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

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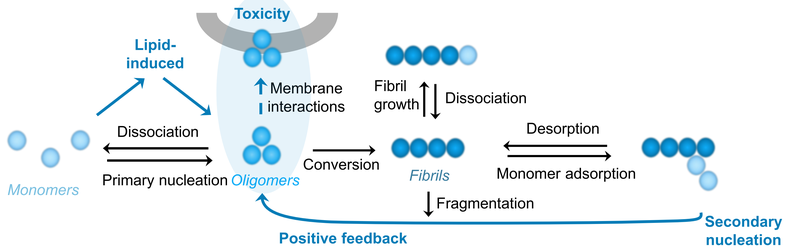
Background and Objective

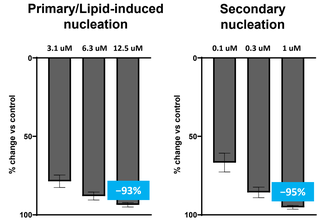
- Oligomeric forms of alpha-synuclein (αS) underlie the onset and progression of Lewy body diseases such as Parkinson's Disease (PD)
- Oligomers bind to membranes, receptors and organelles, resulting in disruption of metabolic and neuronal functional pathways that ultimately lead to neuronal death
- A significant reduction in oligomers is expected to halt disease progression
- We have used a biophysics technology platform for the discovery of small molecule inhibitors of the key source processes generating toxic αS oligomers at the source of the aS aggregation pathway
- WTX-607 is a clinical candidate for the treatment of PD and DLB



WTX-607 exhibits dual pharmacology and excellent PK properties

Precision inhibition of key oligomer-generation mechanisms

- WTX-607 inhibits oligomer 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

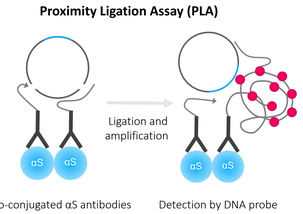


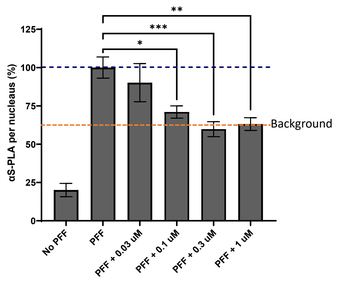
Methods: 20 or 10 μM αS monomer incubated with 100 μM DMPS (pH 6.5, 30 °C) or 0.25% fibril seeds (pH 4.8, 37 °C). Aggregation was monitored using Thioflavin-T fluorescence.

		WTX-607
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-607 targets the source mechanism of aggregation pathway with high potency and efficacy

Oligomer reduction in iPSC Dopaminergic Neurons (seeded)

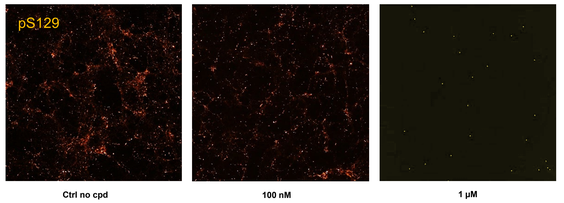


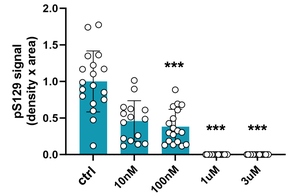


Methods: WT iPSC-derived dopaminergic neurons seeded with 10 μg/ml WT human pre-formed fibrils (PFFs). Data represent the mean ± SD. * P<0.02, ** P<0.002, *** P<0.0008 versus vehicle-treated. One-way ANOVA, Dunnett's multiple comparison test.

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

Complete pS129 Aggregate Reduction in Mouse Primary Neurons (seeded)





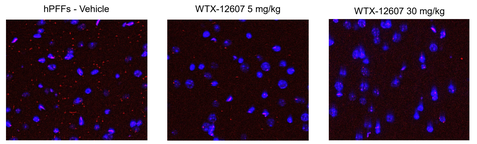
Methods: Mouse primary neurons (MPN) treated with 200 nM WT human PFFs for 8 days.

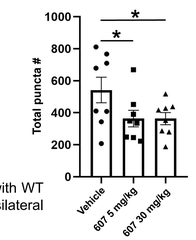
*** P<0.001 vs Veh control, 2-way ANOVA (Dunnett's posthoc)

N=6 technical replicates x 3 independent experiments

WTX-607 delivers efficacy in M83 mouse model

Significant reduction of αS oligomer PLA signal

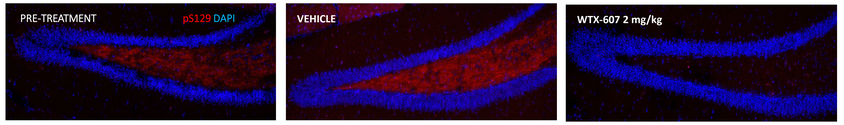


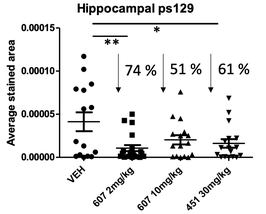


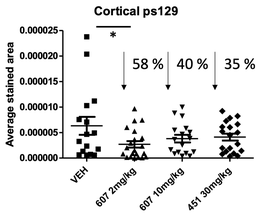
Methods: human A53T αS transgenic mice, unilateral double inoculation with WT human αS PFF into striatum and cortex. α-syn oligomer readout in ipsilateral piriform cortex measured by proximity ligation assay (PLA).

- Background PLA signal also observed in WT non-transgenic mice
- Seeding regime is among the most aggressive.

WTX-607 significantly reduces pS129 aggregates in line 61 Tg mouse model







Methods: Line 61 (Thy-1) mice over-expressing human αS were matured for 3 months with established pathology prior to treatment. Mice were treated with drug WTX-607 and first-gen WTX-11451 via daily oral gavage from week 12 through week 23.

** P<0.01; * P<0.05
One-way ANOVA, Bonferroni

Conclusions

WTX-607 is a small molecule clinical candidate for the treatment of PD and DLB that inhibits the source of α-syn oligomer and aggregate generation, with the potential be a disease-modifying therapeutic. Preparations are underway to initiate a biomarker-driven clinical development later this year.