ATH-1105, a small-molecule positive modulator of the neurotrophic HGF system, is neuroprotective in co-culture of human iPSC-derived motor neurons and muscle

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CONCLUSIONS

1. ATH-1105 enhances MET activation in neuronal models of ALS, including ALS patient-derived motor neurons
2. Neuroprotective activity of ATH-1105 against glutamate excitotoxicity is mediated through the MET receptor
3. ATH-1105 is neuroprotective in a human iPSC-based neuromuscular junction model

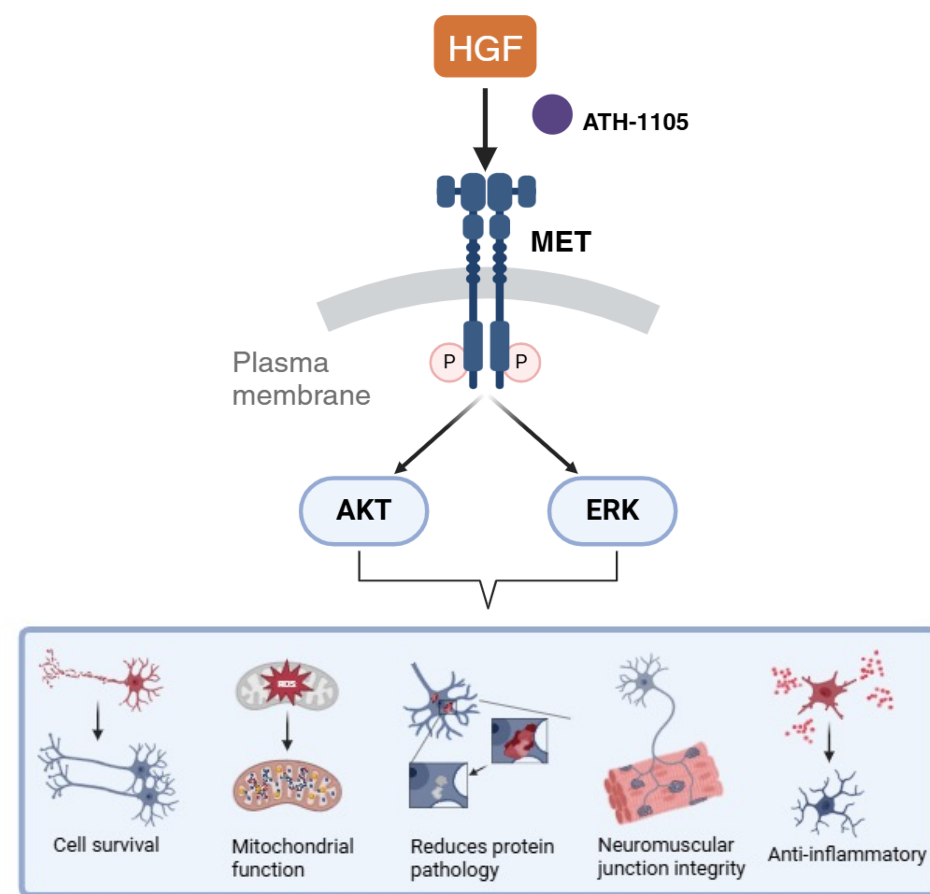
KEY TAKEAWAY

This dataset confirms the engagement of the HGF/MET system in the neuroprotective effects of ATH-1105 and demonstrates the activity of ATH-1105 in human iPSC-based neuronal models of ALS

INTRODUCTION

- ALS is a complex and fatal neurodegenerative disease preferentially impacting motor neurons[1]
- ALS is characterized by progressive neuromuscular junction dysfunction in association with multiple ongoing pathological processes including motor neuron degeneration, glutamate excitotoxicity, mitochondrial dysfunction, oxidative stress, TDP-43 pathology, and inflammation[2]
- Promotion of neurotrophic HGF activity through the MET receptor has been reported to have beneficial effects in preclinical models of ALS through its pleiotropic actions that can counteract various pathological processes of neurodegeneration[3,4]
- ATH-1105 is an oral, brain-penetrant, small-molecule positive modulator of the neurotrophic HGF system that has demonstrated efficacy on ALS-related pathology in vitro and in vivo[5]
- ATH-1105 is currently in a Phase 1a SAD/MAD study for assessment of safety, tolerability, and pharmacokinetics in healthy volunteers (NCT06432647)

