamate-mediated toxicity, oxidative stress, mitochondrial dysfunction, axonal degeneration, TDP-43 extranuclear accumulation, NMJ impairment, and motor neuron death[1-4]
- Up to 97% of people with ALS exhibit TDP-43 proteinopathy[5]
- Promotion of HGF/MET activity has had beneficial effects in preclinical models of ALS through its multimodal neuroprotective and neurotrophic actions[3,4,6-8]
- We sought to evaluate the therapeutic potential of ATH-1105, a small-molecule positive modulator of the neurotrophic HGF system, in several preclinical models of ALS

Figure 1. Positive modulator of the neurotrophic HGF system, ATH-1105, promotes neuroprotective effects through downstream signaling pathways



CONCLUSIONS

Treatment with ATH-1105 in preclinical models of ALS resulted in

1. Improvement in motor and nerve function and mitigation of inflammation, neurodegeneration, and pTDP-43 accumulation
2. Improved outcomes when given either pre– or post–symptom onset and superior preclinical efficacy compared with riluzole under the conditions tested
3. Neuroprotection, metabolic stability, and synaptic integrity in in vitro models of excitotoxicity, including with SOD1^{G93A} spinal motor neurons

KEY TAKEAWAY

These findings highlight the neuroprotective potential of ATH-1105 in preclinical models of ALS and support its further development

Acknowledgments

These studies were sponsored by Athira Pharma, Inc. Editorial support was provided by Apothecom, and funded by Athira Pharma, Inc.

Disclosures

Andrée-Anne Berthiaume, Kayla N. Kleist, Sherif M. Reda, Sharay E. Setti, Robert W. Taylor, Jewel L. Johnston, and Kevin J. Church are employees and stockholders of Athira Pharma, Inc.

Disclaimer

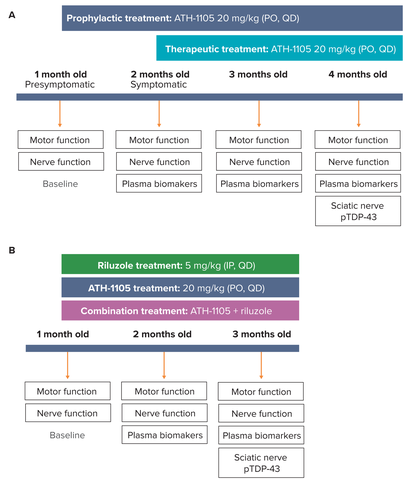
ATH-1105 is an investigational drug and has not received FDA approval nor been demonstrated safe or effective for any use.

Presented at the 2023 Annual NEALS Meeting; October 4-6, 2023; Clearwater Beach, Florida

METHODS

Preclinical efficacy in Prp-TDP43^{A315T} mice

Figure 2. In vivo study designs



- In two independent studies, 1-month-old male mice were sorted into (A) 4 or (B) 5 groups of 10 animals each and given daily treatment according to group assignments
- Both studies contained the following groups:
  - WT + vehicle: WT mice treated with vehicle daily
  - ALS + vehicle: Prp-TDP43^{A315T} ("ALS") mice (JAX #010700) treated with vehicle daily
  - ALS + treatment: Prp-TDP43^{A315T} ("ALS") mice treated as depicted in Figure 2
- Tests of motor and nerve function, and quantification of plasma biomarkers and pTDP-43 in the sciatic nerve were carried out as described in the Supplemental Information (**QR code**)

SOD1^{G93A} spinal motor neuron toxicity assay

- Spinal motor neurons were harvested from E14 SOD1^{G93A} rat embryos and cultured for 13 days
- Cultures were pretreated for 15 minutes with vehicle (containing HGF 0.05 ng/mL) ± ATH-1105 1 µM and then challenged with glutamate 5 µM for 20 minutes
- After 24 hours of incubation in treatment conditions, immunofluorescence analysis via MetaXpress (Molecular Devices) was used to assess neuronal survival (anti–MAP-2), extranuclear TDP-43 (anti–TDP-43), mitochondrial health (MitoTracker), and ER stress (anti-ATF6)

