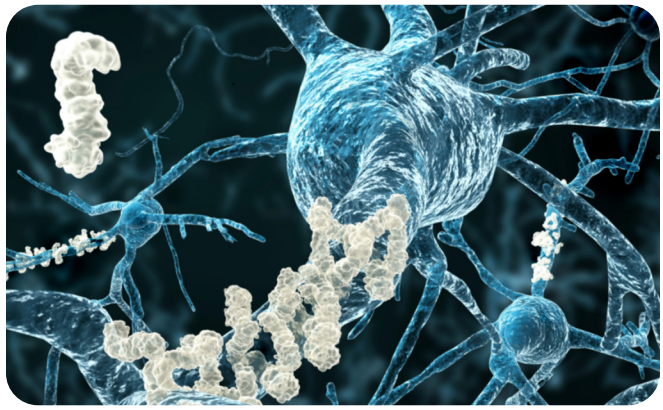
LITERATURE REVIEW

Tau protein aggregation assay relevance to Alzheimer's disease and tauopathies research

Abstract

Tau protein is known to be part of the pathogenesis of several neuronal disorders including Alzheimer's disease, a severe condition that is yet to be met with satisfactory therapies. This note gathers recent published studies that exemplify the use of the HTRF Tau aggregation assay for investigating newly suspected mechanisms of the development of Alzheimer's disease and tauopathies, as well as developing novel immunotherapies that exploit these specific processes to produce promising therapeutic results.



Tau is a soluble cytoplasmic protein found in the human nervous system. In its normal state, its role is believed to be a structural stabilator of microtubules in axons.

In Alzheimer's disease and tauopathies, tau protein becomes hyperphosphorylated (goes from 2-3 phosphorylation sites to at least 7 and up to 85 for the longest Tau isoform, half of which have been confirmed) which causes it to detach from microtubules, misfold and/or associate in Paired Helical Filaments (PHFs). When such filaments become too numerous they aggregate in neurofibrillary tangles which lead to cellular death upon critical accumulation.

Recent studies have suggested the existence of extracellular tau aggregates (seeds) that act as drivers of the pathology by traveling between neurons in a "prion-like" way and spread the disorder across neuronal tissues. This is especially relevant for therapeutic research since such extracellular Tau seeds would be vulnerable to antibody therapies which could prevent the progress of tauopathies.