ATH-1105 mechanism of action in preclinical models of ALS



OBJECTIVE

To demonstrate target engagement and translation of the neuroprotective activity of ATH-1105 from rodent-derived to human-derived models of ALS

METHODS

Phospho-MET assay in rat primary motor neurons

- Rat primary motor neurons were cultured for 13 days and treated with HGF (0.05 ng/ml) or ATH-1105 + HGF (0.05 ng/ml) for 15 minutes, and western blot was used to quantify levels of total MET and pMET (Y1234/Y1235).

Primary rat motor neuron glutamate toxicity assays

- Rat primary motor neurons were cultured for 13 days and 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 neuronal survival (MAP2+ positive neurons) and neurite network (total length of MAP2+ neurites in µm).
- Western blot was used to quantify levels of total MET and pMET (Y1234/Y1235).

siRNA neuroprotection assay

- Rat primary motor neurons were cultured for 13 days, treated with siRNA scramble or siRNA MET for 24 hours, followed by treatment with HGF 5 ng/ml or ATH-1105 100 nM for 15 minutes.
- Cultures then challenged with glutamate 5 µM for 24 hours, and immunofluorescence was used to assess neuronal survival (MAP2+ positive neurons) and neurite network (total length of MAP2+ neurites in µm).

Phospho-MET assay in patient-derived motor neurons

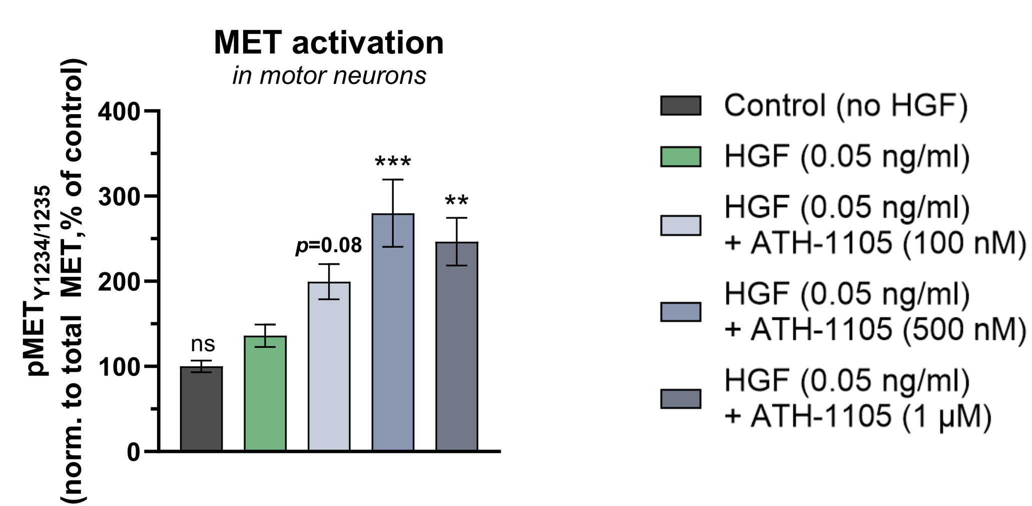
- ALS patient-derived motor neurons were sourced from Cedar Sinai via AnswerALS consortium.
- Motor neurons were plated in 24-well Matrigel plates and maintained for 7 days. On Day 8, cells were treated with vehicle (containing HGF 0.05 ng/ml) or ATH-1105 100 nM for 24 hours.
- After 24 hours, cells were lysed and levels of total MET and pMET (Y1234/Y1235) were quantified via HTRF and ELISA, respectively.

