ATH-1105, a small-molecule positive modulator of the neurotrophic HGF system, attenuates TDP-43 pathology in preclinical models of ALS

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

Athira Pharma, Inc., Bothell, USA

CONCLUSIONS

1. **ATH-1105 promotes motor neuron survival and reduces cytoplasmic TDP-43 accumulation following glutamate injury**
2. **The neuroprotective effects of ATH-1105 are associated with improved autophagic function, which may promote the clearance of pathological TDP-43**
3. **ATH-1105 also promotes mitochondrial health and reduces GSK3β activation, which may mitigate the generation of pathological TDP-43**
4. **In a TDP43-driven mouse model of ALS, ATH-1105 reduces TDP-43 pathology and improves neuromuscular function**

KEY TAKEAWAY

ATH-1105 addresses multiple modes of dysfunction associated with TDP-43 pathology, supporting its ongoing development as a potential treatment for ALS.

Abbreviations: **ALS**, amyotrophic lateral sclerosis; **AKT**, protein kinase B; **ANOVA**, analysis of variance; **CMAP**, compound muscle action potential; **ERK**, extracellular signal regulated kinase; **GAPDH**, glyceraldehyde 3-phosphate dehydrogenase; **GSK3β**, glycogen synthase kinase-3 beta; **HGF**, hepatocyte growth factor; **LAMP2**, lysosomal membrane-associated protein 2; **LC3**, Microtubule-associated proteins 1A/1B light chain 3B, **LSD**, least significant difference; **MAP2**, microtubule-associated protein 2; **NfL**, neurofilament light; **NMJ**, neuromuscular junction; **p**, phosphorylation; **pAKT**, phosphorylated AKT; **PI3K**, phosphoinositide 3-kinase; **pGSK3β**, phosphorylated glycogen synthase kinase-3 beta; **pTDP-43**, phosphorylated TDP43; **ROS**, reactive oxygen species; **SEM**, standard error of the mean; **TDP-43**, transactive response DNA binding protein 43

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Disclosures

Sherif M Reda, Andrée-Anne Berthiaume, Wei Wu, Kayla N Kleist, 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 therapy and has not received FDA approval nor been demonstrated to be safe or effective for any use.

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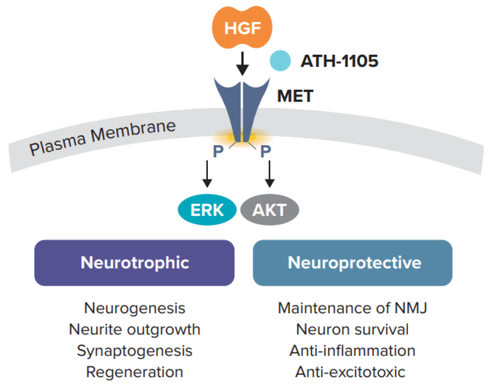
INTRODUCTION

- ALS pathology is associated with progressive motor neuron degeneration, glutamate excitotoxicity, mitochondrial dysfunction, oxidative stress, axonal degeneration, TDP-43 cytoplasmic accumulation, and motor neuron death[1,2]
- TDP-43 is a highly conserved and ubiquitously expressed RNA/DNA-binding protein that has versatile functions in transcription, translation, and mRNA transport and stabilization[3]
- Nuclear depletion and cytoplasmic accumulation of TDP-43 is a critical component of ALS pathology that contributes to the progressive degeneration of motor neurons.
  - Up to ~97% of people with ALS exhibit TDP-43 proteinopathy[4]
- Cytoplasmic TDP-43 can manifest in various pathological forms (TDP-43 aggregates, TDP-43 fragments, and phosphorylated TDP-43), which are associated with several neurodegenerative mechanisms including autophagic impairment, mitochondrial dysfunction, and activation of GSK3β.
- Promotion of neurotrophic HGF activity has been reported to have beneficial effects in preclinical models of ALS through its pleiotropic actions that can counteract various neurodegenerative mechanisms[5,6]
- We sought to assess the impact of ATH-1105, a small molecule positive modulator of HGF, on TDP-43 pathology in preclinical models of ALS.