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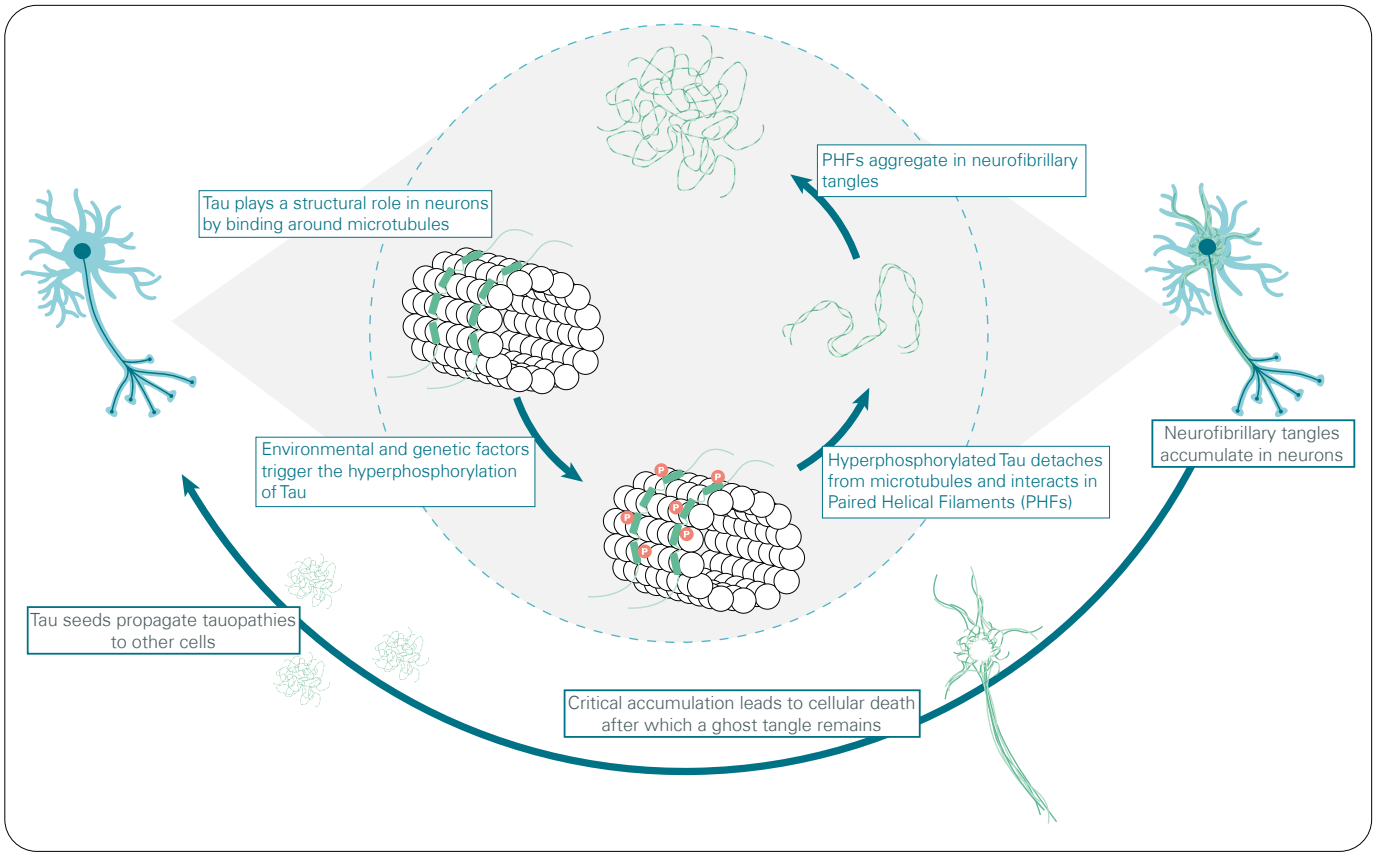

Figure 1: Postulated model for tauopathies pathogenesis and propagation.

Developing screening assays to address Tau seeding propagation

Courade J.P, et al. (2018) Acta Neuropathol. 136(5):729-745

In 2018, Courade *et al.* used the HTRF Tau aggregation kit to develop a robust and quantitative assay that monitors the uptake of Tau aggregates (seeds) in healthy cells, with the ability to screen for antibodies susceptible of binding such seeds to prevent their uptake and tauopathie propagation (fig. 2).

A validation step of this assay was performed by incubating Tau seeds with two different healthy cell populations of HEK293 cells, either transfected with P301S Tau or with an empty vector, before measuring the aggregation (fig. 3).

Results show that Tau seeding triggers Tau aggregation in the Tau-expressing cells. On the other hand, no fluorescence was observed in the non-Tau-expressing cells, which shows the non-persistence of seeds in seeded cells and demonstrates that the fluorescence observed post-seeding is caused by endogenous Tau aggregation. These results not only validate the assay as a relevant tool for monitoring Tau propagation through seeding, but they also provide more evidence to the theory of seed-propagated tauopathies (fig. 4.A).

To further confirm these findings, they used the same Tauaggregation assay to assess the lack of seeds in a healthy human brain sample extract compared to one with Alzheimer's disease (fig. 4.B).