Neuromuscular junction glutamate toxicity assay

- Human iPSC-derived motor neurons expressing SOD1-A4V mutation were cocultured on differentiated human muscle cells for 14 days, a sufficient culture period to allow formation of functional NMJ.
- Mature cocultures were treated for 20 minutes with vehicle, HGF 10 ng/ml, or ATH-1105 10 nM, 100 nM, or 1 µM, and then challenged with glutamate 60 µM for 20 minutes, after which the media was removed, and treatment was reapplied for an additional 48 hours.
- By use of automatic quantification of MAP-2 immunolabeling, cocultures were evaluated to determine motor neuron survival and neurite network (total neurite length).

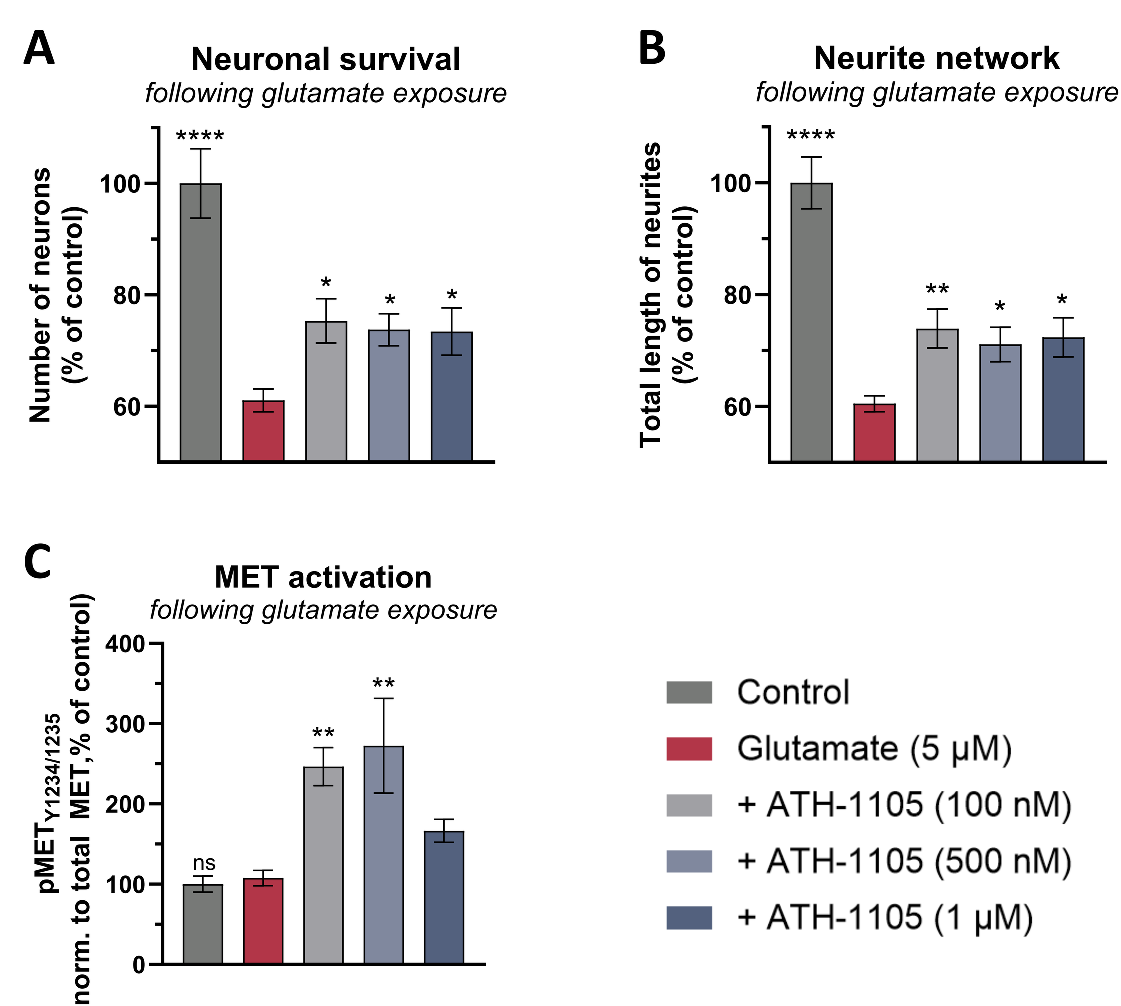
RESULTS

Figure 1. ATH-1105 promotes HGF-mediated activation of the MET receptor in rat primary motor neurons



