Discovery of therapeutic small molecules targeting alpha-synuclein aggregation

Elpida Tsika, PhD | AD/PD™ | 31 March 2023

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Conflict of interest disclosure

Employee of AC Immune SA entitled to stock options.

Parkinson’s disease

Pathological deposition of alpha-synuclein

Most common neurodegenerative movement disorder
Affects ~1% of the population over 65 years

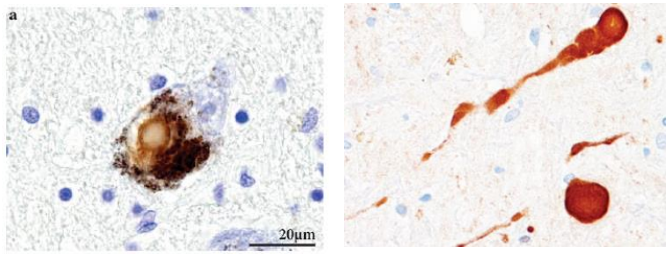
Etiology
5-10% genetic, 90-95% idiopathic, unknown cause

Cardinal motor symptoms
Tremor, rigidity, bradykinesia

Common non-motor symptoms
Sleep disorder, depression, cognitive impairment

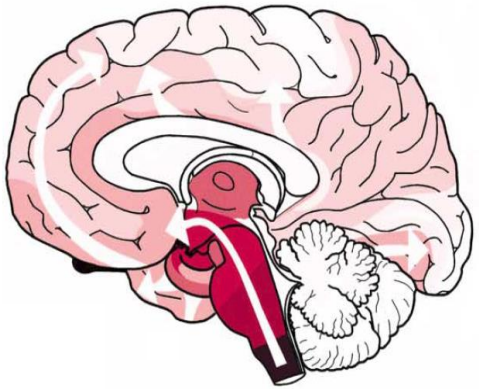
Pathological hallmarks
Neuron loss, alpha-synuclein aggregates – Lewy bodies

Main component of Lewy bodies: Alpha-synuclein



Halliday et al. 2011

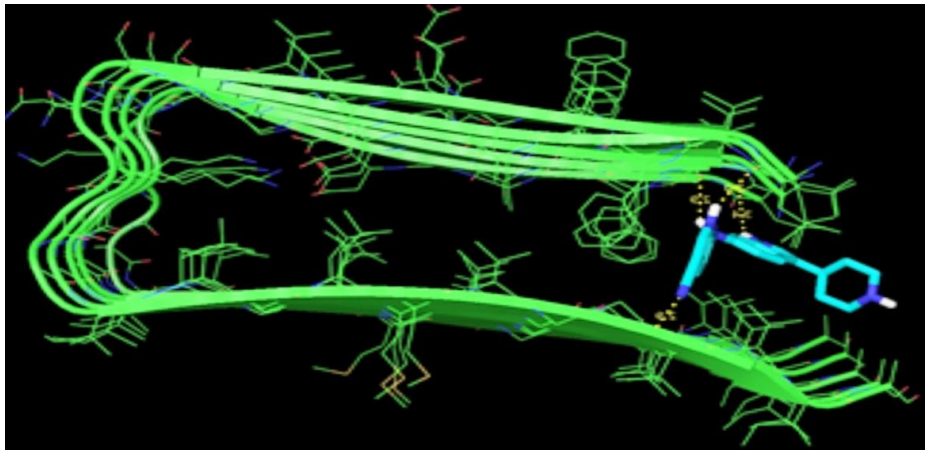
Progression of pathology



Braak et al. 2003

Proprietary Morphomer® platform

Targeting alpha-synuclein aggregation with small molecules



- Robust library of conformation-specific, non-peptidic small molecules with desirable CNS[1] properties constructed and continually refined and expanded over many years
- Comprehensive screening, rational design and early validation processes rapidly generate highly specific hit compounds
- Clinically validated with two diagnostic and one therapeutic candidate in clinical development