The authors then used this assay to test a collection of antibodies generated from a Tau immunization step based on 19 Tau immunogens. The assay was used to assess these antibodies' potency to prevent Tau seeding in healthy cells (fig 5). Their compared performances led the authors to conclude on the Tau epitope importance in developing antibodies suited for blocking Tau aggregate propagation. They found that high-affinity antibodies were clearly superior at blocking Tau seeding. Another relevant finding was that phosphorylation-specific antibodies appeared more suitable to bind pathological Tau than non-phosphorylation-specific ones but not for aggregated Tau binding.

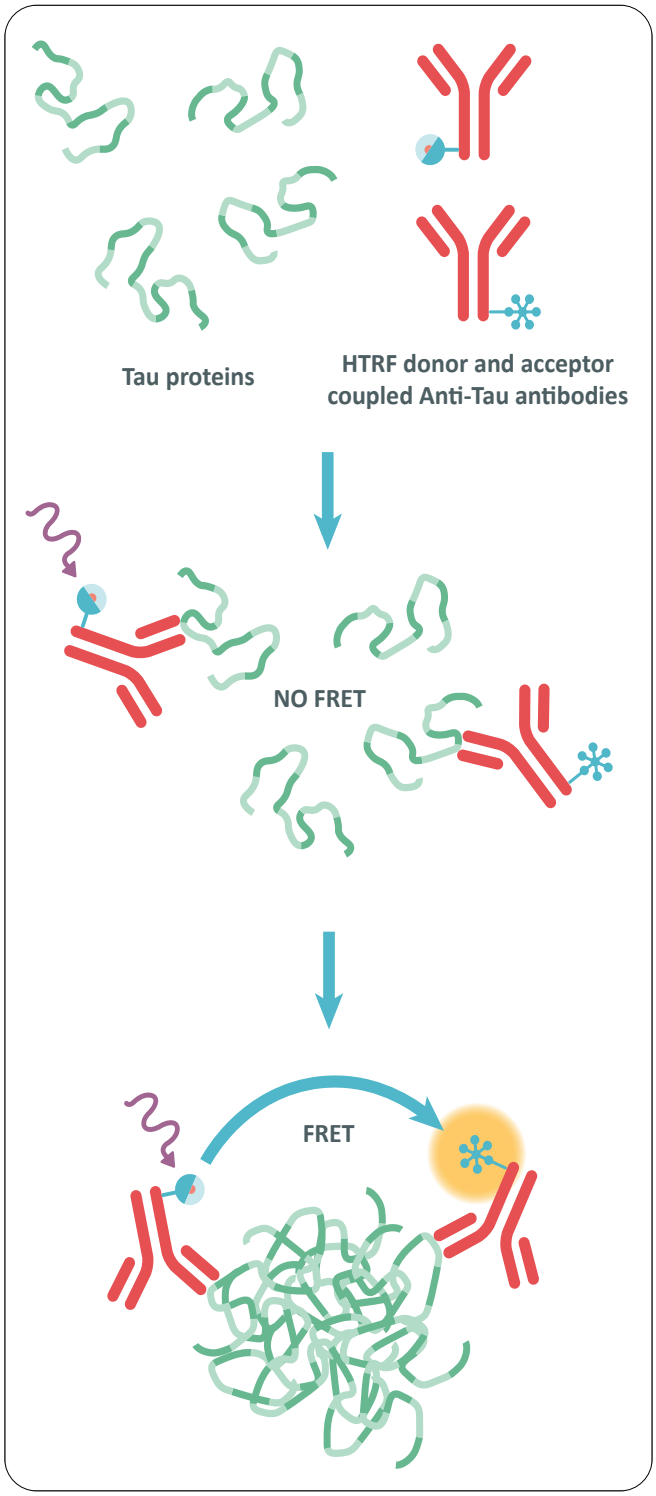

Figure 2: Assay principle – The same Anti-Tau antibodies are coupled with either an HTRF donor or acceptor. They bind to Tau protein on a single epitope, which guarantees that a single antibody binds to one Tau protein. If Tau proteins aggregate, multiple antibodies are brought in proximity with their respective donor or acceptor which triggers FRET.

