Targeting neurotrophic HGF signaling for the treatment of neurodegenerative disorders

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CONCLUSIONS

1. **Positive modulation of neurotrophic HGF signaling is a promising therapeutic strategy that may be beneficial in a range of neurodegenerative disorders**
2. **ATH small molecules promote neurotrophic signaling, protect neurons, reduce protein pathology, and improve function in several models of neurodegenerative disorders**
3. **Preclinical evidence supports continued development of ATH small molecule candidates fosgonimeton, ATH-1020, and ATH-1105 for the treatment of AD, PD, and ALS**

KEY TAKEAWAY

The neurotrophic and neuroprotective effects of HGF positive modulators across diverse neurodegenerative models supports the broad therapeutic potential of this emerging class of compounds

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Abbreviations: 6-OHDA, 6-hydroxy dopamine; **AD**, Alzheimer's disease; **α-syn**, alpha synuclein; **ALS**, amyotrophic lateral sclerosis; **AM**, active metabolite; **ATF6**, activating transcription factor 6; **Aβ**, amyloid beta; **CBE**, conduritol β-epoxide; **CMAP**, compound muscle action potential; **ER**, endoplasmic reticulum; **GBA1**, glucocerebrosidase 1; **HGF**, hepatocyte growth factor; **IND**, investigational new drug; **LAMP2**, lysosomal associated membrane protein 2; **MAP2**, microtubule associate protein 2; **MPP+**, 1-methyl-4-phenylpyridinium; **NeuN**, neuronal nuclei; **PD**, Parkinson's disease; **PFF**, pre-formed fibril; **PM**, positive modulator; **SC**, subcutaneous injection; **SOD1**, superoxide dismutase 1; **TDP43**, transactive response DNA binding protein; **TH**, tyrosine hydroxylase

References: 1. Dugger and Dickson. *Cold Spring Harbor Perspect Biol.* 2017;9(7) **2.** Gontijo et al. *Current Neuropharm.* 2020;18(5):348-407 **3.** Desole et al, *Front Cell Dev Biol.* 2021;9, 683609. **4.** Johnston JL et al. *Neurotherapeutics.* 2023;20(2) 431-451.

Acknowledgments

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Disclosures

Kevin Church, Sherif Reda, Andrée-Anne Berthiaume, Sharay Setti, Kayla Kleist, Wei Wu, Robert Taylor, Jewel Johnston are employees and stockholders of Athira Pharma, Inc.

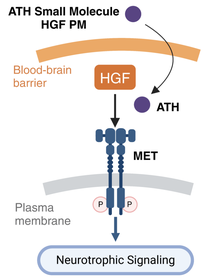
Disclaimer

Fosgonimeton, ATH-1020, and ATH-1105 are investigational therapies and have not received FDA approval nor been demonstrated to be safe or effective for any use.

INTRODUCTION

- Neurodegenerative diseases such as AD, PD, and ALS present diverse symptomology, yet they share common pathological themes, including increased neuroinflammation, protein accumulation, and oxidative, lysosomal, and mitochondrial stress ultimately leading to the death of neurons[1].
- The complex pathology involved in neurodegenerative disorders suggests that multifactorial treatment strategies may be required for disease modification[2,3].
- We have developed a series of small molecule positive modulators (PM) of the neurotrophic HGF system (ATH small molecules) capable of promoting neuroprotective and neurotrophic signaling that may counteract neurodegeneration in a range of diseases[4].

Figure 1: ATH small molecules positively modulate the neurotrophic HGF signaling system to promote neuroprotective signaling pathways to counteract neurodegenerative processes



ATH small molecule HGF PMs promote the phospho-activation of the MET receptor in the presence of endogenous HGF ligand.

ATH small molecules presented here distribute to the CNS.