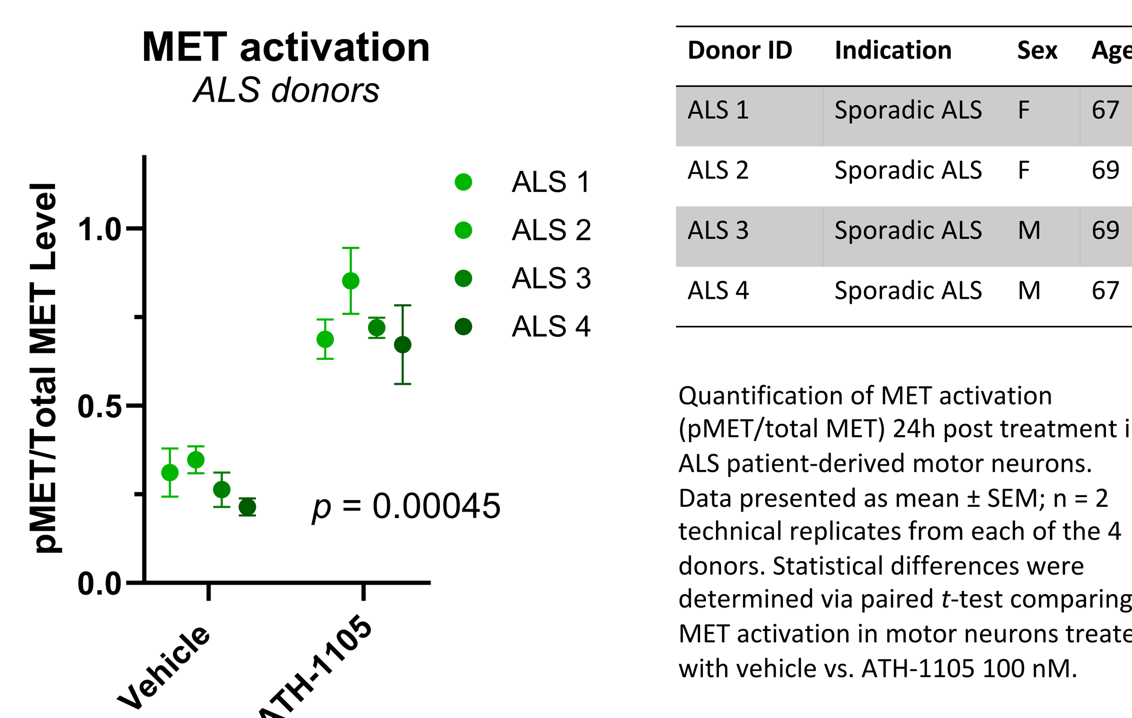
Quantification of MET activation (pMET/total MET) 15 minutes post treatment are expressed as percentage of control (100%). HGF 0.05 ng/ml represents a 'subthreshold' concentration of HGF that is not sufficient to induce MET activation. Data presented as mean ± SEM; n = 4 from 1 culture. Statistical differences were determined by one-way ANOVA followed by Fisher's LSD test versus HGF control. $^{**}p < 0.01$, $^{***}p < 0.001$

Figure 2. ATH-1105 is neuroprotective and promotes MET activation following glutamate injury in rat primary motor neurons



Quantification of (A) neuronal survival, (B) neurite network, and (C) MET activation (pMET/total MET) 24h post glutamate in primary rat motor neurons expressed as percentage of normal control (100%). Data presented as mean ± SEM; n = 5-6 for A, B and n = 3-4 for C from 1 culture. Statistical differences were determined by one-way ANOVA followed by Fisher's LSD test versus glutamate control. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{****}p < 0.0001$

