Fosgonimeton protects against α-synuclein-mediated pathology in preclinical models of Parkinson's disease

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CONCLUSIONS

1. Fosgonimeton improves dopaminergic neuron survival, preserves neurite networks, and decreases lysosomal burden and oxidative stress following α-syn-PFF injury in vitro
2. Fosgonimeton attenuates motor function deficits in the ladder test following α-syn-injury and lysosomal function disruption in vivo
3. Fosgonimeton improves dopaminergic neuron survival and reduces α-syn aggregation and microglial activation in vivo

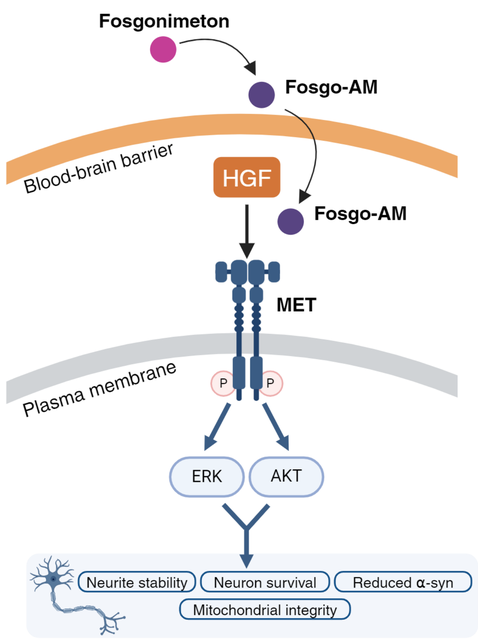
KEY TAKEAWAY

These data highlight the ability of fosgonimeton to mitigate pathological alterations associated with α-synuclein toxicity in vitro and in vivo, supporting the positive modulation of the neurotrophic HGF system as a potential treatment modality for Parkinson's disease

INTRODUCTION

- The aggregation of α-synuclein protofibrils is a major pathological hallmark of Parkinson's disease, associated with lysosomal dysfunction, oxidative stress, and dopaminergic neuron loss, ultimately resulting in motor dysfunction[1]
- Mutations in the lysosomal storage gene GBA1 are among the most common known risk factors for development of PD (affecting 8-14% of diagnosed individuals)[2]
- Positive modulation of the neurotrophic HGF system induces neuroprotective effects that can counteract oxidative stress, lysosomal dysfunction, and neuroinflammation, all of which contribute to dopaminergic neuron degeneration in PD[3-5]
- Fosgonimeton is a small-molecule positive modulator of the neurotrophic HGF system that has demonstrated neuroprotective and anti-inflammatory effects in preclinical models[6]

Figure 1. Fosgonimeton positively modulates the neurotrophic HGF system



Fosgonimeton is converted to the active metabolite (Fosgo-AM) following administration

OBJECTIVE

To assess the impact of fosgonimeton on α-synuclein-mediated pathology in preclinical PD models

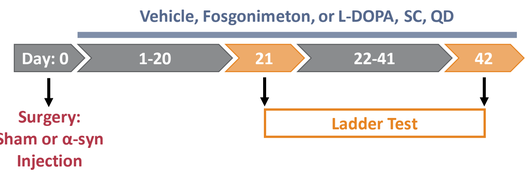
METHODS

α-synuclein dopaminergic neuron assay in vitro

- Rat primary mesencephalic neurons were treated with the active metabolite of fosgonimeton (fosgo-AM) for 20 minutes, and treated with α-synuclein preformed fibrils (α-syn PFF; 250 nM) for an additional 20 minutes. Following a washout, α-syn PFFs were re-applied for 96 hours
- After 96 hours, the cultures were immunostained to assess for dopaminergic neuron survival (number of TH positive neurons), total neurite length (TH positive neurites, µm), mitochondrial reactive oxygen species (overlapping between TH and MitoSox staining, µm²), and lysosomal dysfunction (overlapping between TH and LAMP2 staining in µm²)