ATH-1105 is a positive modulator of the neurotrophic HGF system[5]



OBJECTIVE

To assess the impact of ATH-1105 on neurodegenerative mechanisms associated with TDP-43 pathology

METHODS

In vitro primary motor neuron glutamate toxicity assays

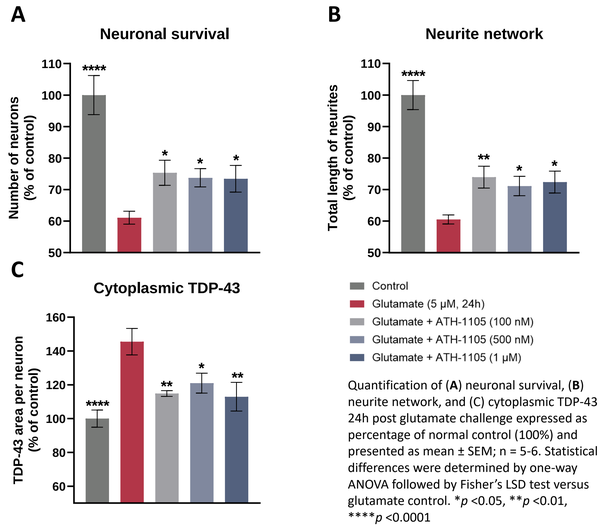
- Rat primary spinal motor neurons were harvested from E14 rat embryos and cultured for 13 days
- Cultures were pretreated for 15 minutes with vehicle (containing HGF 0.05 ng/ml) or ATH-1105, and then challenged with glutamate 5 µM for 24 hours
- After 24 hours, immunofluorescence was used to assess the following metrics:
  - Neuronal survival (MAP2+ positive neurons)
  - Neurite network (total length of MAP2+ neurites in µm)
  - Cytoplasmic TDP-43 (overlap area of TDP-43 and MAP2+ neurons in µm^2)
  - LC3+ autophagosomes (overlap area of LC3 and MAP2+ neurons in µm^2)
  - LAMP2+ lysosomes (overlap area of LAMP2 and MAP2+ neurons in µm^2)
  - Autophagosome-lysosome co-localization (overlap area of LC3-LAMP2 in µm^2)
  - Functional mitochondria (overlap of MitoTracker and MAP2+ neurons in µm^2)
  - Active caspase-3 (overlap between caspase-3 and MAP2+ neurons in µm^2)
- Western blot analysis was used to evaluate pTDP-43 (Ser409/410), pGSK3β (Ser9), total GSK3β, and GAPDH (reference protein)

In vivo assays in the Prp-TDP43^{A315T} mouse model of ALS

- 1-month-old male mice were used in the study. Mice were divided into four groups (n=10) and treated once daily for 2 months as follows:
  1. WT mice treated with oral vehicle
  2. Prp-TDP43^{A315T} mice treated with oral vehicle
  3. Prp-TDP43^{A315T} mice treated with oral ATH-1105 10 mg/kg
  4. Prp-TDP43^{A315T} mice treated with oral ATH-1105 20 mg/kg
- Quantification of pTDP-43 was performed in homogenized sciatic nerve using the AlphaLISA SureFire Ultra Human Phospho-TDP-43 (Ser409/410) detection kit
- Quantification of plasma NfL was performed in duplicate for each animal by NfL ELISA kit: Novus Biologica Ref. NBP2-80299
- Neuromuscular function was evaluated by measuring CMAP amplitude from the intrinsic foot muscles of anesthetized mice using steel-needle electrodes (MLA1302; AD Instruments)

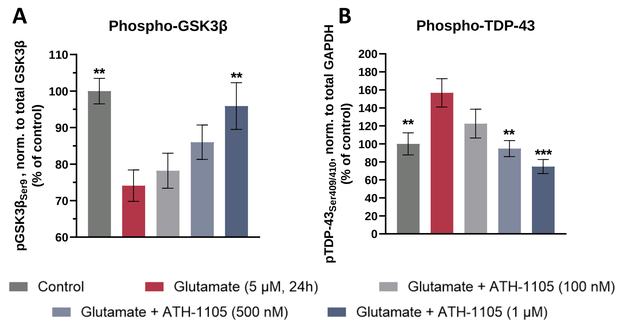
RESULTS

Figure 1. ATH-1105 promotes motor neuron survival and reduces cytoplasmic TDP-43 following glutamate injury



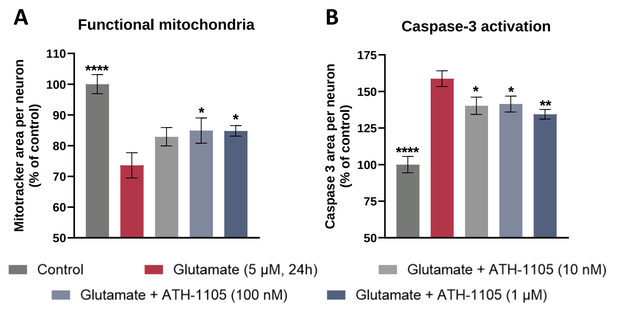
Quantification of (**A**) neuronal survival, (**B**) neurite network, and (C) cytoplasmic TDP-43 24h post glutamate challenge expressed as percentage of normal control (100%) and presented as mean ± SEM; n = 5-6. Statistical differences were determined by one-way ANOVA followed by Fisher's LSD test versus glutamate control. *p <0.05, **p <0.01, ****p <0.0001