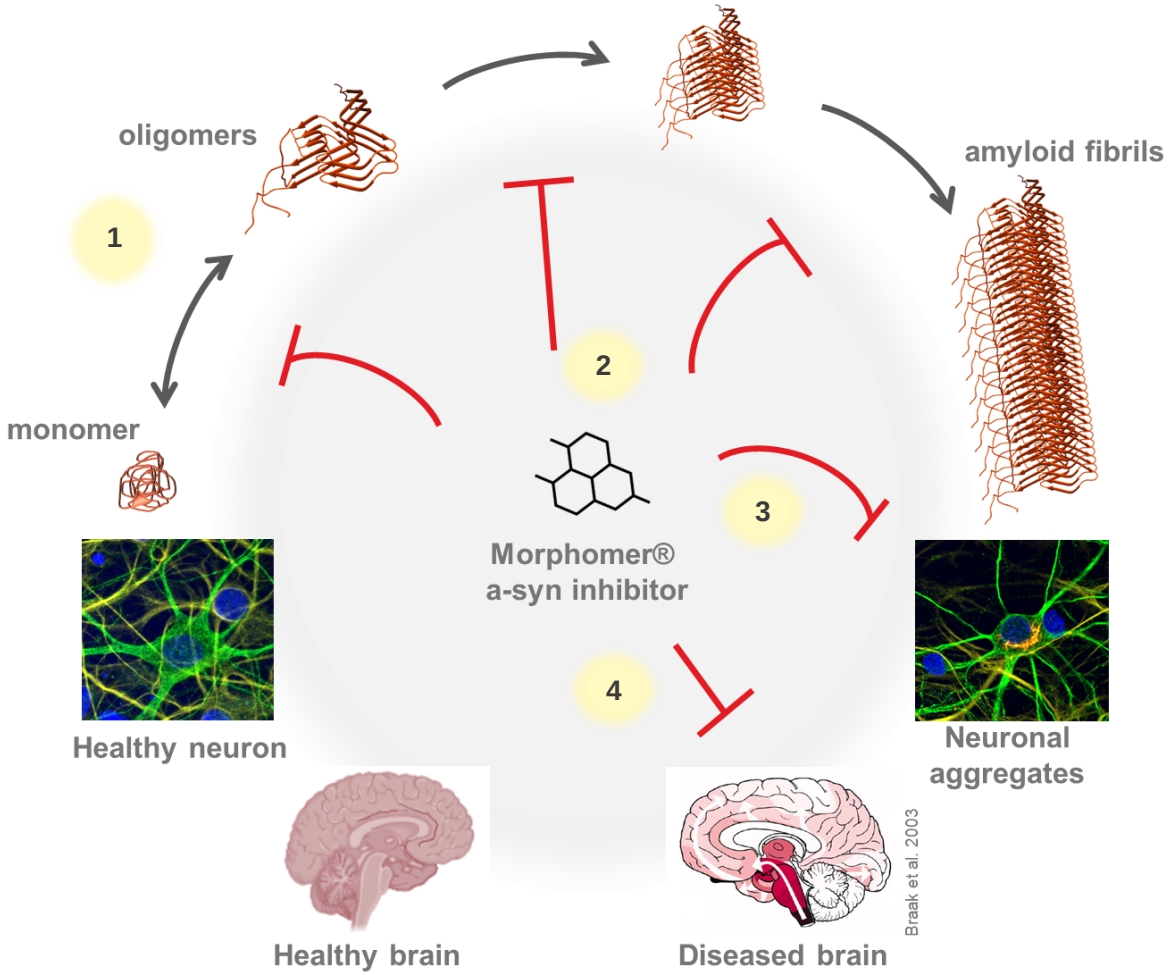
(1) CNS: Central Nervous System

Discovering inhibitors of alpha-synuclein (a-syn) aggregation

Morphomers® target intracellular pathological aggregates and intraneuronal spreading

1 A-syn aggregation process

2 Morphomer® a-syn mode of action

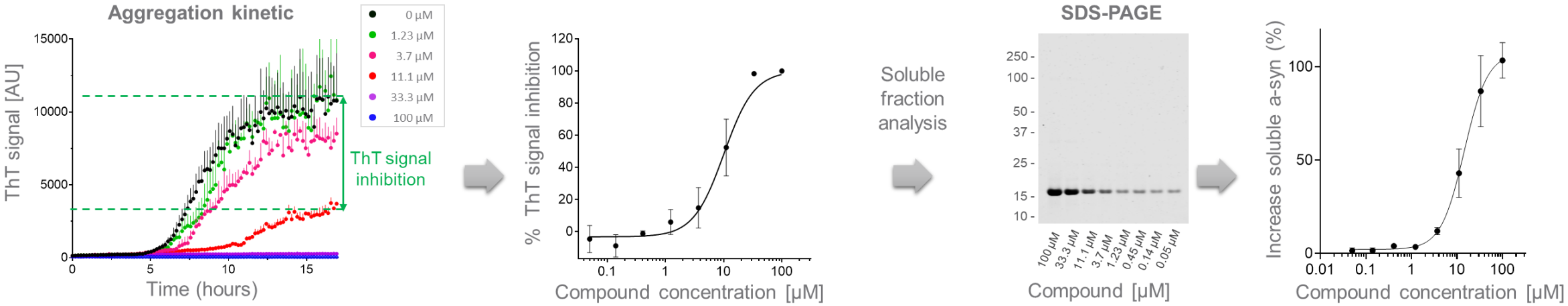


3 Stop accumulation of pathological a-syn

4 Slow down disease progression

Inhibition of a-syn aggregation *in vitro*

Thioflavin T monitored β-sheet content and conversion to insoluble aggregates



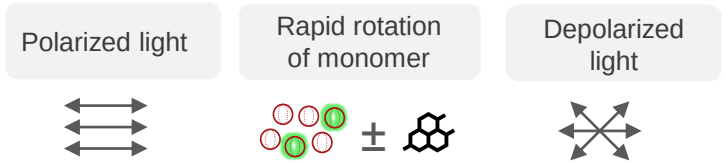
Ref: Tsika et al. MDS 2022

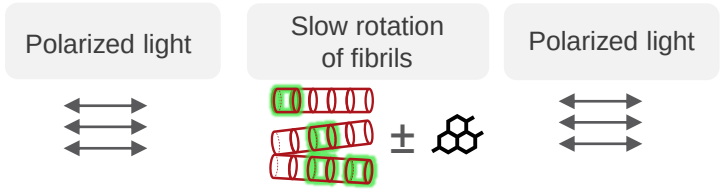
Morphomer® compounds:

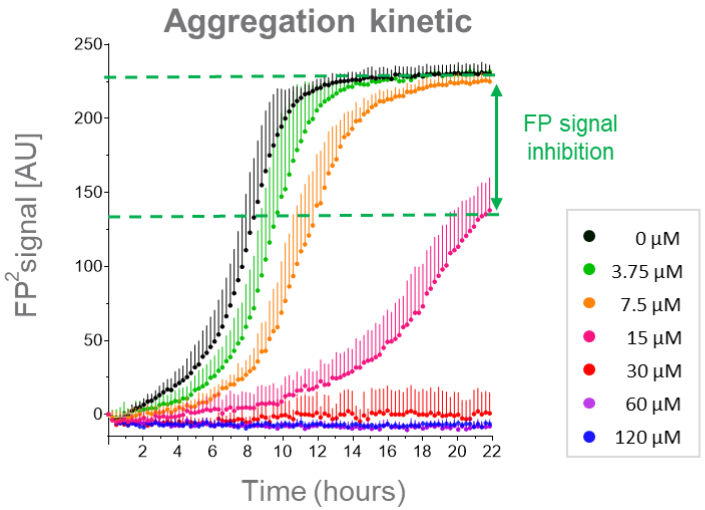
- inhibit formation of β-sheet-rich structures, monitored by Thioflavin T
- prevent the conversion of a-syn into fibrillar, insoluble conformations, shown by sedimentation analysis

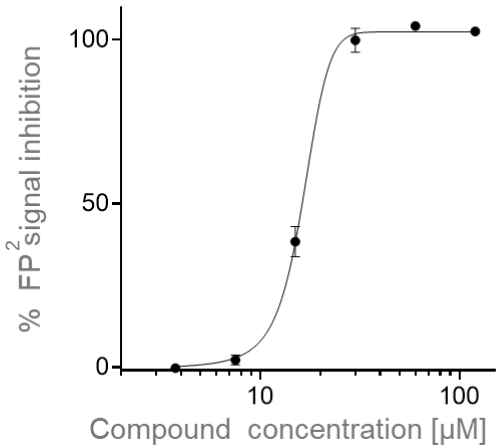
Inhibition of a-syn aggregation *in vitro*

Fluorescence polarization-monitored effects on size and conversion to insoluble aggregates

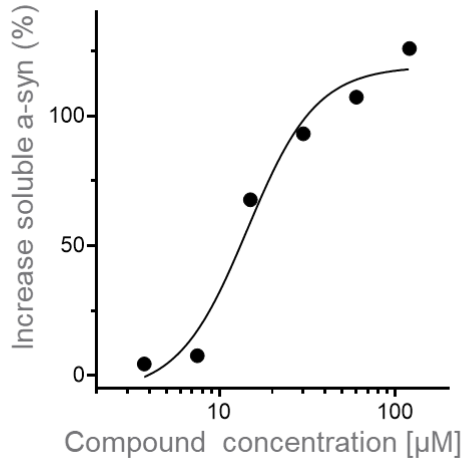








Soluble fraction analysis



Ref: Tsika et al. MDS 2022

Morphomer® compounds:

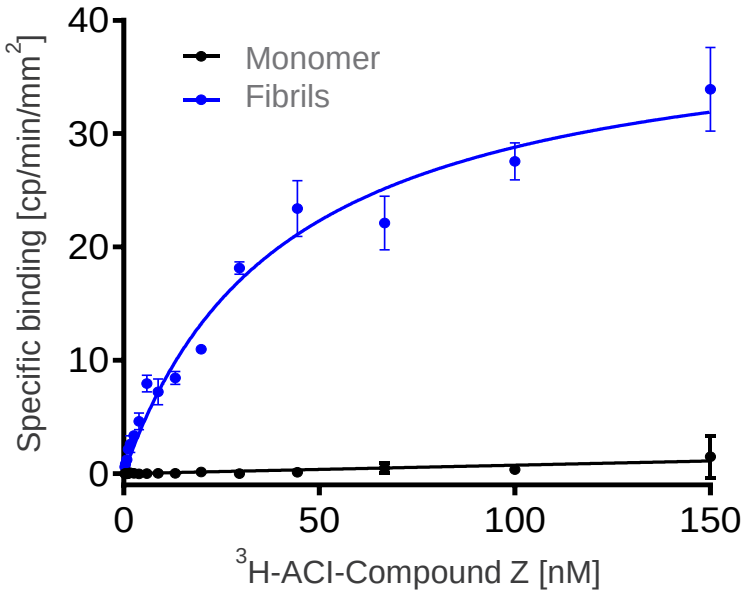
- inhibit a-syn aggregation into high molecular weight species, monitored by fluorescence polarization
- prevent the conversion of a-syn into fibrillar, insoluble conformations, shown by sedimentation analysis

