

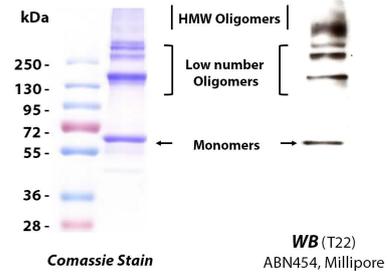
# HUMAN TAU OLIGOMERS INDUCE NEURODEGENERATION: TAUOPATHY MODELS FOR TARGET VALIDATION AND DRUG DEVELOPMENT

T. Pillot, Y. Terroire, A. Allouche, P. Goetghebeur, N. Fischer, P. Housset, S. Lemoine, A. Köpke, V. Koziel

SynAging SAS, Vandoeuvre-Lès-Nancy, France. Contact: T. Pillot, SynAging SAS for further information, +33 (3) 835 961 45, thierry.pillot@synaging.com

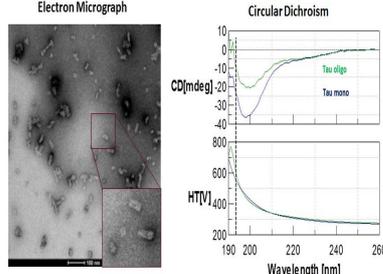
Soluble oligomeric forms of human Tau protein (hTO) play an important role in AD and other tauopathies. Translational models are important to enable target validation, drug discovery and increase the chances for success in clinical trials. Here we report newly developed *in vitro* and *in vivo* neurodegenerative models, induced by a single minute dose of hTO, simulating sporadic tauopathies.

## hTO of Recombinant Full-Length Human Tau Protein (Tau-441, 2N4R)



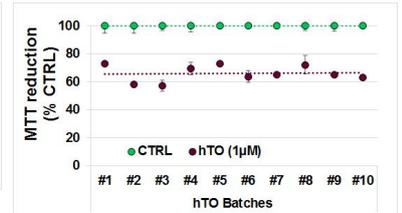
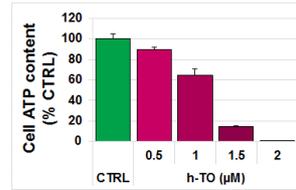
Recombinant Human Tau monomers (>97% purity), are treated to generate hTO in a highly reproducible manner. These oligomers were detected by Coomassie-stained (left), and immunoblot (SDS-PAGE) using anti-Tau (T22) antibody (right).

## Characterization of Human Tau Oligomers



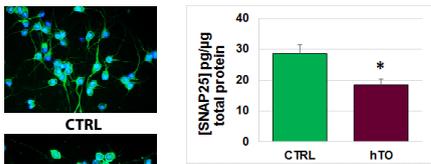
CD measurements suggest a random coil 2D structure for monomers and a more ordered structure in oligomers. Electron micrographs show small duplex shaped forms, different to tau filaments (hTF).

## hTO-Induced Neurotoxicity in Rodent Primary Neurons



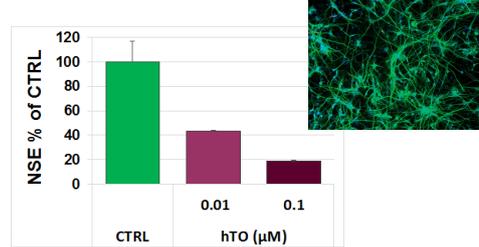
hTO induce dose-dependent cell death in primary cortical neurons (DIV 6) after 24 h of treatment. Read-out: CellTiter-Glo® (left-up) and Live/Dead™ (left-down) assays. All highly reproducible hTO batches induce neurodegeneration *in vitro*. The average percentage of cell death after incubation with hTO (1 μM) for 24 h was 66 ± 2.5%

## hTO-Induce Cytoskeletal Distruption & Synaptic Protein impairment *In Vitro*



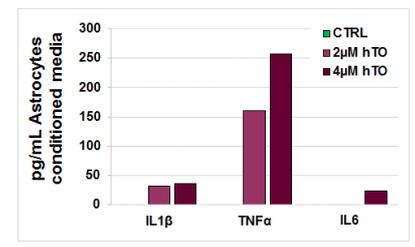
Cortical neurons were incubated with hTO (1 μM) for 24 h. Cytoskeleton network disruption in the presence of hTO was visualized by MAP-2 labelling (left). Synaptic impairment was analyzed by presynaptic marker SNAP-25 ELISA (up)

