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| IMI Scientific Symposium 2018 Abstract title: Comparative assessment of seed extraction methods relevant for the study of Alzheimer disease (IMPRiND)Session: New targets, tools and pathwaysIMI project: IMPRIND |

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Filamentous tau aggregates are one of the hallmark lesions in Alzheimer’s disease (AD). Therefore, therapeutic intervention that targets the build-up of tau aggregates is considered a promising approach to prevent and treat AD. Up until recently, most Tau aggregation models over-expressed large amounts of mutated Tau to study aggregation, but recent developments allow us to build cellular and animal models of aggregation of endogenous wild type (WT) Tau. These models are generated by seeding with Tau assemblies purified from post-mortem brain tissue (Guo et al. 2016) and it is believed that these WT Tau aggregation models mimic more closely what happens in AD.

One of the principal objectives of IMPRiND (Grant Agreement No. 116060) is to map and target critical steps in the propagation, proteostatic response and protection against aggregated tau. To do so, it is essential to identify disease-relevant seeds which induce pathology in cellular and *in vivo* models. Within the IMPRiND consortium, several partners had successfully induced aggregation of WT endogenous Tau with AD derived seeds purified using different extraction methods (classical method1, enhanced method and Guo et al. method2). However, it was unclear which method would yield the highest amounts of seeding competent material so that screening with these AD derived seeds would be feasible. Therefore, in this study, 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 and finally total and aggregated Tau levels were assessed. All seeds were characterized by the presence of filaments but the size of these filaments depended on the extraction method used and probably reflects the difference in sonication of the three extraction methods. Total and aggregated Tau levels were much lower in seeds extracted by the classical method compared to the two other methods while Tau purity was highest in seeds prepared according to the Guo et al. method. When seeds were added to cell cultures (two WT rodent primary neuronal models and one human P301S Tau-Venus HEK293 clonal cell line), we observed that all seeds could induce Tau aggregation in all models, although to different extent. Finally, while assessed in all models, seeds prepared according to the classical and enhanced method induced toxicity at high concentration only in the mouse primary neuronal model.

Taken together, we concluded that seeds prepared using both the enhanced and Guo et al. method contained the highest amount of Tau and were very potent in inducing aggregation. However, adding toxicity and purity into the equation made us select the Guo et al. method as the best method to purify AD relevant seeds. We have also established that it is feasible to use this type of seeds for medium throughput screening purposes.

1. Greenberg, S. G., & Davies, P. (1990). A preparation of Alzheimer paired helical filaments that displays distinct tau proteins by polyacrylamide gel electrophoresis. *Proceedings of the National Academy of Sciences*, *87*(15), 5827–5831.

2. Guo, J. L., Narasimhan, S., Changolkar, L., He, Z., Stieber, A., Zhang, B., … Lee, V. M. Y. (2016). Unique pathological tau conformers from Alzheimer’s brains transmit tau pathology in nontransgenic mice. *The Journal of Experimental Medicine*, *213*(12), 2635 LP-2654.