(1) Fluorescence polarization

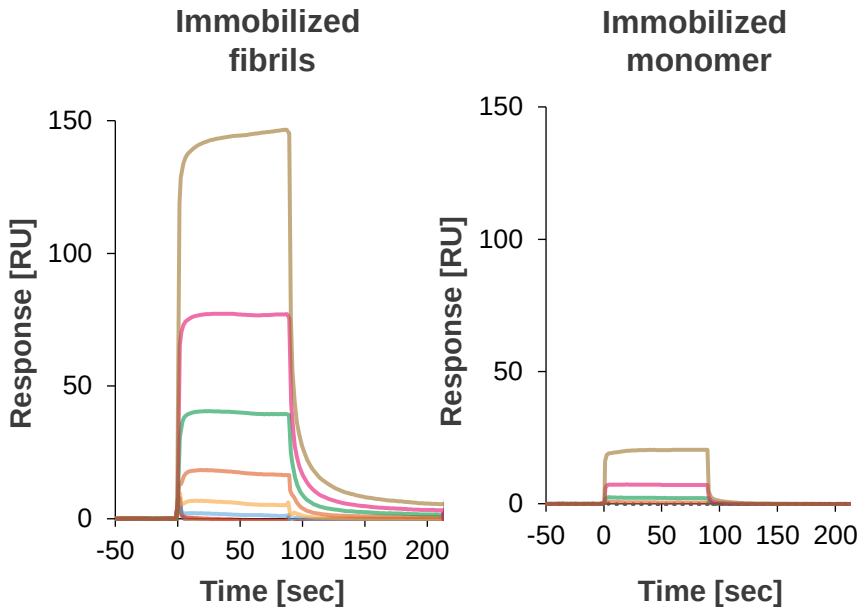
Aggregate binding specificity

Recombinant a-syn monomer vs fibrils

Compound Z



Compound Z



Direct immobilization of a-syn monomer or fibrils to the SPR sensor chip

Ref: Ait-Bouziad et al., Synuclein 2022

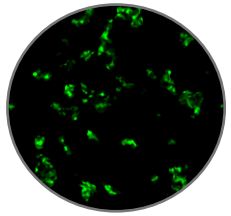
Morphomer® compound Z, shown as example, demonstrates specific binding to aggregated a-syn:

- Radiobinding assay on a-syn fibrils vs a-syn monomer
- Label-free binding assessment by SPR[1]

(1) Surface plasmon resonance

Inhibition of a-syn aggregation in cells

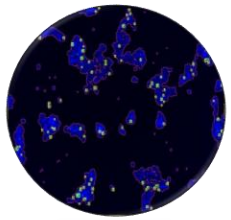
PFF[1]-seeded HEK[2] cells overexpressing human a-syn with eGFP[3] reporter



HEK a-syn-eGFP

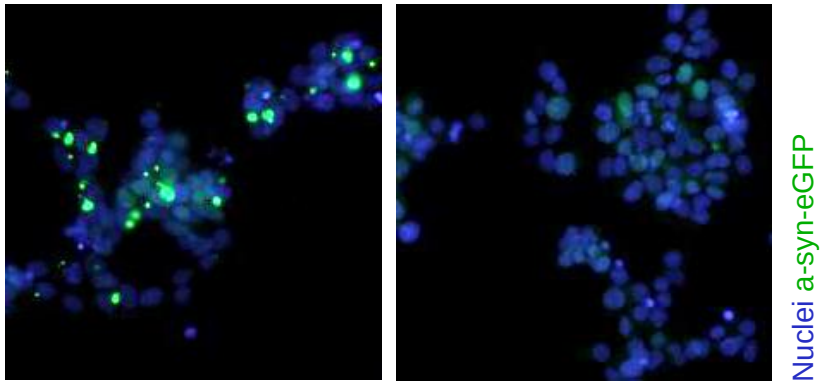
+/- compound treatment

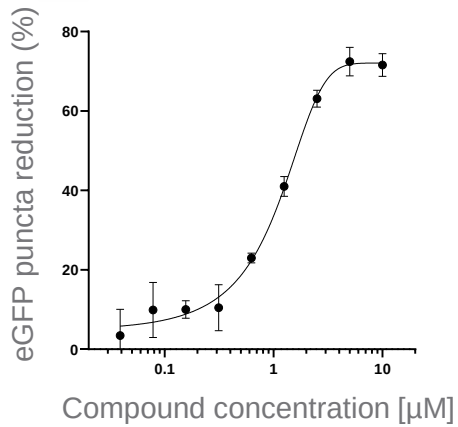
+ human a-syn preformed fibrils (PFF)



