

Comparative assessment of seed extraction methods relevant for the study of Alzheimer's disease

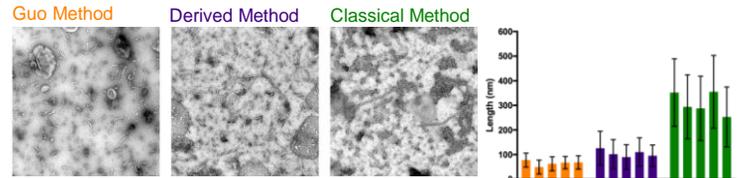
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Facts & Figures

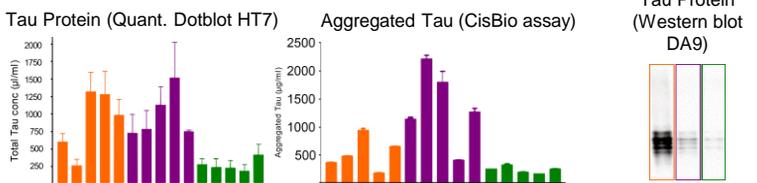
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Contributions	
IMI funding:	4 684 998 €
EFPIA in kind:	6 365 900 €
Other:	312 500 €
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Project website:	www.imprind.org
Social media:	twitter.com/imprind

Results

All seeds are characterized by the presence of filaments but the size of these filaments depends on the extraction method.



Total and aggregated Tau levels are respectively 2 to 6 and 2 to 8 times lower in seeds extracted by the classical method compared to the two other methods. Tau purity is highest in seeds prepared according to the Guo et al. method.



Challenge

One of the principal objectives of IMPRIND is to map and target critical steps in the propagation, proteostatic response and protection against aggregated tau, one of the hallmarks of Alzheimer's disease (AD). To do so, it is essential to identify disease-relevant seeds which induce AD-like pathology in cellular and in vivo models.

Approach & Methodology

We performed a systematic comparison between seeds extracted using different protocols. Tau seeds from five different patients were extracted using three different extraction methods. Seed morphology, total protein levels, electrophoretic signature, total and aggregated Tau levels as well as toxicity were assessed.

LMB	Lilly	Janssen
Classical Greenberg-Davies method	Derived Greenberg-Davies method	Guo method
Sarkosyl extraction	Sarkosyl extraction	Sarkosyl extraction
Sucrose gradient	-	-
No sonication	Sarkosyl pellet resuspended by sonication	Different purification and sonication steps after sarkosyl pellet step

Greenberg, S. G., & Davies, P. (1990).
Guo, J. L. *et al.* (2016).

Value of IMI collaboration

Four IMPRIND partners have worked together, each contributing with their own expertise:

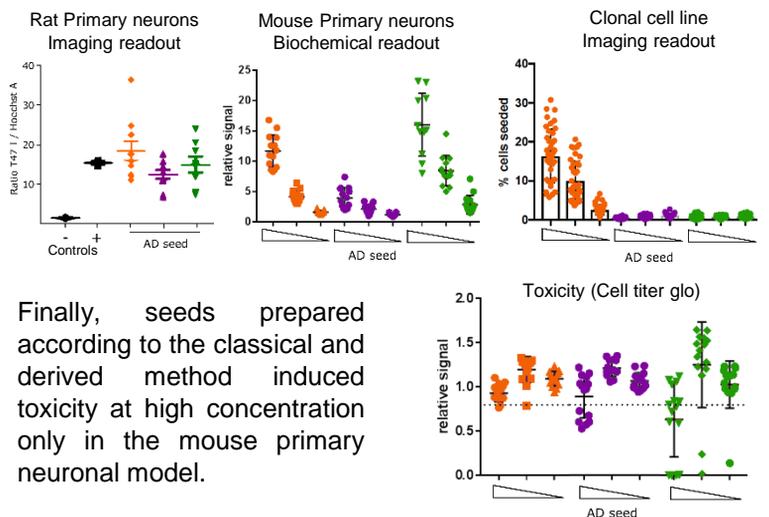
Janssen: Guo seed extraction, aggregate quantification, mouse primary culture seeding with biochemical readout, toxicity readout.

Eli-Lilly: Derived G-D seed extraction, Western blot, aggregate quantification, rat primary culture seeding with imaging readout.

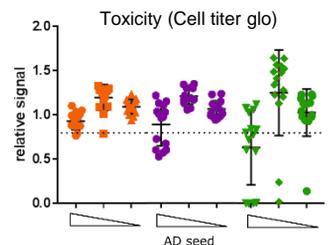
LMB: Classical G-D seed extraction, EM, quantitative dot blot.

UCAM: Clonal cell line seeding.

When seeds are added to cell cultures (two WT rodent primary neuronal models and one human P301S Tau-Venus HEK293 clonal cell line), we observe that all seeds induce Tau aggregation in all models, although to a different extent.



Finally, seeds prepared according to the classical and derived method induced toxicity at high concentration only in the mouse primary neuronal model.



Impact & take home message

Therapeutic intervention that targets the build-up of tau aggregates is considered a promising approach to prevent and treat AD. Recently, new models of WT Tau aggregation have been generated by seeding with Tau assemblies purified from post-mortem brain tissue. These are believed to mimic more closely what happens in AD compared to previous cellular models. Four partners of the IMPRIND consortium investigated together which Tau seed extraction method would yield the highest and most potent amount of seeds, able to induce 1) WT Tau aggregation in neurons and 2) Tau P301S aggregation in a clonal screening model. We concluded that the Guo method was the best option.

This extraction method will be used to generate seeds for high throughput screening in the P301S screening model and medium throughput screening in neurons to identify genes targeting the build-up of endogenous Tau aggregates.

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