Figure 2. ATH-1105 promotes phospho-inactivation of GSK3β and reduces pTDP-43 following glutamate injury



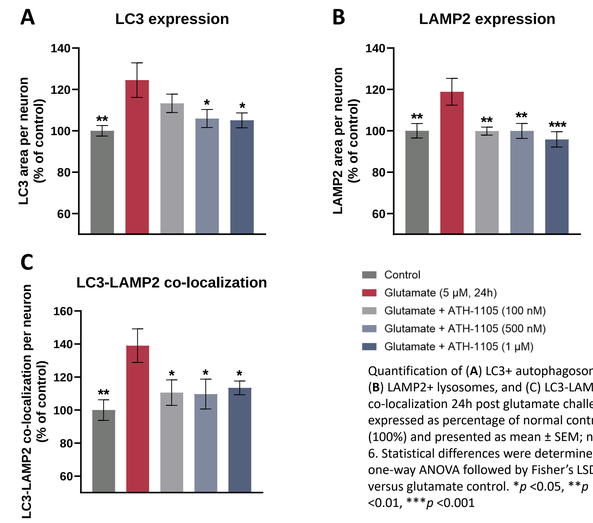
Quantification of (**A**) Phospho-GSK3β (Ser9) and (**B**) Phospho-TDP-43 (Ser409/410) 24h post glutamate challenge expressed as percentage of normal control (100%). Data presented as mean ± SEM; n = 3-4. Statistical differences were determined by one-way ANOVA followed by Fisher's LSD test versus glutamate control. **p <0.01, ***p <0.001

Figure 3. ATH-1105 supports mitochondrial health and reduces caspase-3 activation following glutamate injury



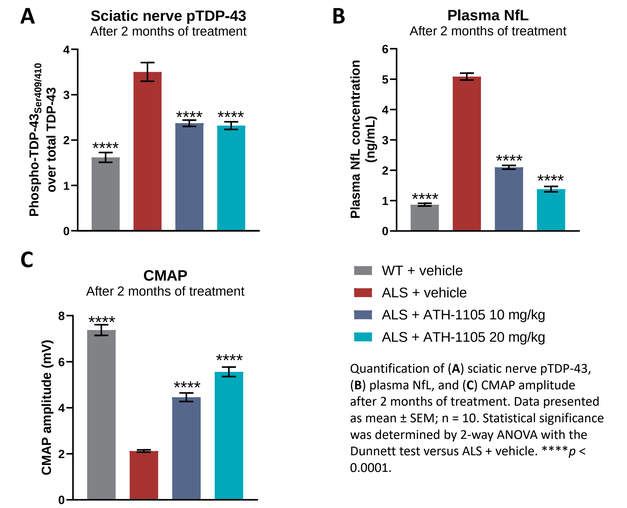
Quantification of (**A**) functional mitochondria (via MitoTracker) and (**B**) caspase-3 activation 24h post glutamate challenge expressed as percentage of normal control (100%). Data presented as mean ± SEM; n = 4-6. Statistical differences were determined by one-way ANOVA followed by Fisher's LSD test versus glutamate control. *p<0.05, **p <0.01, ****p <0.0001

Figure 4. ATH-1105 restores autophagic function following glutamate injury



Quantification of (**A**) LC3+ autophagosomes, (**B**) LAMP2+ lysosomes, and (C) LC3-LAMP2 co-localization 24h post glutamate challenge expressed as percentage of normal control (100%) and presented as mean ± SEM; n = 5-6. Statistical differences were determined by one-way ANOVA followed by Fisher's LSD test versus glutamate control. *p <0.05, **p <0.01, ***p <0.001

Figure 5. ATH-1105 reduces pTDP-43 and NfL, and improves neuromuscular function in the Prp-TDP43^{A315T} mouse model of ALS



Quantification of (**A**) sciatic nerve pTDP-43, (**B**) plasma NfL, and (**C**) CMAP amplitude after 2 months of treatment. Data presented as mean ± SEM; n = 10. Statistical significance was determined by 2-way ANOVA with the Dunnett test versus ALS + vehicle. ****p < 0.0001.

Figure 6. Proposed avenues by which ATH-1105 attenuates neurodegenerative mechanisms associated with TDP-43 pathology