α-synuclein-induced motor impairment and pathophysiology in aged mice

Experimental timeline:



- Eighteen-month-old male mice received bilateral intranigral (AP -0.3, ML ± 0.12, DV -0.45) injections of α-syn-PFF (50 µM, 2.5 µL/side) or sham surgery on day 0. α-syn mice also were administered the GBA1 inhibitor, CBE, 3 times per week (50 mg/kg, IP) from the day of surgery
- Daily subcutaneous treatment with vehicle, fosgonimeton (0.5 or 1 mg/kg, SC) or L-DOPA (20 mg/kg, SC) began on day 1
- Two separate assessments of motor function via the ladder test were conducted on days 21 and 42
- Termination took place following the final ladder test on day 42.
- Levels of DA and its metabolites HVA and DOPAC were assessed in the right striatum via LC-MS/MS methodology
- DAT levels were assessed in the left substantia nigra and striatum via western blotting methodology
- Total number of TH positive neurons, α-synuclein aggregation, and microglial activation (Iba1) were assessed in the substantia nigral area via immunofluorescence methodology

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Disclosures

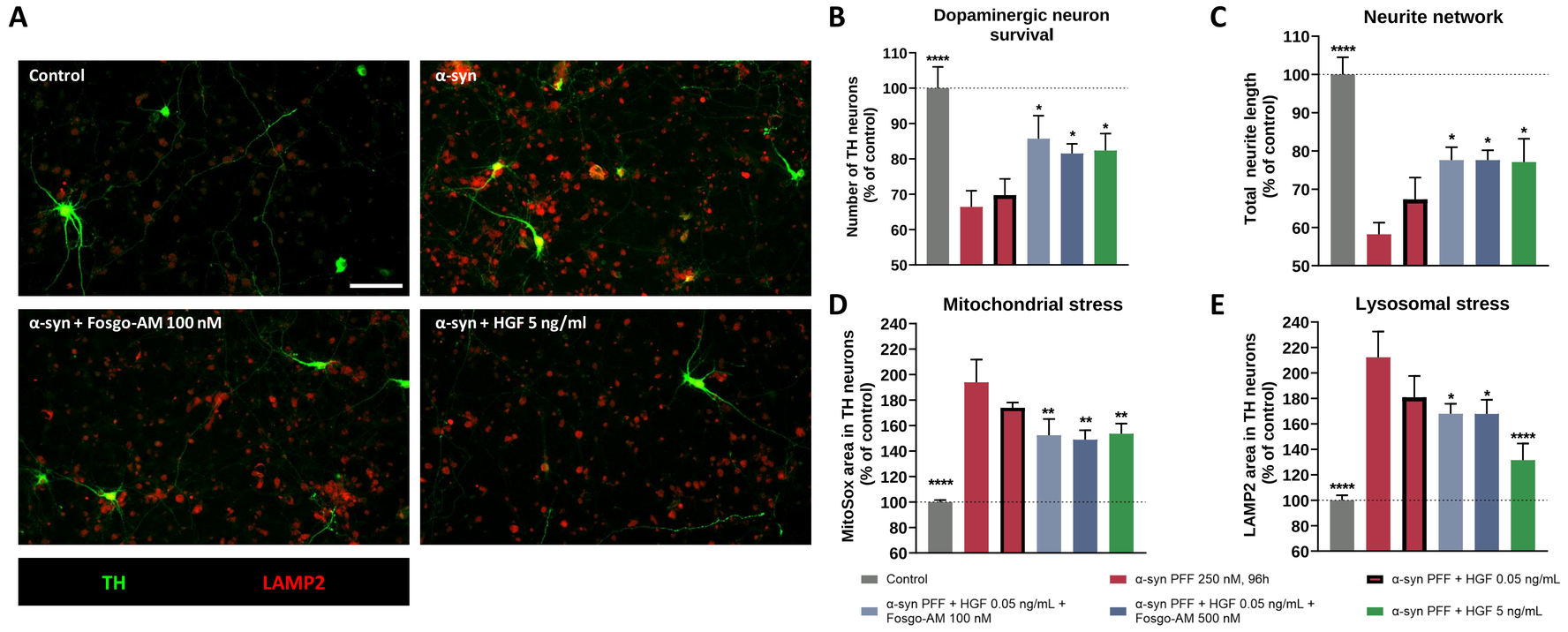
Sharay E Setti, Sherif M Reda, Andrée-Anne Berthiaume, Wei Wu, Jewel L Johnston, Robert W Taylor, Kevin J Church are employees and stockholders of Athira Pharma, Inc.