Nerve-muscle coculture impairment assay

- Whole spinal cord sections, including 4 DRGs, were harvested from E13 Wistar rat embryos and cocultured on a monolayer of human muscle cells for 27 days, a sufficient culture period to allow formation of functional NMJs
- Mature cocultures were pretreated for 20 minutes with vehicle (DMSO, 0.1%) or ATH-1105 10 nM, 100 nM, or 1 µM and then challenged with glutamate 60 µM for 20 minutes, after which treatment was reapplied for an additional 48 hours
- By use of automatic quantification (Edison Developer; GE Healthcare) of anti–NF-200 immunolabeling of motor neurons and α-bung labeling of AChRs, cocultures were evaluated to determine motor neuron survival (number of neurons), AChR clustering (α-bung staining area), and number of motor units (number of α-bung staining clusters)

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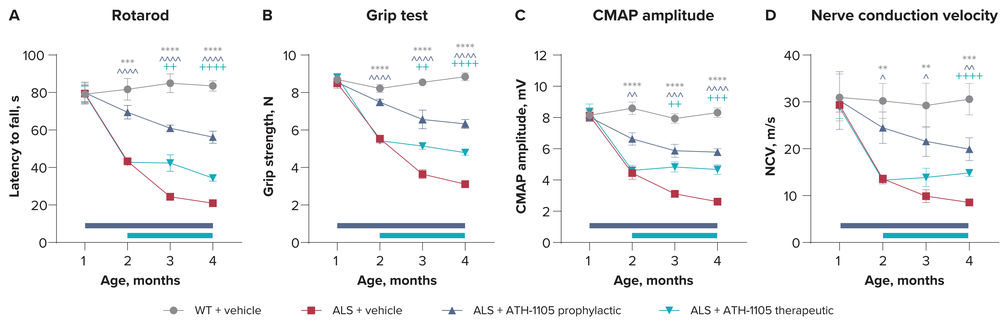
RESULTS

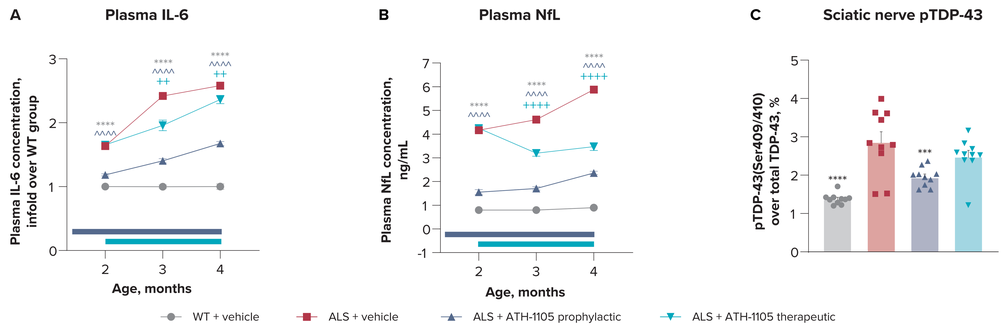
Figure 3. ATH-1105 dosed pre– or post–symptom onset improves motor and nerve function in ALS mice



Graphical representation of (A) rotarod latency to fall, (B) grip strength force, (C) CMAP amplitude, and (D) nerve conduction velocity. Solid bars along *x* axis depict treatment duration for the prophylactic (top, dark blue) and therapeutic (bottom, teal) ATH-1105 groups. Data are presented as mean ± SEM; n = 10 each. Statistical significance was determined by mixed-effects analysis with Dunnett's test vs the ALS + vehicle group. +•+ represents WT + vehicle vs ALS + vehicle. ^•^ represents ALS + prophylactic ATH-1105 vs ALS + vehicle. "•" represents ALS + therapeutic ATH-1105 vs ALS + vehicle.

