10 YEARS OF BREAKTHROUGHS A HEALTHIER FUTURE

IMPRiND Blocking propagation of protein aggregates in neurodegenerative diseases

SERVIER

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Zebrafish Proteinopathy Models for Target Validation

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Facts & Figures

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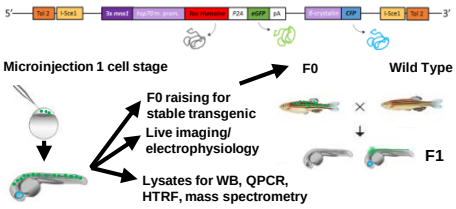
Contributions	
IMI funding:	4 684 998 €
EFPIA in kind:	6 365 900 €
Other:	312 500 €
Total Cost:	11 363 398 €
Project website:	www.imprind.org
Social media:	twitter.com/imprind

Challenge

Accumulation and toxic gain of function of misfolded protein assemblies, such as α-synuclein and Tau, are major pathological features of Parkinson's and Alzheimer's disease, respectively. The IMPRiND project (grant agreement No. 116060) aims to map critical steps in the aggregation and propagation of these misfolded proteins. To address these features, Servier is developing zebrafish models for the stable expression of human wild type and mutated variants of α-synuclein and Tau. Zebrafish models are well poised to bridge the gap between *in vitro* models and traditional *in vivo* rodent models, providing an *in vivo* vertebrate system amenable to accelerated throughput. To generate the stable transgenic animals, we designed plasmids for the expression of these proteins in zebrafish motor neurons (with and without fluorescent reporters) and use biochemical, imaging and electrophysiological based approaches to characterize the lines.

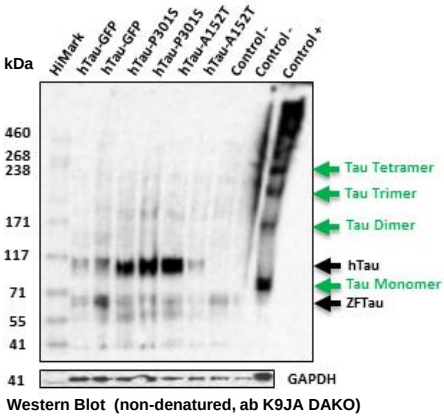
Approach & Methodology

Plasmids were individually injected into embryos at the single cell stage and subsequently integrated into the zebrafish genome resulting in stable transgenic zebrafish overexpressing human Tau and α-synuclein protein. Transgenic animals underwent biochemical evaluation to assess Tau and α-synuclein expression levels and the presence of pathogenic aggregates (via qPCR, Western blot, mass spectrometry and HTRF).



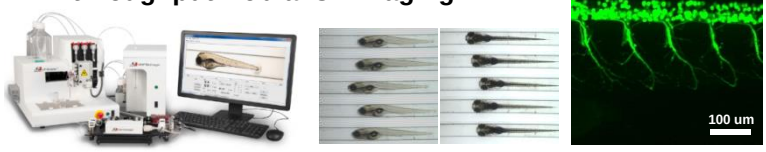
The coexpression of either Tau or α-synuclein with GFP allows for the analysis of changes in neuronal morphology due to the presence of these proteins.

C Tau aggregate formation is not observed in WB or HTRF



Western Blot (non-denatured, ab K9JA DAKO)

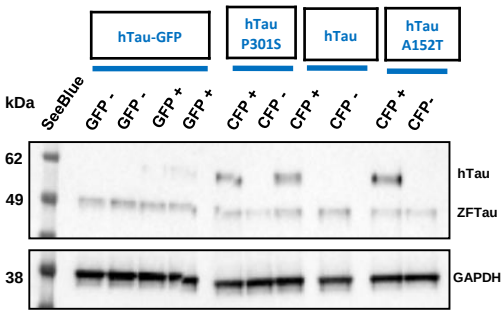
D Automated positioning for medium throughput zebrafish imaging



Using the VAST BioImager can accelerate screening efforts *in vivo*. This system will be used to monitor aggregation with immuno histochemistry and neuronal morphology.

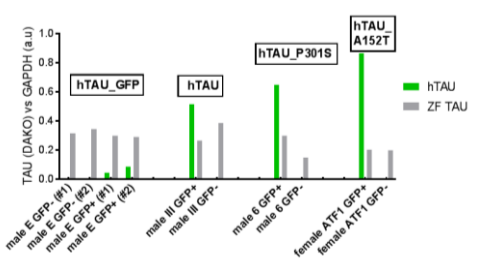
Results

A Human Tau in zebrafish larvae

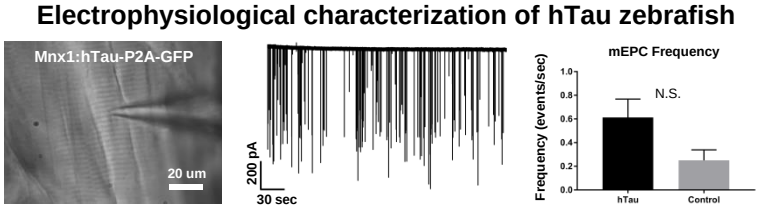


Western Blot (denatured, ab K9JA DAKO)

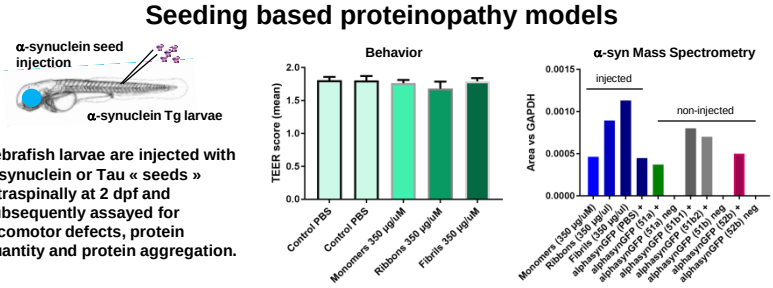
B hTau vs. zebrafish Tau



E Electrophysiological characterization of hTau zebrafish



F Seeding based proteinopathy models



Zebrafish larvae are injected with α-synuclein or Tau « seeds » intraspinally at 2 dpf and subsequently assayed for locomotor defects, protein quantity and protein aggregation.

Value of IMI collaboration:

The IMPRiND Consortium provides access to expertise and material to accelerate model standardization and drug discovery. Servier's role is to develop and share new zebrafish based models for proteinopathy research. As this model is not considered an animal model according to EU standards, it's use can significantly contribute to a reduction in traditional animal testing.

Impact & take home message:

The zebrafish model is well poised to bridge the gap between *in vitro* assays and traditional rodent based *in vivo* studies. These animal models, once fully developed may constitute an important stepping stone on the path to novel therapeutics.

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