Disclaimer

Fosgonimeton is an investigational therapy that has not received FDA approval nor been demonstrated to be safe or effective for any use.

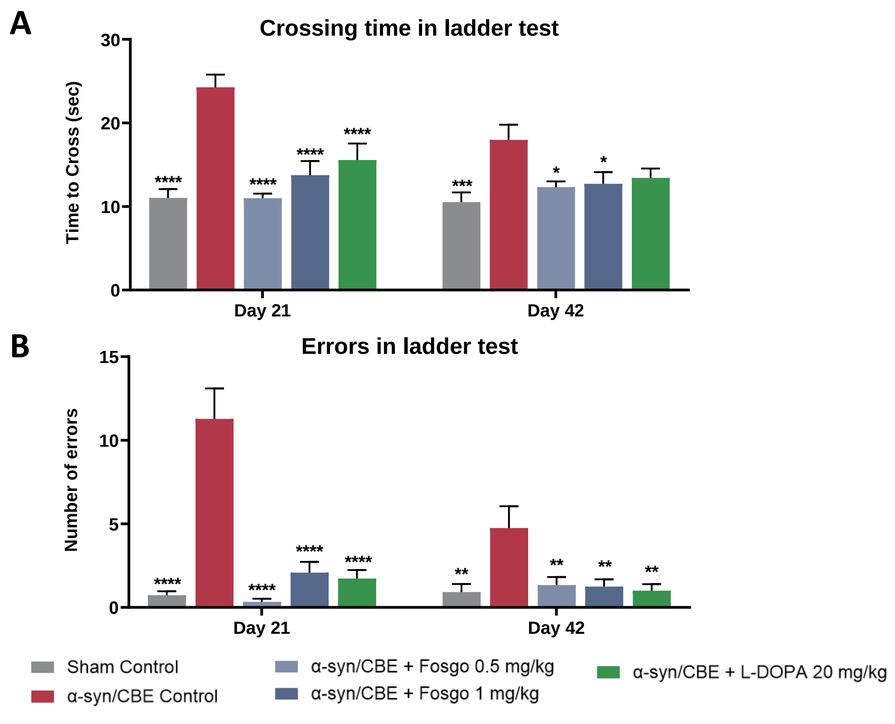
RESULTS

Figure 2. Fosgo-AM promotes neuronal survival and network connectivity, and reduces mitochondrial and lysosomal stress following α-synuclein PFF injury in primary culture of dopaminergic neurons



