SMALL MOLECULE INHIBITORS FOR PRECISE INHIBITION OF A-SYNUCLEIN OLIGOMER GENERATION IN PARKINSON'S DISEASE

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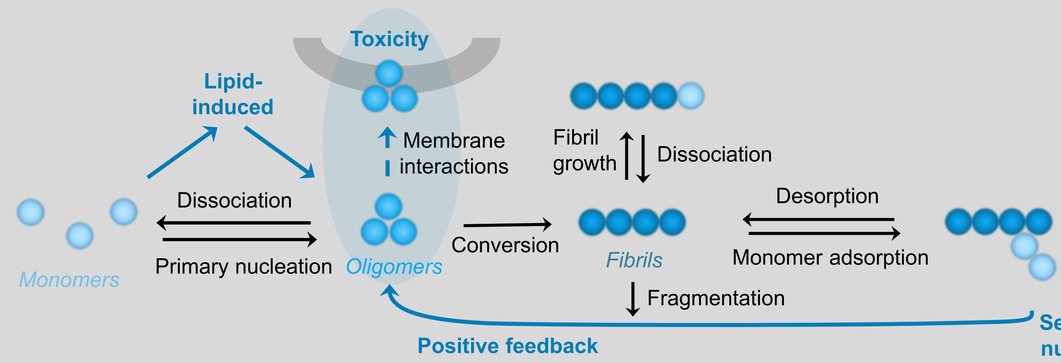
Background and Objective

- Oligomeric forms of alpha-synuclein (αS) underlie the onset and progression of Parkinson's Disease (PD)
- Oligomers bind to membranes, receptors and organelles, disrupt metabolic and neuronal functional pathways and ultimately cause neuronal death
- A significant reduction in oligomers is expected to halt disease progression
- Here, we present a platform for the discovery and development of inhibitors of the key processes generating toxic αS oligomers

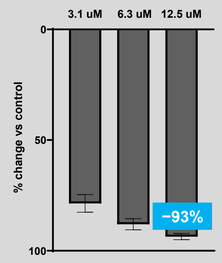


WTX-A exhibits dual pharmacology and excellent PK properties

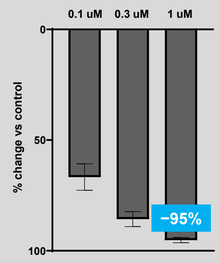
Precision inhibition of key oligomer-generation mechanisms

- WTX-A inhibits oligomers generation via both lipid-induced and secondary nucleation processes
- Inhibition is specific for αS; no inhibition was observed in tau and Aβ42 amyloid aggregation assays

Primary/Lipid-induced nucleation



Secondary nucleation



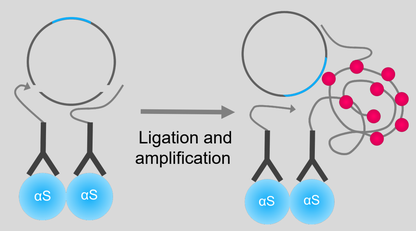
Methods: 20 or 10 μM αS monomer incubated with 100 μM DMPS (pH 6.5, 30 °C) or 0.25% fibril seeds (pH 4.8, 37 °C). Aggregation was monitored using Thioflavin-T fluorescence.

		WTX-A
Primary/lipid-induced nucleation K_D (nM)		850
Secondary nucleation K_D (nM)		50
Pharmacokinetics (mouse, 10 mg/kg)	CSF C_{max} (nM)	840
	Bioavailability (%)	119
	Clearance (ml/min/kg)	0.04

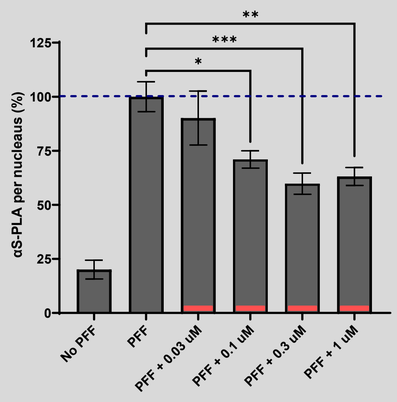
WTX-A targets the core mechanisms with good potency

Oligomer reduction in iPSC Dopaminergic Neurons (seeded)

Proximity Ligation Assay (PLA)