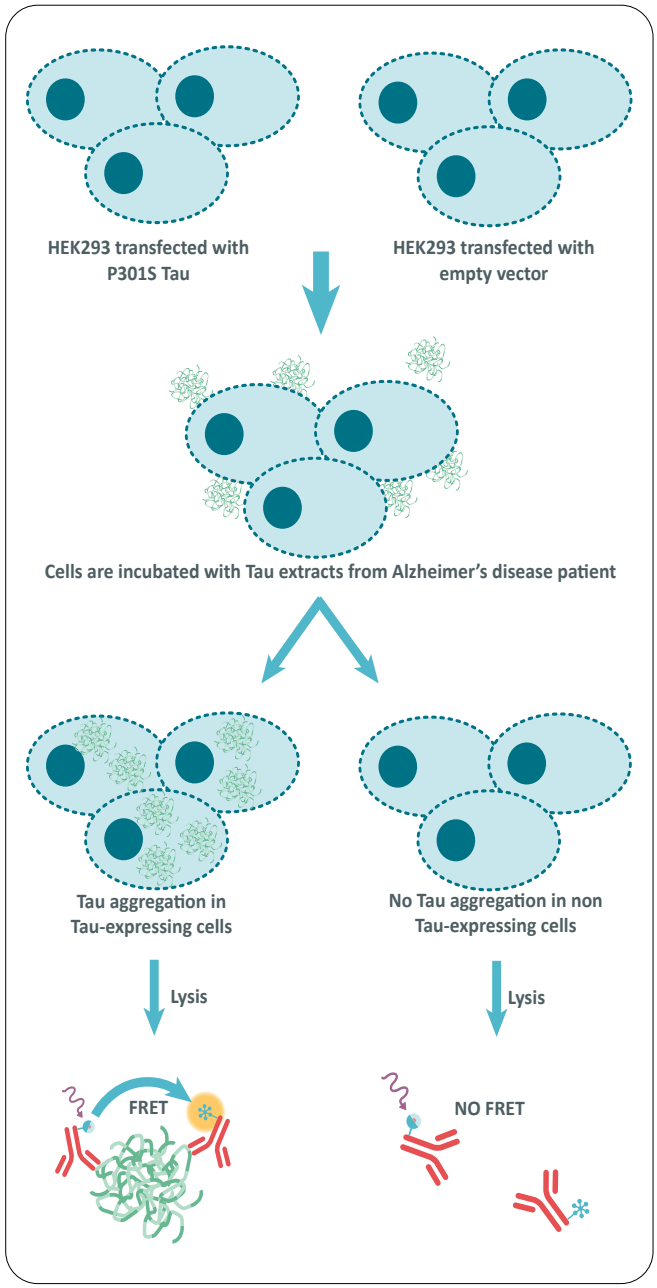

Figure 3: Development of a quantitative Tau seeding cell model. Adapted from Courade J.P, *et al.* (2018) Acta Neuropathol. 136(5):729-745.