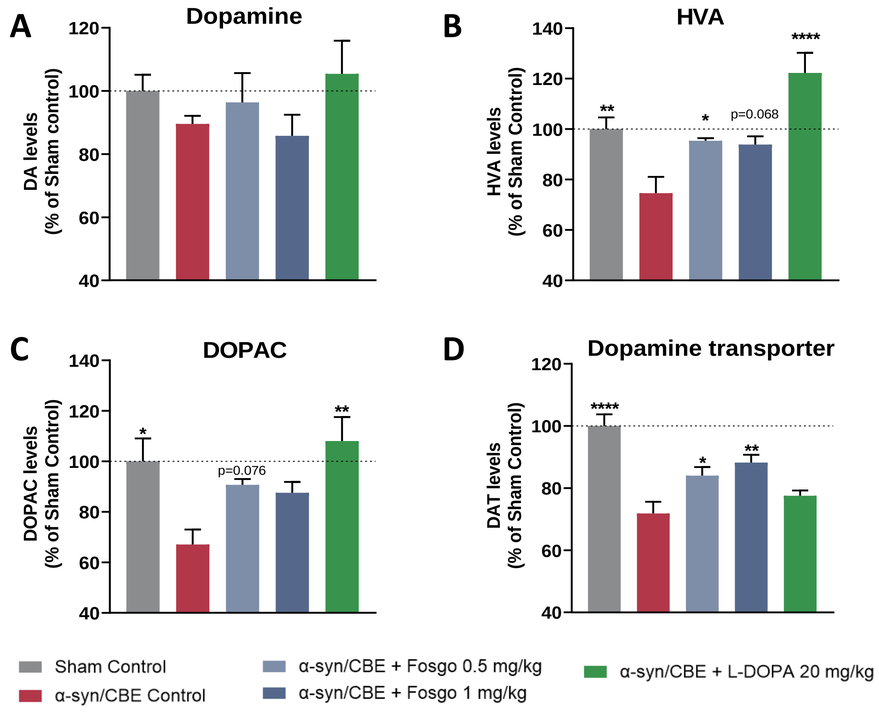
(A) Representative images highlighting the effect of α-syn PFF on TH+ neurons and LAMP2 in the presence and absence of fosgo-AM (supplemented with 0.05 ng/mL HGF). Scale bar = 100 µm. Quantification of (B) neuronal survival (i.e., number of TH+ neurons), (C) neurite network (i.e., total length of TH+ neurites in µm), (D) mitochondrial stress (overlap of TH and MitoSox area in µm²), and (E) lysosomal stress (overlap of TH and LAMP2 area in µm²) expressed as percentage of normal control (100%). Data presented as mean ± SEM; n = 5-6. Statistical differences were determined by one-way ANOVA followed by Fisher's LSD test versus α-syn control. *p <0.05, **p <0.01, ****p <0.0001

Figure 3. Fosgonimeton protects against α-synuclein-induced motor impairment in the ladder test



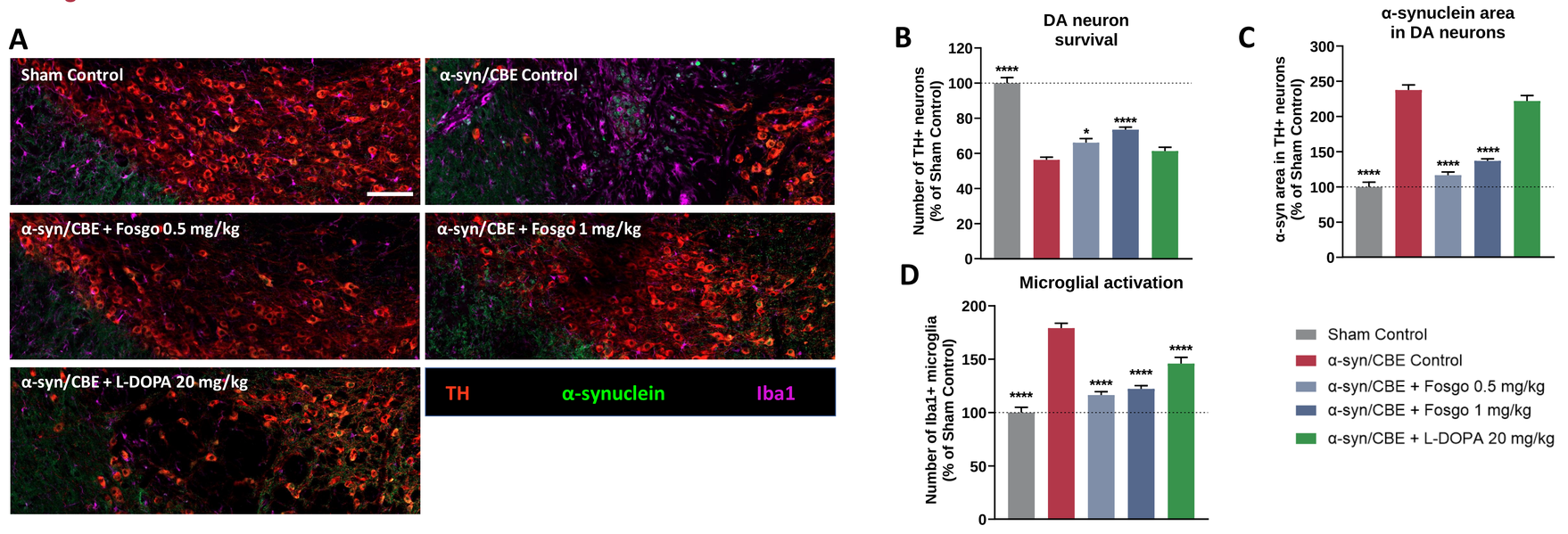
Graphical representation of motor function in the ladder test as measured by (A) time to cross in seconds and (B) number of errors, on study days 21 and 42. Data are presented as mean ± SEM; n=10-12 mice each. Statistical significance was determined via mixed-effects analysis with Dunnett's multiple comparisons test vs α-syn/CBE + Vehicle. *p <0.05; **p <0.01; ***p <0.001; ****p <0.0001