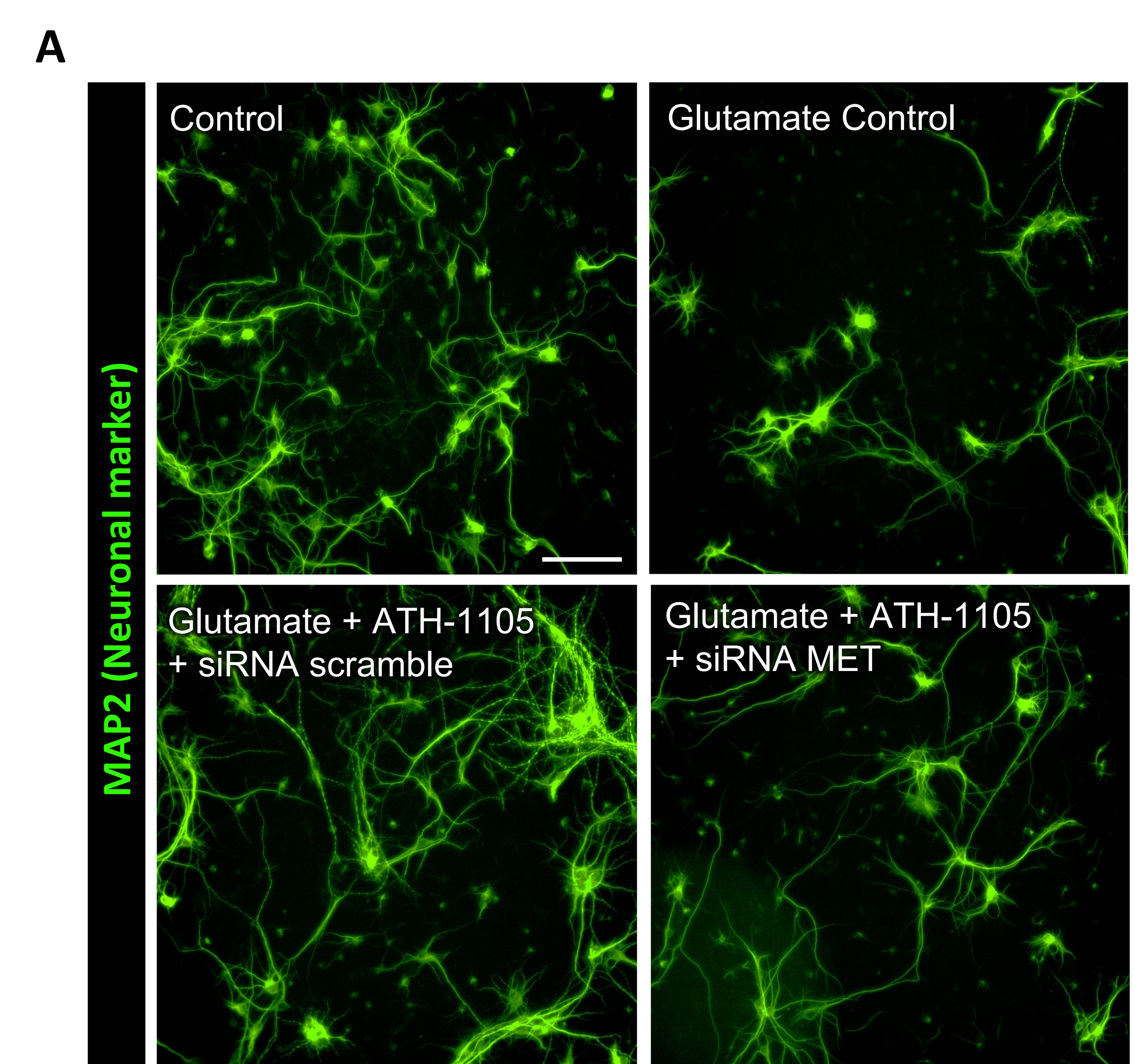
Figure 4. ATH-1105 promotes MET activation in ALS patient-derived motor neurons