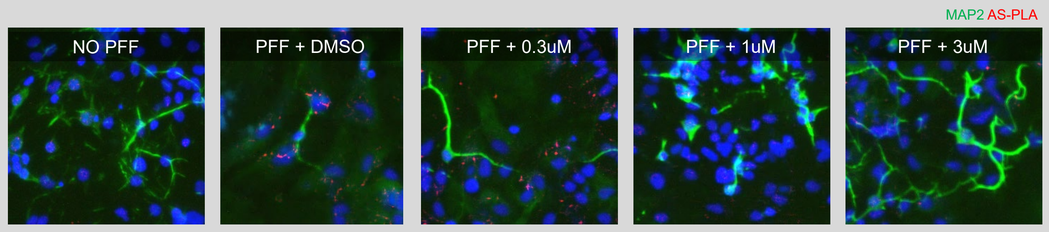


PLA-conjugated αS antibodies Detection by DNA probe

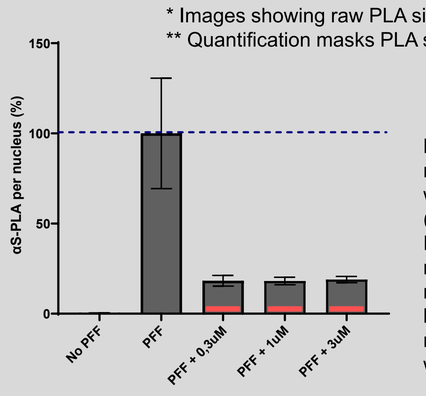


Methods: WT iPSC-derived dopaminergic neurons seeded with 10 μg/ml WT pre-formed fibrils (PFF). Data represent the mean ±SD. * P<0.02, ** P<0.002, *** P<0.0008 versus vehicle-treated. Ordinary one-way ANOVA, Dunnett's multiple comparison test. Data relating to WTX-A marked with orange.

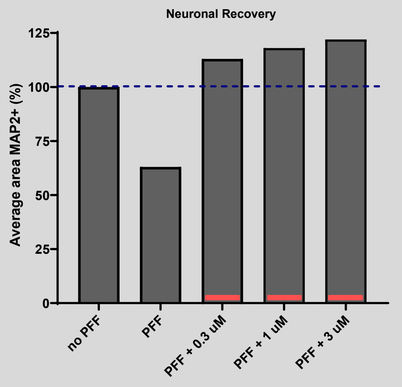
Potent Oligomer Reduction in Mouse Primary Neurons (seeded)



* Images showing raw PLA signal without mask
** Quantification masks PLA signal overlapping with nuclei



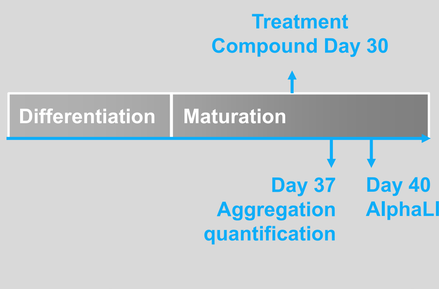
Methods: Mouse primary neurons (MPN) seeded with 10 μg/ml mouse PFFs (left; technical variability on FOVs). Data represent the mean ±SEM. Orthogonal readout: neuronal recovery by MAP2 area (right). Data relating to WTX-A marked with orange.

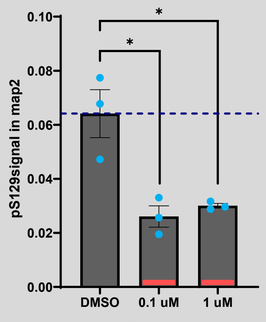


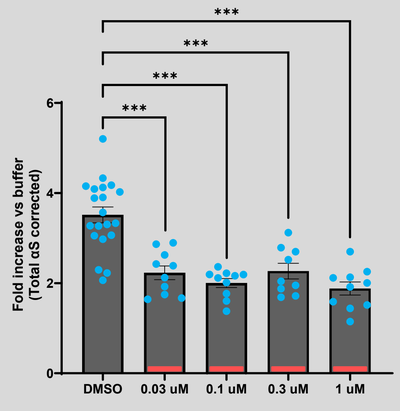
- Cell potency <300 nM total drug concentration in both MPNs and iPSCs
- Estimated <1 nM free drug

WTX-A shows efficacy in a range of biological systems

Decrease in aggregation in SNCA triplication iPSC-derived cortical neurons



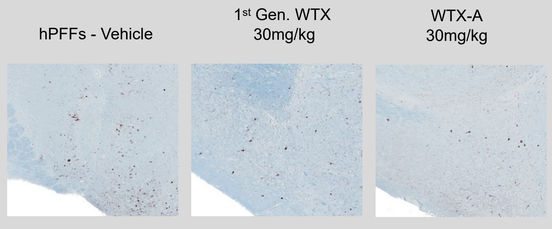


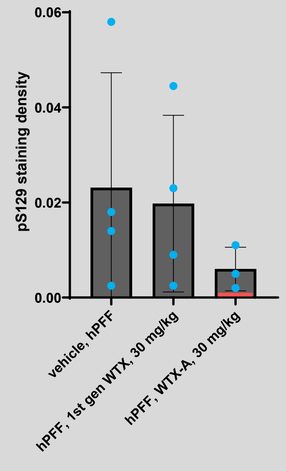


Methods: Immunocytochemistry staining pS129 aggregate signal in MAP2 (above). Separate orthogonal AlphaLISA measures total aggregated αS (right), Data represent the mean ±SD. * P<0.05, *** P<0.001 versus vehicle-treated. Ordinary one-way ANOVA, Dunnett's multiple comparison test. Data relating to WTX-A marked with orange.

WTX-A delivers efficacy in M83 mouse model

Reduces pS129 αS aggregates ~70% (seeded)





Methods: human WT αS transgenic mice, double bilateral inoculation with human A53T mutant αS PFF into striatum and cortex. pS129 αS aggregate readout in AON region. Data relating to WTX-A marked with orange.

Conclusions

We are developing disease-modifying small molecules that inhibit the source of oligomer and aggregate generation, and preparing to initiate a biomarker-driven clinical development program with the initial trials in PD.