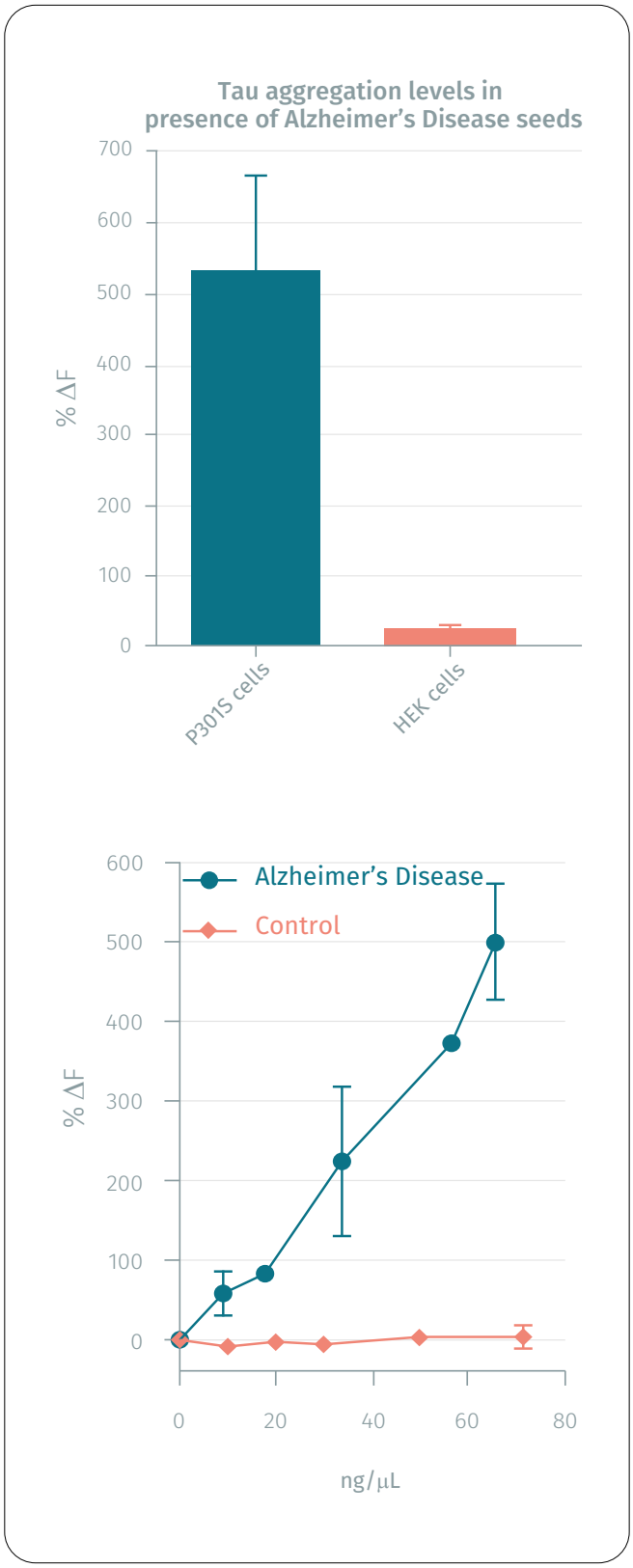

Figure 4: (A) Tau aggregation signal in HEK293 cells transfected with either P301S Tau or the empty vector. (B) Tau aggregation signal in Alzheimer's disease and healthy human brain extracts.

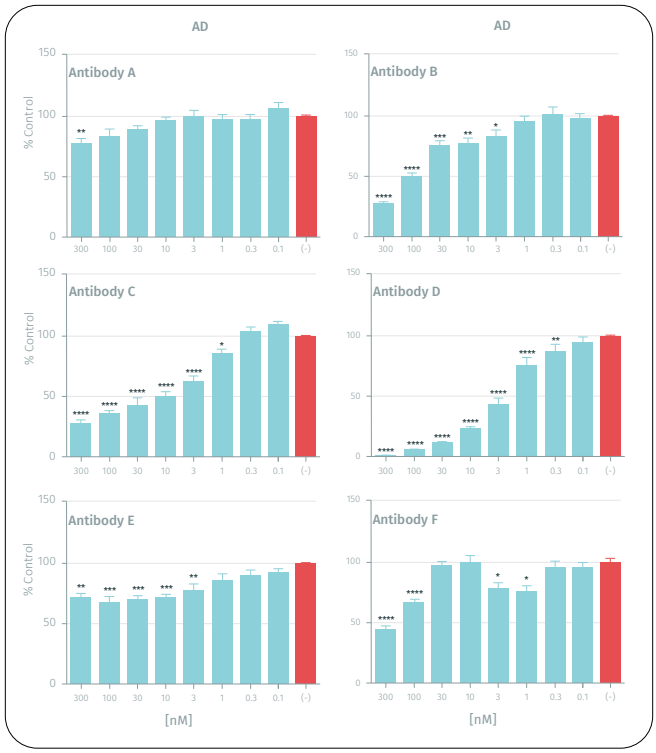

Figure 5: Post-seeding aggregation in presence of various antibodies.