ATH HGF PMs in the presented studies:
- **Fosgonimeton**
  - Prodrug delivered via SC injection rapidly converts to active metabolite fosgo-AM
- **ATH-1020**
  - Orally bioavailable
- **ATH-1105**
  - Orally bioavailable

Figure 2: ATH small molecules are under clinical investigation or development for the treatment of multiple neurodegenerative diseases

		PRECLINICAL		CLINICAL		
Program	Indication	Early Discovery	IND-Enabling Studies	Phase 1	Phase 2	Phase 3
Fosgonimeton (Prodrug administered via SC injection)	AD (LiftAD, ActAD)			Phase 2/3 Clinical Trial		Open-Label Extension
					Phase 2 Clinical Trial	Open-Label Extension
	PD (Shape-PD)			Exploratory Phase 2 Clinical Trial		
ATH-1020 (oral)	Neurodegenerative Disease			Phase 1 Clinical Trial		
ATH-1105 (oral)	ALS			IND-Enabling studies	IND Filing in 2024	

*These trials are complete with topline data reported in June 2022 and December 2023 respectively.

METHODS

In Vitro

- MET activation: HEK293 cells were treated with the indicated ATH molecule in vehicle containing a low dose of HGF (1 ng/ml). MET phosphorylation (Y1234/1235) was quantified via ELISA
- Neurotrophic activity: Primary hippocampal neurons isolated from newborn rats were treated with the indicated ATH compound, and in some cases in the presence of exogenous HGF protein for 3-4 days (neurite assay) or 8-9 days (synapse assay). Cultures were immunostained against βII-tubulin to measure neurite length and synaptobrevin II to count synapses.
- AD models: Primary cortical neurons isolated from embryonic rats were incubated with $Aβ_{1-42}$ oligomers for 24 hours and assessed for survival, accumulation of pTau, and mitochondrial stress by automated image acquisition of cultures immunostained for MAP2, AT100, and MitoSox respectively.
- PD models: Primary cultures of rat dopaminergic neurons were treated with α-synuclein PFF. Neuron survival and lysosomal stress were assessed by automated image acquisition of cultures immunostained for MAP2 and LAMP2 respectively. Neuron survival was assessed by cell titer glow.
- ALS models: Primary motor neurons with a $SOD1^{G93A}$ genotype in culture were treated with glutamate to induce excitotoxicity. Neuron survival, ER stress, and mitochondrial stress were assessed by immunostaining for MAP2, ATF6, and MitoTracker respectively.

In Vivo

- AD model: $Aβ_{1-42}$ oligomers were administered via intrahippocampal injection to induce neuron death and cognitive impairment in mice. After 7 days of treatment with fosgonimeton, cognitive performance was evaluated in the Y-maze. After 28 days of treatment, neuron survival was assessed by quantifying NeuN+ cells in the CA1 layer of the hippocampus.
- PD models: In one model, Parkinson's disease-related phenotypes were elicited by single intranigral injection of α-synuclein and CBE (lysosomal disruptor) administration 3 times per week in mice. Motor function was assessed by the ladder test after 6 weeks of treatment. α-synuclein area in dopaminergic neurons was assessed in the substantia nigra via immunostaining for tyrosine hydroxylase (TH) and α-synuclein. In another model in rats, unilateral injection of 6-OHDA was used to produce PD-related deficits. Grip strength was measured after 6 weeks of treatment.
- ALS model: Transgenic Prp-$TDP43^{A315T}$ mice were used as a model of ALS. Motor function was assessed by latency to fall in rotarod, and nerve function was assessed by CMAP-amplitude after 2 months of treatment. In a separate study, survival was assessed in animals treated with ATH-1105 from 1-5 months of age.

