REDUCING TOXIC ALPHA-SYNUCLEIN OLIGOMERS IN PD THROUGH PRECISE TARGETING OF THE MOLECULAR MECHANISMS OF OLIGOMER FORMATION WITH SMALL MOLECULE INHIBITORS

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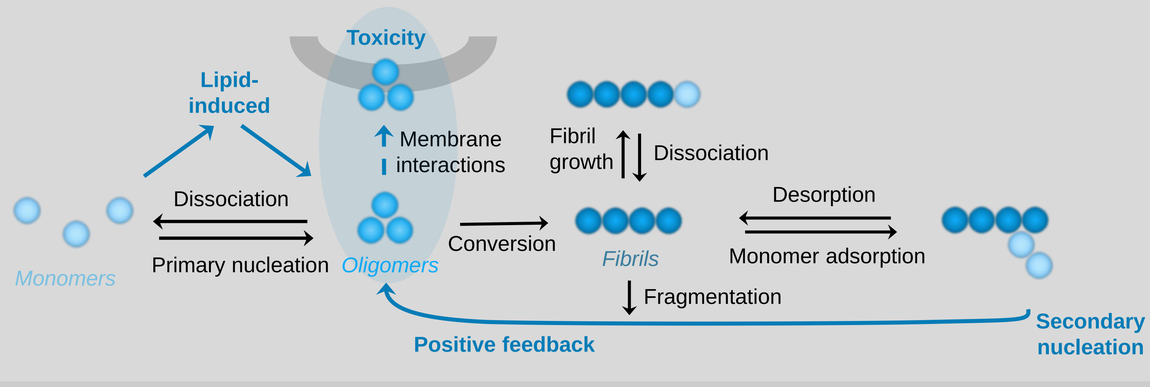
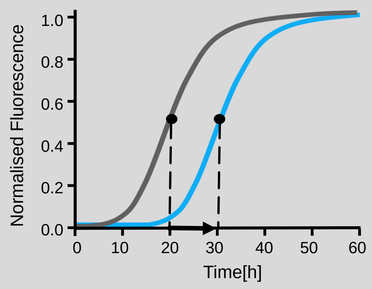
Background and Objective

- 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
- Here, we present a platform for the discovery and development of inhibitors of the key processes generating toxic αS oligomers



WTX-A inhibits the generation of oligomers through the precise targeting of lipid-induced and secondary nucleation processes

KIC_{50}: [Molecule] to cause 50% delay in aggregation

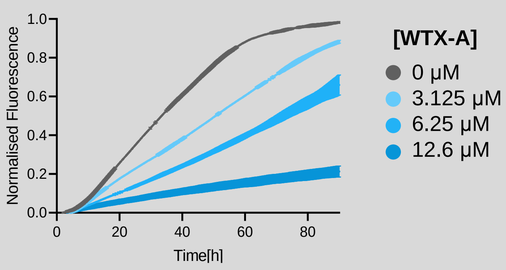


KIC_{50} = Quantitative comparison between molecules

- WTX-A developed as a MedChem prototype compound
- Inhibition is specific for αS– no inhibition was observed in tau and Aβ42 amyloid aggregation assays
- Compound potency has been optimised *in vitro* using KIC_{50} values extracted from aggregation kinetics curves

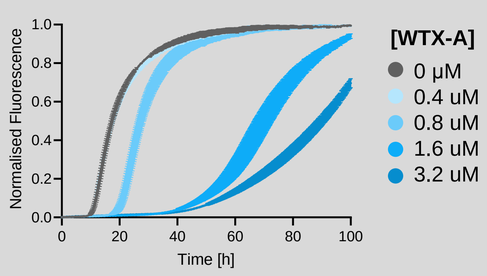
Lipid induced – kinetic assay

KIC_{50} = 14.5 μM



Secondary nucleation – kinetic assay

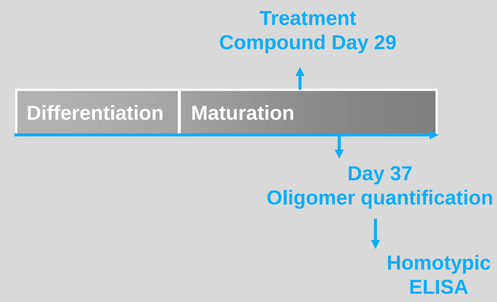
KIC_{50} = 1.3 μM

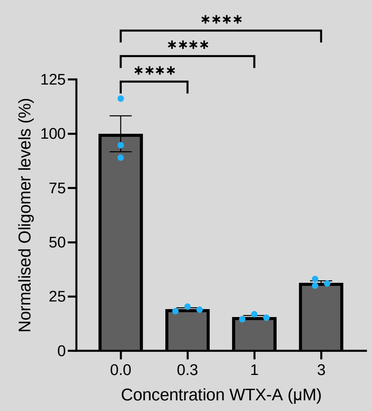


Methods: 20 or 10 μM αS monomer incubated with 100 μM DMPS (pH 6.5, 30 °C) or 0.25% fibril seeds (pH 4.8, 37 °C). Aggregation was monitored using Thioflavin-T fluorescence.