Fixation

High content imaging





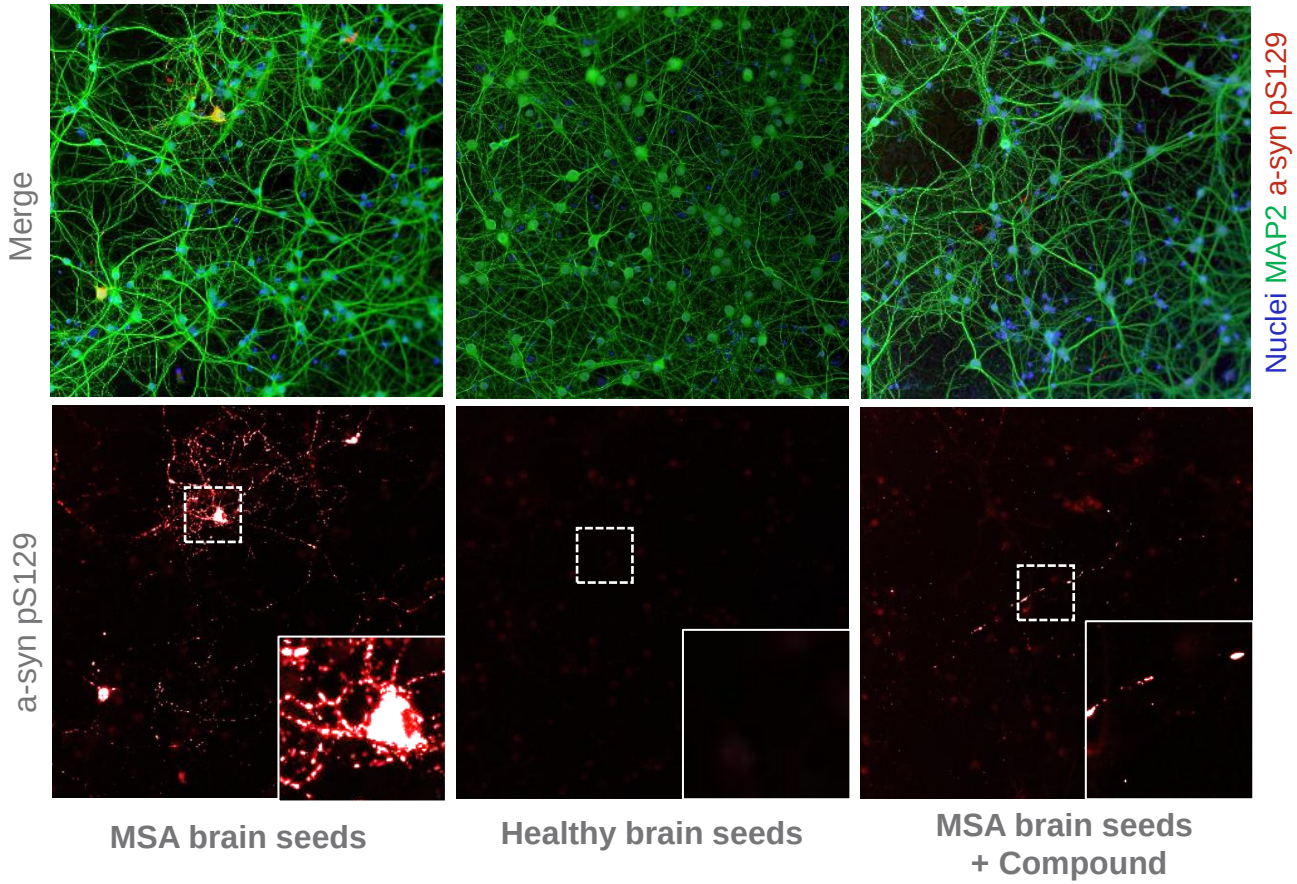
Ref: Tsika et al. MDS 2022

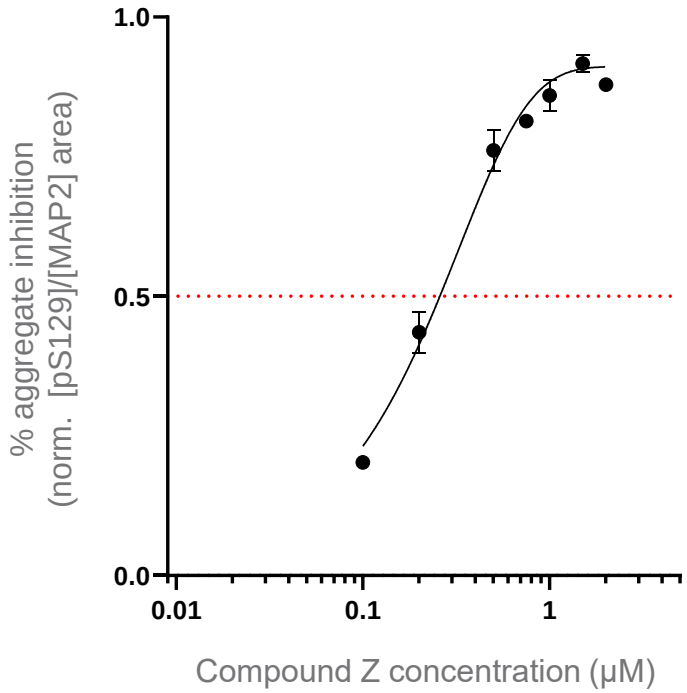
- PFF addition to HEK-a-syn-eGFP cells leads to accumulation of detergent-insoluble, intracellular aggregates
- Treatment of cells with Morphomer® compounds results in reduction of intracellular aggregates

(1) Preformed fibrils; (2) Human embryonic kidney; (3) enhanced green fluorescent protein