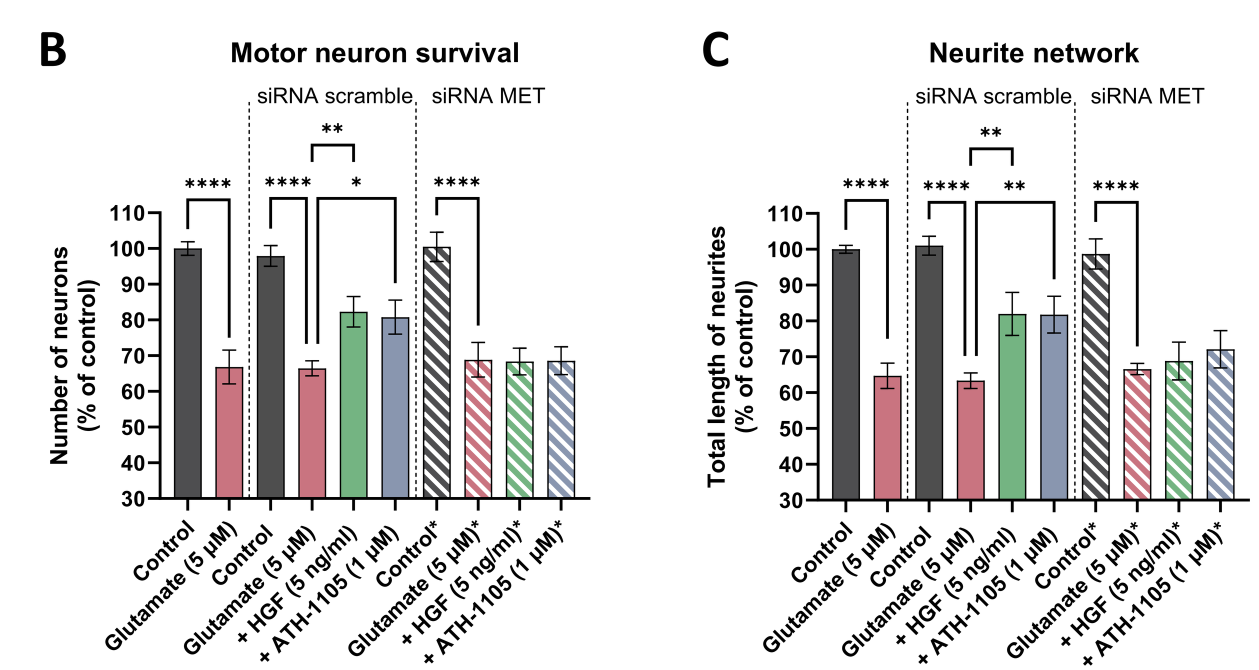


Donor ID	Indication	Sex	Age
ALS 1	Sporadic ALS	F	67
ALS 2	Sporadic ALS	F	69
ALS 3	Sporadic ALS	M	69
ALS 4	Sporadic ALS	M	67

Quantification of MET activation (pMET/total MET) 24h post treatment in ALS patient-derived motor neurons. Data presented as mean ± SEM; n = 2 technical replicates from each of the 4 donors. Statistical differences were determined via paired *t*-test comparing MET activation in motor neurons treated with vehicle vs. ATH-1105 100 nM.