The following applies to all symbols: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

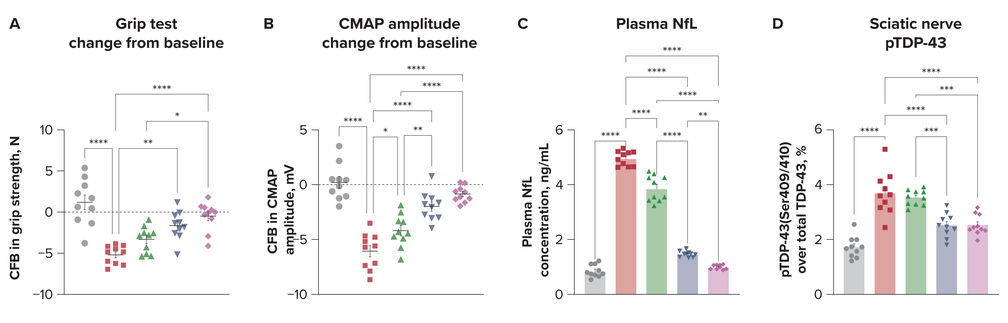
Figure 4. ATH-1105 dosed pre– or post–symptom onset reduces biomarkers of inflammation, neurodegeneration, and protein pathology in ALS mice



Graphical representation of plasma (A) IL-6 and (B) NfL levels from time of treatment initiation. (C) pTDP-43 levels in homogenized sciatic nerve at 4 months of age. Solid bars along *x* axis depict treatment duration for the prophylactic (top, dark blue) and therapeutic (bottom, teal) ATH-1105 groups. Data are presented as mean ± SEM in (A, B) and + SEM in (C); n = 10 each. Statistical significance was determined by (A, B) mixed-effects analysis or (C) one-way ANOVA with Dunnett's vs the ALS + vehicle group. (A, B) •+• represents WT + vehicle vs ALS + vehicle. ^•^ represents ALS + prophylactic ATH-1105 vs ALS + vehicle. "•" represents ALS + therapeutic ATH-1105 vs ALS + vehicle.

The following applies to all symbols: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

Figure 5. ATH-1105 alone or in combination with riluzole improves motor and nerve function, and reduces plasma NfL and pTDP-43 accumulation in ALS mice

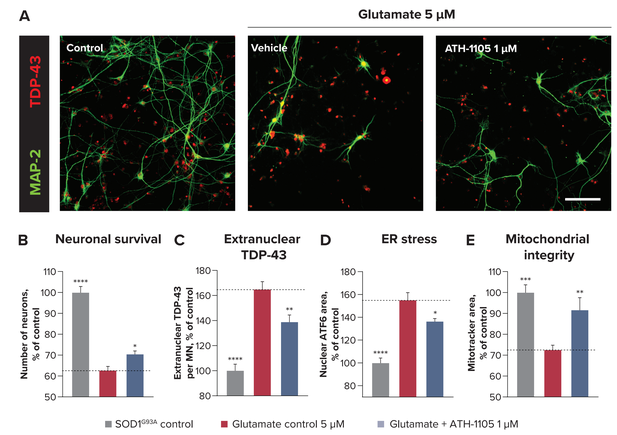


Graphical representation of change from baseline at 3 months of age in (A) grip test performance and (B) CMAP amplitude. (C) Plasma NfL levels and (D) pTDP-43 over total TDP-43 levels in homogenized sciatic nerve at 3 months of age.

Data are presented as mean ± SEM in (A, B) and + SEM in (C, D); n = 10 mice each. Statistical significance determined by one-way ANOVA with Dunnett's test. Comparisons vs WT + vehicle included in analyses but not shown.

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

Figure 6. ATH-1105 attenuates glutamate-mediated toxicity in SOD1^{G93A} spinal motor neurons

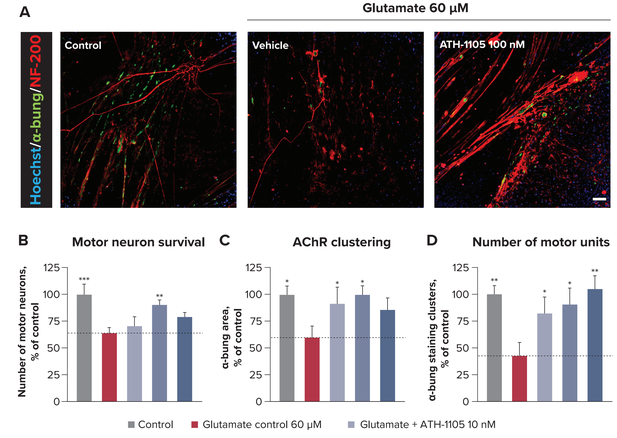


(A) Representative images of SOD1^{G93A} spinal motor neurons challenged with glutamate (scale bar = 100 µm). Graphical representation of the effect of ATH-1105 on (B) neuronal survival, (C) extranuclear TDP-43, (D) ER stress, and (E) mitochondrial integrity in SOD1^{G93A} motor neurons challenged with glutamate.