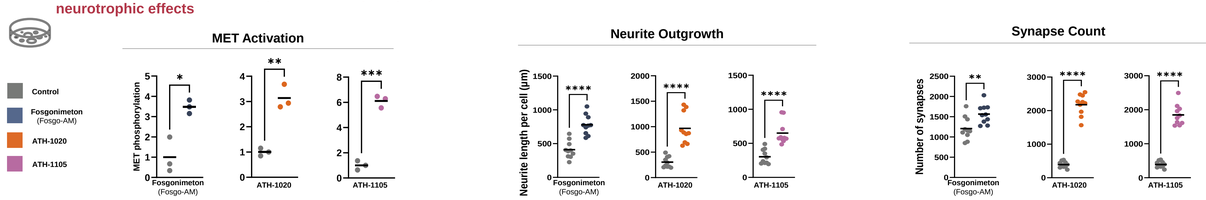
RESULTS

ATH small molecules enhance neurotrophic HGF signaling

ATH small molecule HGF PMs

Fosgonimeton	ATH-1020	ATH-1105
SC Injectable	Oral	Oral

Figure 3: ATH small molecules promote MET phosphorylation and stimulate neurotrophic effects



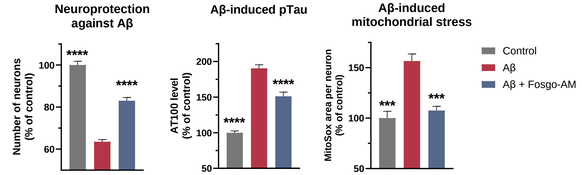
ATH small molecules enhance MET activation (phosphorylation) in HEK cells after 15 minutes of treatment. Fosgo-AM: 0.01 nM, ATH-1020: 1 nM, ATH-1105: 0.1 nM. Statistics: Student's t-test, * p< 0.05, * p<0.01, *** p< 0.001.

ATH small molecules promote extension of neurites in cultured primary hippocampal neurons after 3-4 days treatment. Fosgo-AM: 10 nM, ATH-1020: 1 nM, ATH-1105: 1 nM. Statistics: Student's t-test, * p< 0.05, * p<0.01, *** p< 0.001.

ATH small molecules promote the formation of synapses in cultured primary hippocampal neurons after 8-9 days of treatment. Fosgo-AM: 1 nM, ATH-1020: 1 nM, ATH-1105: 1 nM. Statistics: Student's t-test, * p< 0.05, * p<0.01, *** p< 0.001.

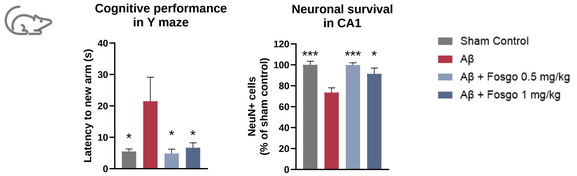
Fosgonimeton in preclinical models of AD

Figure 4: Fosgo-AM is neuroprotective and reduces pTau and mitochondrial stress in AD models in vitro



Primary rat cortical neurons in culture were incubated with $Aβ_{1-42}$ oligomers and treated with fosgo-AM (100 nM) and processed for imaging with the following assessments: Neuron survival by number of MAP2+ cells, pTau accumulation by AT100 staining, and mitochondrial stress by MitoSox.
Statistics: One-way ANOVA with Fisher's LSD, *** p< 0.001, **** p < 0.0001 vs Aβ

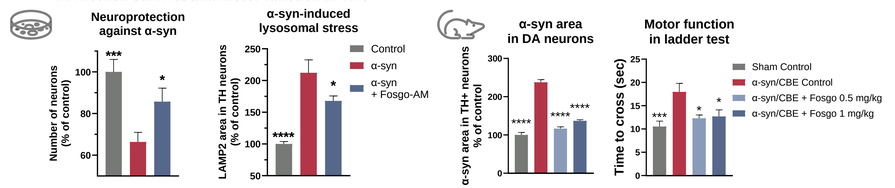
Figure 5: Fosgonimeton improves cognitive performance and protects hippocampal neurons in animals challenged with Aβ1-42



$Aβ_{1-42}$ oligomers were delivered to the hippocampus of aged (18 month) male mice as a model of AD-related pathology. Spatial memory performance was assessed by latency to enter the new arm of a Y-maze and neuronal survival was assessed by NeuN+ cells in the CA1 area of the hippocampus.
Statistics: One-way ANOVA with Dunnett's post test, * p< 0.05, *** p< 0.001 vs Aβ, outliers by Grubb's test removed from Y-maze.