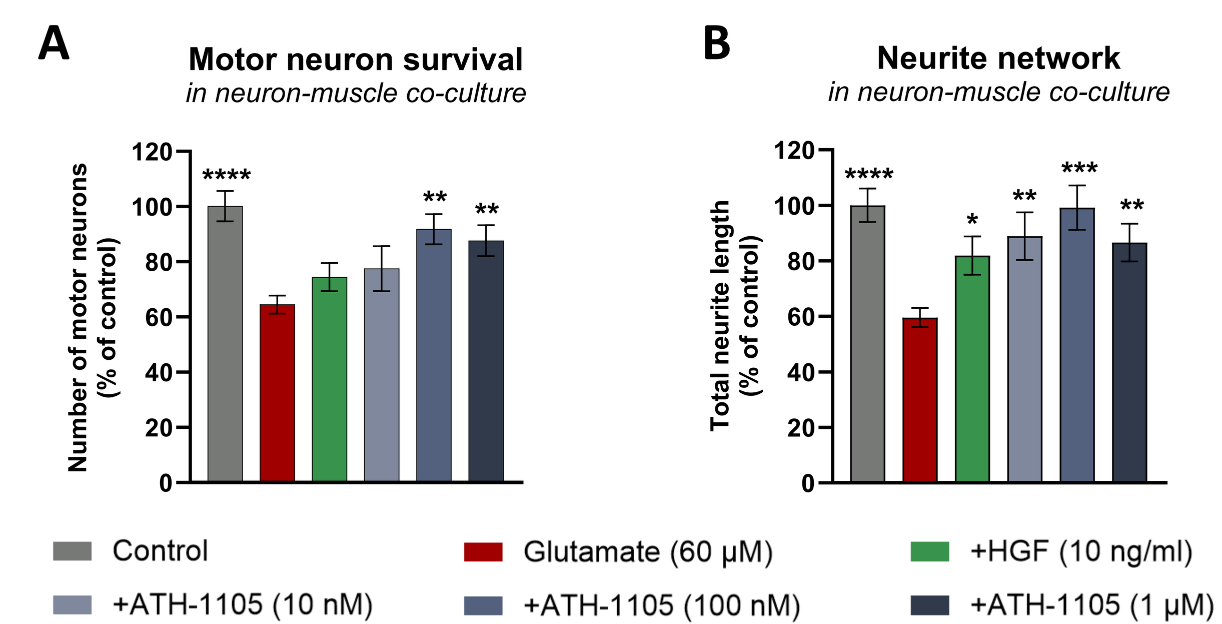
Figure 3. Neuroprotective effects of ATH-1105 against glutamate injury are abolished following siRNA-mediated knockdown of MET





(A) Representative images highlighting the effect of ATH-1105 in primary rat motor neurons challenged with glutamate in the presence of siRNA scramble (control) or siRNA MET. Scale bar = 100 µm. Quantification of (B) neuronal survival and (C) neurite network expressed as percentage of normal control (100%). HGF 5 ng/ml was included as a positive control. Data presented as mean ± SEM; n = 5-6 from 1 culture. Statistical differences were determined by one-way ANOVA followed by Fisher's LSD test versus glutamate control as indicated. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{****}p < 0.0001$

Figure 5. ATH-1105 enhances neuronal survival and preserves neurite networks following glutamate injury in human iPSC-derived SOD1^{A4V} NMJ model



Quantification of (A) neuronal survival and (B) neurite network 48h post glutamate in human iPSC-derived motor neuron - muscle co-cultures. HGF 5 ng/ml was included as a positive control. Data expressed as percentage of normal control (100%) and presented as mean ± SEM; n = 12 from 2 independent cultures. Statistical differences were determined by one-way ANOVA followed by Fisher's LSD test versus glutamate control. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$

Abbreviations: **AKT**, protein kinase B; **ALS**, Amyotrophic lateral sclerosis; **ANOVA**, analysis of variance; **ERK**, extracellular regulated kinase; **HGF**, hepatocyte growth factor; **HTRF**, homogenous time-resolved fluorescence; ; **iPSC**, induced pluripotent stem cells; **LSD**, least significant difference; **MAP-2**, microtubule-associated protein 2; **NMJ**, neuromuscular junction; **p**, phosphorylation; **pMET**, phosphorylated MET; **SEM**, standard error of the mean; **siRNA**, small interfering RNA ; **SOD1**, superoxide dismutase 1

References: **1.** Hulisz D. *Am J Manag Care.* 2018;24(15):S320-S326. **2.** Tortelli R et al. *Front Neurol.* 2020;11:552295. **3.** Ishigaki A et al. *J Neuropathol Exper Neurol.* 2007;66:1037-1044. **4.** Vallarola A et al. *Int. J. Mol. Sci.* 2020;21(22):8542. **5.** Berthiaume AA et al. *Front Neuro.* 2024;18.

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Disclosures

Sherif M. Reda, Wei Wu, Robert W. Taylor, Jewel L. Johnston, and Kevin J. Church are employees and stockholders of Athira Pharma, Inc. Andrée-Anne Berthiaume was a former employee of Athira Pharma, Inc.

Disclaimer

ATH-1105 is an investigational therapy that has not received FDA approval and has not been demonstrated to be safe or effective for any use.