Data are presented as mean + SEM; n = 4-6 each. One-way ANOVA with Fisher's LSD vs glutamate alone.

*$p < 0.05$; **$p < 0.01$; ****$p < 0.0001$.

Figure 7. ATH-1105 protects neuromuscular junction integrity against glutamate-mediated toxicity in nerve-muscle cocultures



(A) Representative images (scale bar = 100 µm) and graphical representation of motor neuron muscle coculture showing the effects of ATH-1105 on (B) motor neuron survival, (C) AChR clustering, and (D) number of motor units after challenge with glutamate.

Data are presented as mean + SEM; n = 6 each. One-way ANOVA with Fisher's LSD vs glutamate alone.

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

Abbreviations: **α-bung**, α-bungarotoxin; **AChR**, acetylcholine receptor; **AKT**, protein kinase B; **ALS**, amyotrophic lateral sclerosis; **ANOVA**, analysis of variance; **ATF6**, activating transcription factor 6; **CFB**, change from baseline; **CMAP**, compound muscle action potential; **DMSO**, dimethylsulfoxide; **DRG**, dorsal root ganglia; **ER**, endoplasmic reticulum; **ERK**, extracellular signal–related kinase; **HGF**, hepatocyte growth factor; **IL-6**, interleukin 6; **IP**, intraperitoneal; **LSD**, least significant difference; **MAP-2**, microtubule-associated protein 2; **NCV**, nerve conduction velocity; **NF-200**, neurofilament-200; **NfL**, neurofilament light chain; **NMJ**, neuromuscular junction; **P**, phosphorylation; **PO**, oral gavage; **pTDP-43**, phosphorylated TDP-43; **QD**, once daily; **SEM**, standard error of the mean; **SOD1^{G93A}**, superoxide dismutase 1 G93A mutation; **TDP-43**, TAR DNA-binding protein 43; **WT**, wild type

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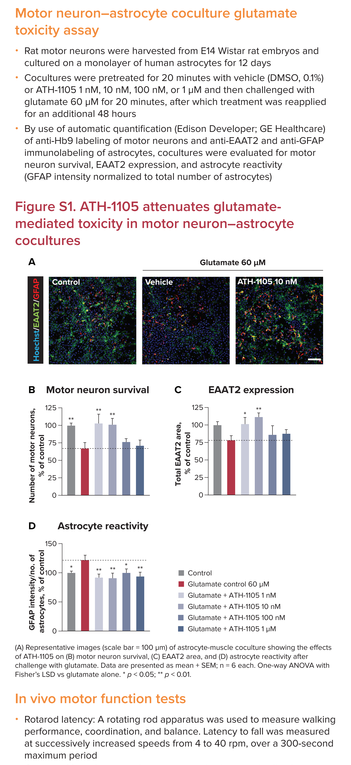
SUPPLEMENTAL INFORMATION

Motor neuron–astrocyte coculture glutamate toxicity assay

- Rat motor neurons were harvested from E14 Wistar rat embryos and cultured on a monolayer of human astrocytes for 12 days
- Cocultures were pretreated for 20 minutes with vehicle (DMSO, 0.1%) or ATH-1105 1 nM, 10 nM, 100 nM, or 1 µM and then challenged with glutamate 60 µM for 20 minutes, after which treatment was reapplied for an additional 48 hours
- By use of automatic quantification (Edison Developer; GE Healthcare) of anti-Hb9 labeling of motor neurons and anti-EAAT2 and anti-GFAP immunolabeling of astrocytes, cocultures were evaluated for motor neuron survival, EAAT2 expression, and astrocyte reactivity (GFAP intensity normalized to total number of astrocytes)

Figure S1. ATH-1105 attenuates glutamate-mediated toxicity in motor neuron–astrocyte cocultures



(A) Representative images (scale bar = 100 µm) of astrocyte-muscle coculture showing the effects of ATH-1105 on (B) motor neuron survival, (C) EAAT2 area, and (D) astrocyte reactivity after challenge with glutamate. Data are presented as mean ± SEM; n = 6 each. One-way ANOVA with Fisher's LSD vs glutamate alone. * $p < 0.05$; ** $p < 0.01$.