## hTO-Induced Neurodegeneration in Human HIP iPSC-Derived Neurons



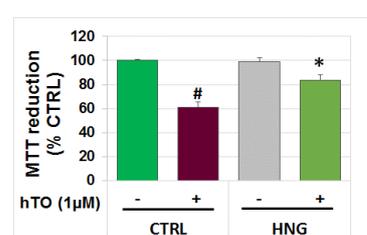
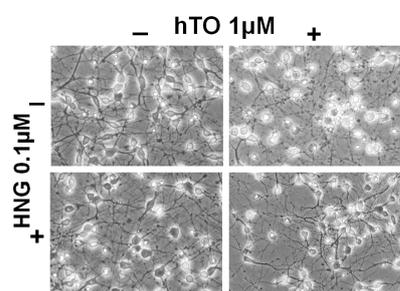
Human HIP iPSC cells were differentiated for five weeks and treated with hTO (0, 0.01 & 0.1 μM) for 72 h. Neuronal survival was determined by NSE ELISA (neuron specific enolase).

## hTO-Induce pro-inflammatory cytokines release of rodent primary astrocytes



Primary astrocytes were incubated with hTO (0, 2 & 4 μM) for 1 h. Conditioned media from astrocytes were analyzed by ELISA to quantify pro-inflammatory cytokines levels (IL1β, TNFα & IL6).

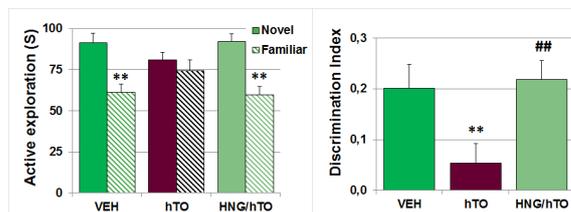
## Humanin (HNG) prevents hTO-Induced Neurotoxicity *In Vitro*



Rat primary cortical neurons were treated with 0.1 μM HNG and 1 μM hTO/vehicle for 24 h.

Up: Phase-contrast images of treated neurons. Down: Cell viability (MTT assay).

## hTO-Induced Cognitive Deficits, Reverted by Humanin



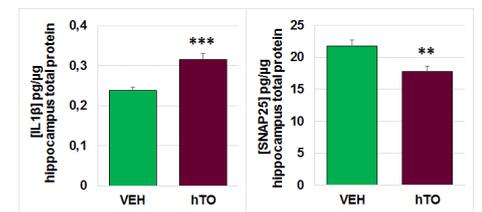
Mice were injected bilaterally into the CA1 region of the hippocampus with hTO (2 μg in 2 μl). 15 days post injection, the animals were assessed in the hippocampal dependent Spatial Recognition Test (SRT).

Animal were first exposed for 5 min to one compartment of the two compartment box (picture below). After an inter trial interval of 30 min, animals were placed back into the apparatus with both compartment available for exploration for 5 min. Active exploration was assessed by an observer blind to the treatment.

\*\* p < 0.01 vs. vehicle  
## p < 0.01 vs. hTO



## hTO-Induced Neuro-Inflammation and Synaptic Protein Decrease *In-Vivo*



ELISA analysis of mouse hippocampal lysate showed increased proinflammatory cytokine production (IL1β, TNFα) in mice inoculated hTO, and decreased levels of synaptic markers.

## Summary

- SynAging report novel tauopathy models induced by highly reproducible human tau oligomers (hTO)
- hTO induce neuronal cell death in rodent primary neurons and in human HIP iPSC cells (*in vitro* models of tauopathies)
- hTO induce release of pro-inflammatory cytokines of primary astrocytes
- In wild-type mice, a bilateral injection hTO into the hippocampus (CA1) results in dramatic impairment of cognitive functions, increased proinflammatory cytokine production and synaptic impairment. This non-inherited tauopathies model can validate disease modifying and symptomatic candidates for clinical development