ATH small molecules in preclinical models of PD

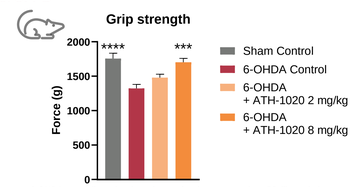
Figure 6: Fosgonimeton promotes neuron survival and reduces lysosomal stress in vitro and improves DA neuron survival and motor function in vivo



α-synuclein PFF were used to model PD-related phenotypes in primary dopaminergic neuron culture. Treatment effects of fosgo-AM (100 nM) were assessed in imaging experiments using TH+ cell counts and staining for LAMP2. Statistics: One-way ANOVA with Fisher's LSD, * p< 0.05 *** p< 0.001, **** p< 0.0001 vs α-syn

Aged (18 month) mice received a bilateral intranigral injection of α-synuclein PFF and injections of CBE 3 times a week to model PD-related phenotypes. α-synuclein area was determined in TH+ neurons in the substantia nigra. Motor behavior was assessed in the ladder test. Statistics: One-way ANOVA with Dunnett's post test, * p< 0.05, *** p< 0.001, **** p< 0.0001 vs α-syn/CBE

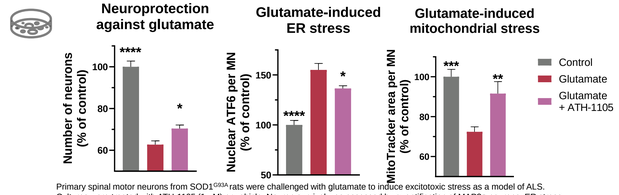
Figure 7: ATH-1020 improves grip strength in the 6-OHDA model of PD



6-OHDA was delivered to the striatum of male rats to produce PD-like pathology. Preclinical efficacy of ATH-1020 via oral gavage was assessed by measurement of grip strength. Statistics: One-way ANOVA with Dunnett's post test, *** p< 0.001, **** p< 0.0001 vs 6-OHDA

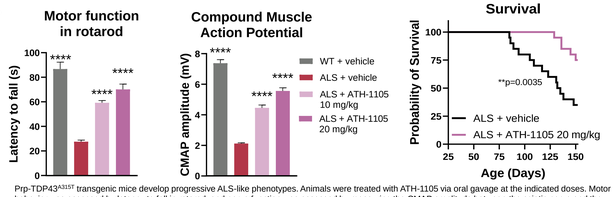
ATH-1105 in preclinical models of ALS

Figure 8: ATH-1105 promotes neuronal survival, reduces ER stress, and improves mitochondrial function following excitotoxic glutamate injury



Primary spinal motor neurons from $SOD1^{G93A}$ rats were challenged with glutamate to induce excitotoxic stress as a model of ALS. Cultures were treated with ATH-1105 (1 μM) or vehicle. Neuron survival was assessed by quantification of MAP2+ neurons. ER stress and mitochondrial stress were quantified by ATF6 and MitoTracker, respectively. Statistics: One-way ANOVA with Fisher's LSD, * p< 0.05 , *** p< 0.001, **** p < 0.0001 vs glutamate

Figure 9: ATH-1105 improves motor function, nerve function, and survival in the Prp-$TDP43^{A315T}$ transgenic mouse model of ALS.



Prp-$TDP43^{A315T}$ transgenic mice develop progressive ALS-like phenotypes. Animals were treated with ATH-1105 via oral gavage at the indicated doses. Motor behavior was assessed by latency to fall in rotarod, and nerve function was assessed by measuring the CMAP amplitude between the sciatic nerve and the muscles of the foot. Statistics: One-way ANOVA with Dunnett's post test, **** p < 0.0001 vs ALS + vehicle. In a separate experiment, survival was assessed in animals treated with ATH-1105 from 1 to 5 months of age. Statistics: Log-rank (Mantel-Cox) test.