Inhibition of pathological a-syn aggregation in neurons

MSA[1]-seeded rat cortical neurons





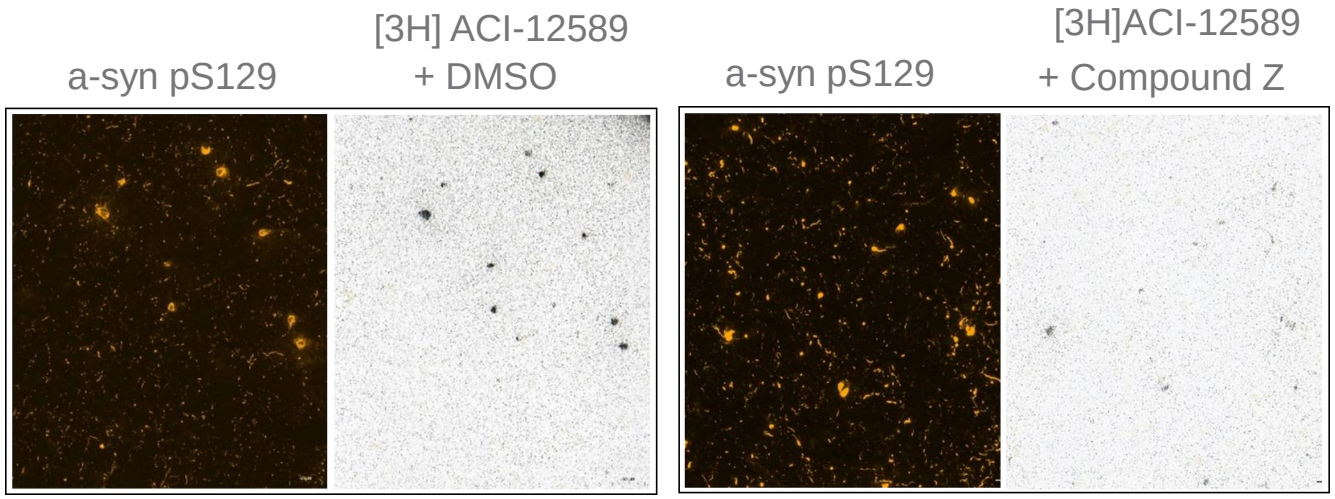
Ref: Tsika et al. MDS 2022

- Rat primary neurons seeded by brain-derived a-syn develop pS129[2]-positive inclusions
- Morphomer® treatment reduces burden of intracellular a-syn aggregates with IC_{50} in nanomolar range

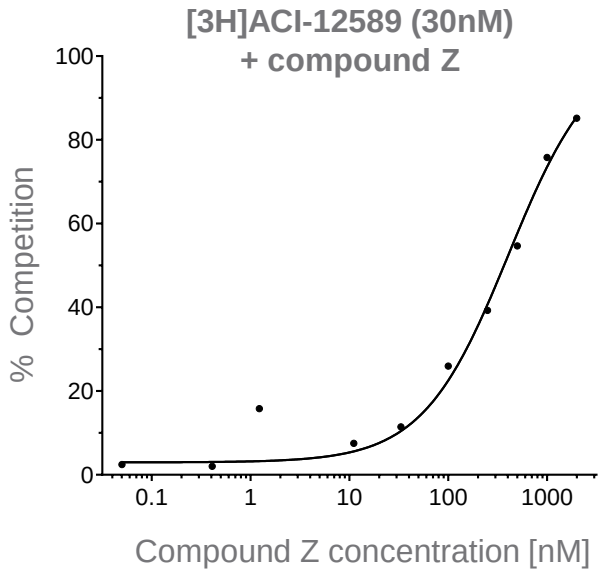
(1) Multiple system atrophy; (2) Phospho-Serine 129

Target engagement on PD[1]-derived aggregates

High-resolution autoradiography, radiobinding assessment



Binding to PD brain-enriched a-syn aggregates - radiobinding



Ref: Ait-Bouziad et al. AD/PD™ 2021

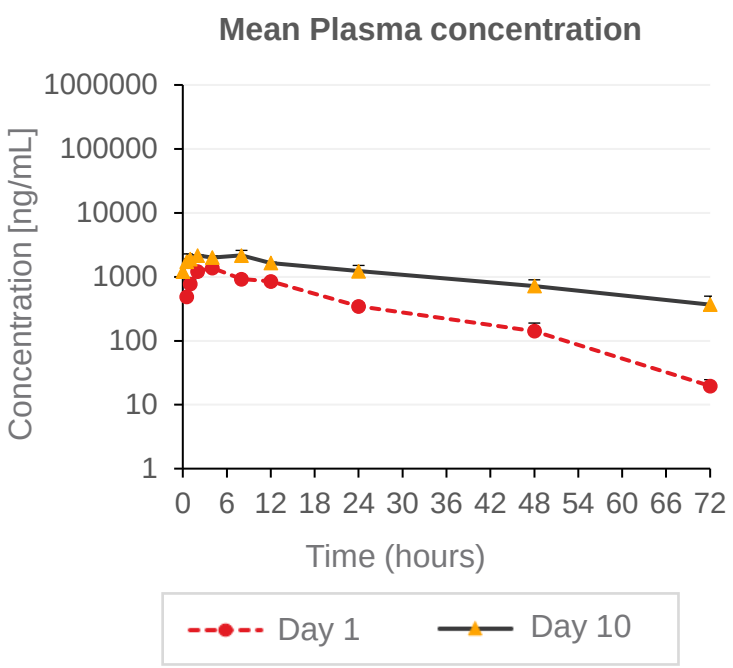
- Compound Z partially displaces [3H]ACI-12589[2] binding to a-syn aggregates on PD amygdala tissue
- Ki in sub-µM range for the displacement of [3H]ACI-12589 binding to a-syn aggregates extracted from PD brain

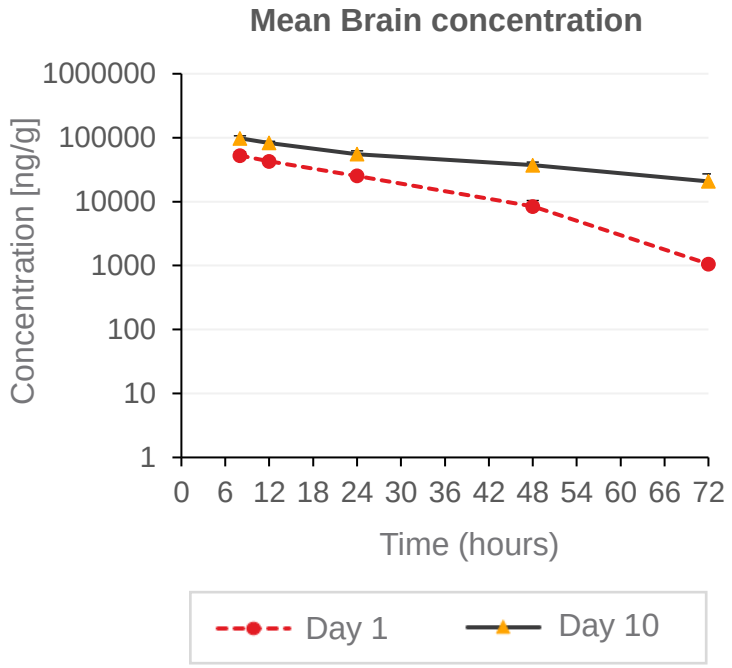
(1) Parkinson's disease; (2) a-syn PET tracer candidate