In vivo motor function tests

- 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 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

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 two sites of stimulation was measured alongside the skin surface with the animal's legs fully extended, and NCV was calculated from latency measurements

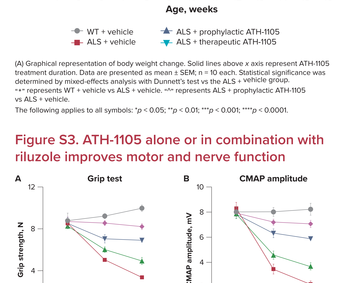
Plasma biomarkers

- Quantification of IL-6 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)

pTDP-43 quantification

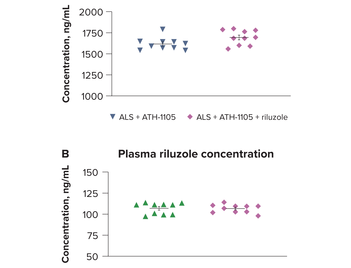
- Quantification of pTDP-43 was performed in homogenized sciatic nerve using the AlphaLISA SureFire Ultra Human Phospho-TDP-43 (Ser409/410) detection kit (Perkin Elmer Ref. ALSU-PTDP43) following manufacturer's instructions

Figure S2. ATH-1105 dosed pre–symptom onset protects against body weight loss in ALS mice



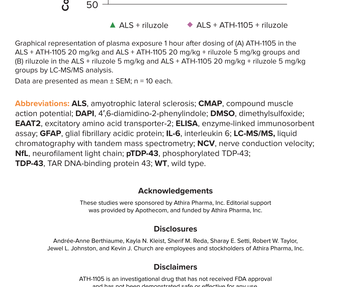
(A) Graphical representation of body weight change. Solid lines above x axis represent ATH-1105 treatment duration. Data are presented as mean ± SEM; n = 10 each. Statistical significance was determined by mixed-effects analysis with Dunnett's test vs the ALS + vehicle group.
^^ represents WT + vehicle vs ALS + vehicle. ^^^ represents ALS + prophylactic ATH-1105 vs ALS + vehicle.
The following applies to all symbols: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

Figure S3. ATH-1105 alone or in combination with riluzole improves motor and nerve function



Graphical representation of (A) grip test performance and (B) CMAP amplitude over time with riluzole and ATH-1105 treatment alone or in combination for a total of 2 months, from 1 to 3 months of age in ALS mice. Data are presented as mean ± SEM; n = 10 each.

Figure S4. Concentration of ATH-1105 and riluzole in plasma 1 hour after dosing



Graphical representation of plasma exposure 1 hour after dosing of (A) ATH-1105 in the ALS + ATH-1105 20 mg/kg and ALS + ATH-1105 20 mg/kg + riluzole 5 mg/kg groups and (B) riluzole in the ALS + riluzole 5 mg/kg and ALS + ATH-1105 20 mg/kg + riluzole 5 mg/kg groups by LC-MS/MS analysis.
Data are presented as mean ± SEM; n = 10 each.

Abbreviations: **ALS**, amyotrophic lateral sclerosis; **CMAP**, compound muscle action potential; **DAPI**, 4',6-diamidino-2-phenylindole; **DMSO**, dimethylsulfoxide; **EAAT2**, excitatory amino acid transporter-2; **ELISA**, enzyme-linked immunosorbent assay; **GFAP**, glial fibrillary acidic protein; **IL-6**, interleukin 6; **LC-MS/MS,** liquid chromatography with tandem mass spectrometry; **NCV**, nerve conduction velocity; **NfL**, neurofilament light chain; **pTDP-43**, phosphorylated TDP-43; **TDP-43**, TAR DNA-binding protein 43; **WT**, wild type.

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Disclaimers

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