WTX-A *in vitro* potency translates into efficacy in a range of biological systems

Decrease in oligomer levels in WT iPSC-derived dopaminergic neurons

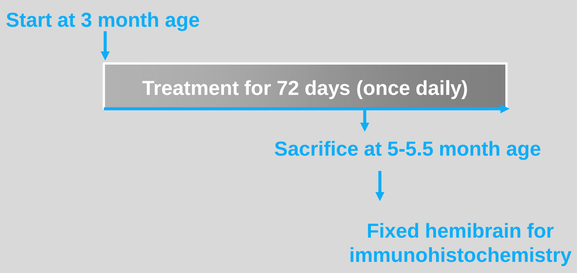


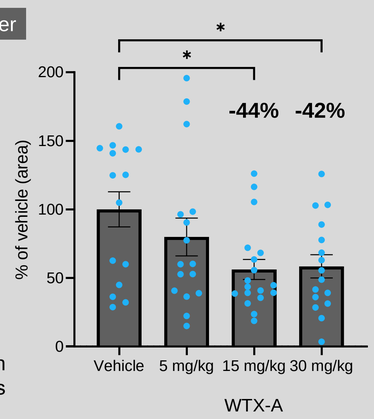


Methods: Homotypic ELISA is a proprietary assay developed to detect specifically oligomeric, but not monomeric and fibrillar, species. These results have been validated with orthogonal methods, including Super Resolution microscopy. Data represent the mean ±SD. **** P<0.0001 versus vehicle-treated. Ordinary one-way ANOVA, Dunnett's multiple comparison test.

Decrease in ProK resistant phosphorylated αS aggregates in Line 61 transgenic mice

Overexpress human WT αS under the regulatory control of the Thy-1 promoter



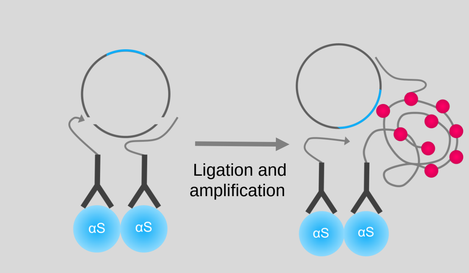


Methods: Immunohistochemistry quantification. Data represent the mean ±SEM. * P<0.05 versus vehicle-treated. Ordinary one-way ANOVA, Dunnett's multiple comparison test. (in collaboration with Eisai)

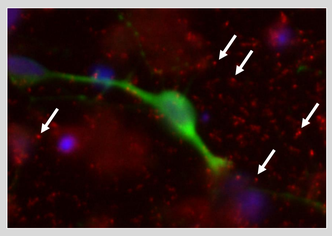
2nd generation inhibitors target core mechanisms, with greater potency and improved PK properties

	WTX-A	WTX-B
KIC_{50}		
Secondary nucleation (μM)	1.3	**0.314**
Lipid induced (μM)	14.5	**13.5**
Pharmacokinetics (10 mpk mouse)		
CSF Cmax (μM)	0.41	**0.84**
Clearance (ml/min/kg)	0.09	**0.04**

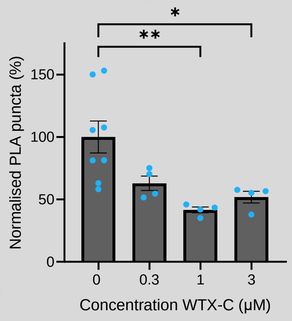
Implementation of αS proximity ligation assay to validate 2nd generation molecules



PLA-conjugated αS antibodies | Detection by DNA probe



Presence of puncta (red) indicates αS oligomerization



Methods: WT iPSC-derived dopaminergic neurons. Data represent the mean ±SD. * P<0.05 ** P<0.01 versus vehicle-treated. Ordinary one-way ANOVA, Dunnett's multiple comparison test.

Conclusions

We are developing a new generation of small molecules that target the source of αS oligomer and aggregate generation, with a biomarker-driven development program for the treatment of the α-synucleinopathies: PD, DLB, and MSA.