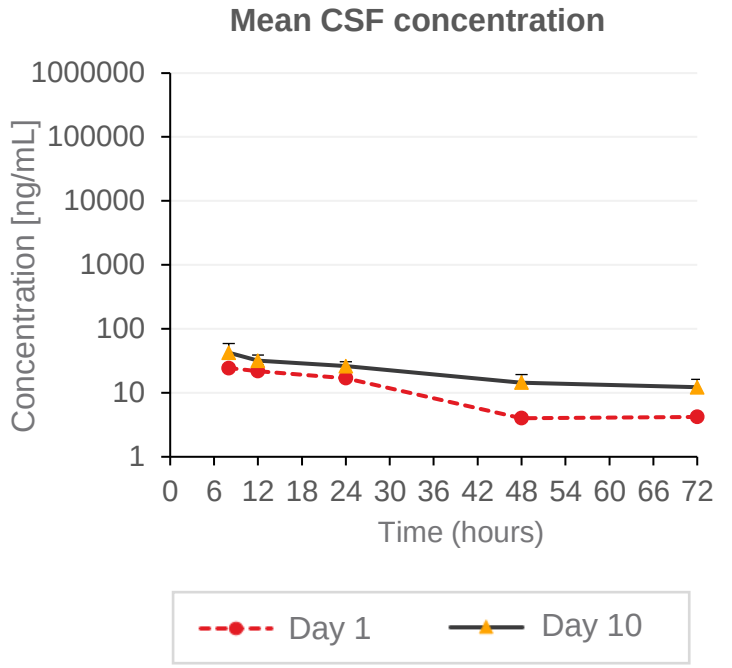
Pharmacokinetic studies

Oral administration (20mg/kg) in mice

Compound Z administered p.o.[1] once or repeatedly over 10 days; total concentration measured over 72 hours post-dose







Ref: Tsika et al. MDS 2022

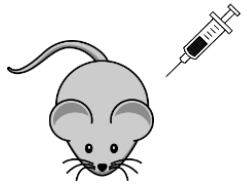
- Accumulation observed in plasma, brain and CSF[2]
- Compound Z highly exposed in the CNS[3]

(1) Per os; (2) Cerebrospinal fluid; (3) Central Nervous System

Compound Z evaluation in proof-of-concept *in vivo* study

Via food administration in the a-syn hPFF[1] model on the M83 line

Tg M83$^{+/-}$
8 weeks



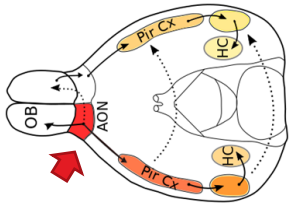
Human A53T a-syn expressed in neurons under the PrP promoter

Unilateral hPFF inoculation & dietary administration of compound

14 weeks in-life

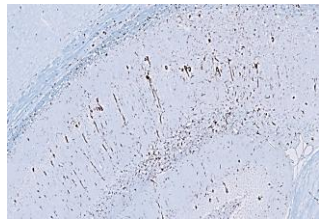
Started dosing 48h following surgeries

Projections from injection site to interconnected brain areas



Injection site

pS129 a-syn/ NeuN[2] IHC[3]



Group	Inoculum	Treatment
A	PBS	Vehicle (normal chow)
B	hPFF	Vehicle (normal chow)
C	hPFF	100mg/kg Compound Z
D	hPFF	60mg/kg Compound Z

- 3 months post-hPFF injection pathological a-syn detected in brain regions anatomically connected to the injection site (AON[4]): cortical areas, hippocampus and brainstem, in both ipsilateral and contralateral hemispheres.
- Neuronal loss, mainly in ipsilateral hemisphere, can confound interpretation of treatment effects so analysis is focused on regions with no cell death

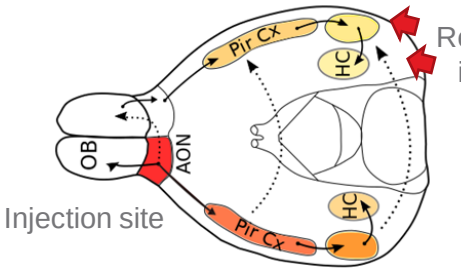
Ref: AC Immune unpublished data

- Readout: pS129 a-syn aggregates in brain regions interconnected to injection site

(1) hPFF: human preformed fibrils; (2) NeuN: neuronal marker; (3) IHC: immunohistochemistry; (4) AON: anterior olfactory nucleus