Overall this study provides evidence for the high epitope-dependent nature of Tau seed antibodies-based therapies, as well as suggesting that Tau seeds exhibit a variety of different epitopes which "questions the potential efficacy of some of the Tau antibodies that are currently in clinical development" the authors concluded.

Developing antibody inhibitors for Tau aggregation propagation

Rosenqvist N, et al. (2018) Alzheimers Dement (N Y). 4:521-534

Building upon the Tau seeding theory and the prevalent role of hyperphosphorylated Tau in the rise of Tau aggregates and neurofibrillary tangles (seeds), Rosenqvist *et al.* sought to generate antibodies against a specific phosphorylated form of Tau (pS396-Tau) and investigate their *in-vitro* and *in-vivo* potency for preventing Tau seeding and tauopathies propagation.

The *in-vitro* tests relied on the HTRF Tau-aggregation assay and were performed in the same format described by Courade *et al.* Antibodies were generated by immunization of mice with peptide P30-pS396/pS404-tau and tested for their Tauaggregation inhibitory potential in Tau-expressing rTg4510

neurons cultures. The material used for seeding and inducing the propagation of Tau aggregation was either pooled brain of tauopathie advanced rTg4510 mice or of non-human-tauexpressing transgenic tTA mice for control (table. 1).

Table 1: Inhibiton of Tau-aggregation in rTg4510 mice neurons seeded with advanced tauopathie rTg4510 brain extracts in presence of selected antibodies. Non tau-expressing P3 tTA mice neurons for control.

Tissues seeded	Antidboy tested	% Tau aggregation
P3 rTg4510 (tau-expressing)	Control IgG	100%
	C10.2	55%
	D1.2	83%
	C10.1	100%
P3 tTA (non tau-expressing)	No antibody	<5%

The results allowed for the identification The results allowed for the identification of C10.2 as a promising antibody for Tau aggregate immunotherapy and further *in-vivo* investigations of these antibodies confirmed the potential of C10.2 as a Tau seeding inhibitor (table 2).

Table 2: *In-vivo* seeding model format for antibody potency on Tau aggregation inhibition.

Model	Mice
Seeding material	rTg4510 crude brain extracts
Control seeding material	tTA crude brain extracts
Seeding method	Extra-hippocampal injection
Analysis method	AT8 immuno-histochemistry & Gallyas silver stain

The authors concluded on the relevance of the novel anti-Tau antibody C10.2 for Tau seeding inhibition and were even able to demonstrate the inability of Alzheimer's disease brain extracts to propagate Tau aggregation *in-vitro* and *in-vivo* when previously incubated with C10.2, which emphasize the specificity of that antibody for pathological Tau seeds.

Conclusion

While Alzheimer's disease remains a largely unmet medical condition, the role of Tau protein in its pathogenesis and expansion becomes increasingly prevalent and investigated. The latest evidence suggests that its propagation in the form of aggregates from pathologic cells to healthy ones could be the key driver in the disease evolution. The publications gathered in this note exemplify how HTRF assays and technology can provide relevant tools to further improve the understanding we have of these mechanisms and develop new, potent therapies to address such disorders.

Works cited

1. J.P, *et al.* (2018). Epitope determines efficacy of therapeutic anti Tau antibodies in a functional assay with human Alzheimer Tau. Acta Neuropathologica.

2. Rosenqvist N, *et al.* (2018). Highly specific and selective anti-pS396-tau antibody C10.2 targets seeding-competent tau. Alzheimer's & Dementia.

HTRF assays cited

Product	Tests	Cat. No.#
Tau aggregation kit	500	6FTAUPEG
Tau aggregation kit	10000	6FTAUPEH

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