Figure 4. Fosgonimeton mitigates α-synuclein-induced deficits in nigrostriatal dopaminergic signaling components



Graphical representation of nigrostriatal level of (A) DA, (B) HVA, (C) DOPAC, as measured by LC/MS, and (D) DAT, as measured by Western blot. Data are presented as mean ± SEM; n=4-5 mice each. Statistical significance was determined via one-way ANOVA with Dunnett's multiple comparisons test vs α-syn/CBE + Vehicle. *p <0.05; **p <0.01; ****p <0.0001

Figure 5. Fosgonimeton protects against α-synuclein induced dopaminergic neuron loss, α-synuclein aggregation, and microglial activation



(A) Representative images of dopaminergic neurons (TH, in red), α-synuclein (green), microglial activation (Iba-1, in purple) Scale bar = 100 µM. Graphical representation of (B) DA neuron survival, (C) α-synuclein area in DA neurons, and (D) microglial activation. Data are presented as mean ± SEM; n=6-7 mice each. Statistical significance was determined via one-way ANOVA with Dunnett's multiple comparisons test vs α-syn/CBE + Vehicle. *p <0.05; ****p <0.0001

References: **1.** Mhyre et. al. (2012). *Subcell Biochem*, 65, 389-455. **2.** Clark et. Al. (2013). *Neurology,* 69, 1270-1277. **3.** Matsumoto K et al.(2014). Biomedicines, 2(4):275-300. **4.** Maina et. al. (1999) Nat Neurosci. 1999;2(3):213-217. **5.** Kitamura et al. (2019). Int J. Mol. Sci, 20(5), 1054. **6.** Johnston JL et al. *Neurotherapeutics*. 2023;20(2):431-451

Abbreviations: **AKT**, protein kinase B; **ANOVA**, analysis of variance; **α-syn**, alpha-synuclein, **CBE**, conduritol B epoxide; **DA**, dopamine; **DAT**, dopamine transporter; **DOPAC**, 3,4-dihydroxyphenylacetic acid; **ERK**, extracellular signal-regulated kinase; **fosgo-AM**, active metabolite of fosgonimeton; **GBA1**, glucocerebrocidase-1; **HGF**, hepatocyte growth factor; **HVA**, homovanillic acid; **LAMP2**, lysosomal-associated membrane protein 2; **LC-MS/MS**, liquid chromatography with tandem mass spectrometry; **L-DOPA**, levodopa; **PD**, Parkinson's disease; **PFF**, preformed protofibrils **SEM**, standard error of the mean; **TH**, tyrosine hydroxylase.