Decrease of pS129 a-syn in various brain areas

Compound Z evaluation in proof-of-concept *in vivo* study

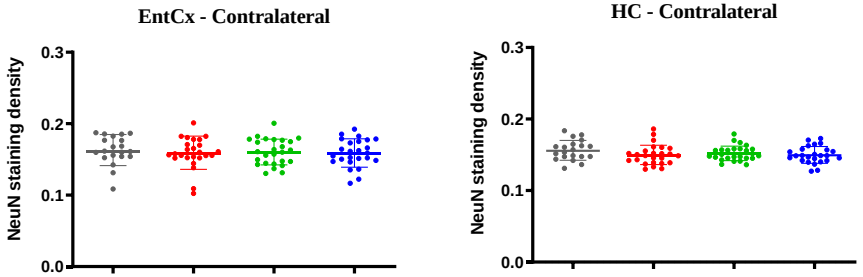


Injection site

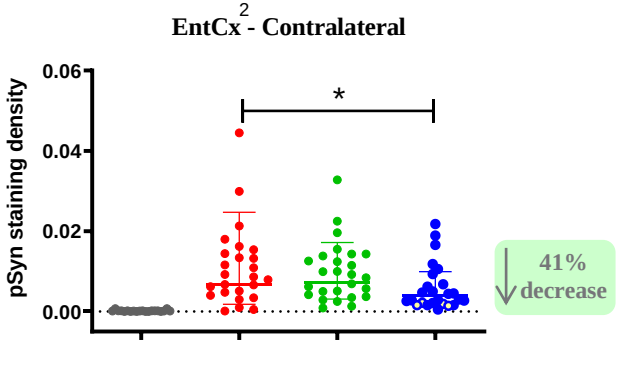
Regions of interest

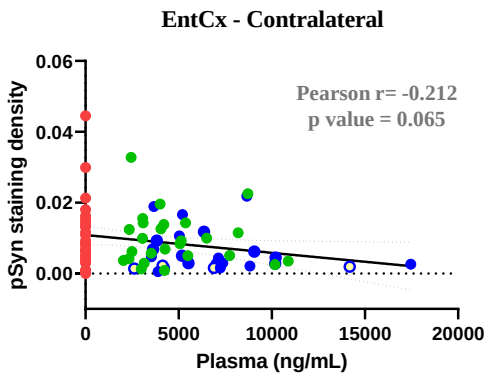
- PBS – Vehicle
- hPFF – Vehicle
- hPFF – Compound Z 60 mg/kg
- hPFF – Compound Z 100 mg/kg
- hPFF – Compound Z 100 mg/kg, <Day 105

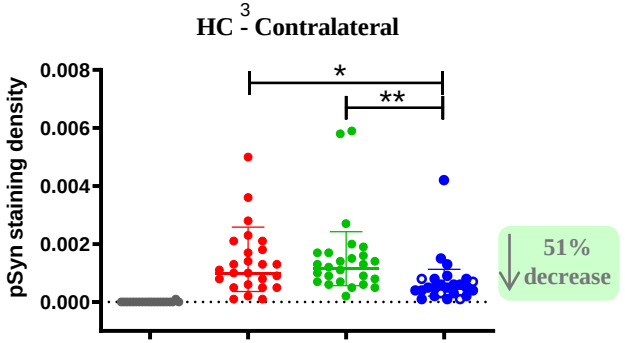
NeuN[1] staining – neuronal marker

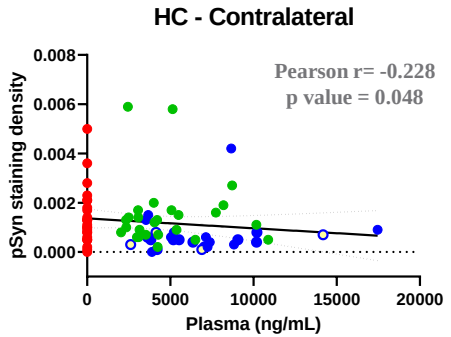


pS129 a-syn levels









2-way ANOVA, Group A excluded. Transformation log(1+x)

Ref: AC Immune unpublished data

- Morphomer® treatment significantly reduces pathological a-syn in the brain

(1) NeuN: neuronal marker; (2) EntCx: entorhinal cortex; (3) HC: hippocampus

Summary

Discovered novel a-syn aggregation inhibitors using our Morphomer® platform which:

- inhibit formation of β-sheet-rich, insoluble aggregates
- reduce burden of intracellular aggregates
- show target engagement to pathological species

First orally available and CNS penetrant Morphomer® which significantly reduces pathology in an animal model of Parkinson's disease

Several chemical series identified; Medicinal chemistry optimization ongoing to identify lead candidate

Acknowledgements

Andrea Pfeifer
Marie Kosco-Vilbois
Sonia Poli
Heiko Kroth
Nicolas Fournier
Clarisse Schumer
Thomas Jaquier
Alexis Fenyi
Ruth Luthi-Carter
Nadine Ait-Bouziad
Nicolas Dreyfus
Coralie Vallet
Leonida Maliqi
Lorène Aeschbach
Sylvain Pautet
Sebastien Menant
Johannes Brune
Irina Borovko

Brain tissue samples:

Netherlands Brain Bank

The University of Miami's Brain Endowment Bank

All the donors and their families for their indispensable contributions to research!

HEK-a-syn-eGFP cell line provided by Prof. Outeiro University Medical Center Goettingen, Germany

The work was supported by grant MJFF-021171 from the Michael J. Fox Foundation for Parkinson's Research

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Shifting the treatment paradigm for neurodegenerative disease towards precision medicine and disease prevention

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