Impact of epitope location on Tau antibody efficacy in inhibiting Tau-seeded aggregation

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Background

- In Alzheimer's disease (AD), Tau pathology follows a well-defined spatiotemporal pattern which closely correlates with cognitive decline seen in patients.
- It is hypothesized that Tau pathology spreads cell-to-cell to different brain regions through neuronal connections. Immunotherapy strategies are designed to slow disease progression by binding extracellular Tau seeds, preventing neuronal seed uptake and propagation of Tau pathology.
- Monoclonal antibodies (mAbs) targeting different Tau regions have been or are currently being evaluated in clinical trials.
- The extent to which antibody epitope location and binding to AD brain-derived Tau aggregates predict functional inhibition of Tau seeding remains unclear.

Objective

To determine if epitope location and binding to AD brain-derived Tau aggregates predict functional inhibition of Tau-seeded aggregation, a panel of Tau mAbs targeting different regions of the protein (Figure 1) was evaluated in a neuronal Tau seeding model.

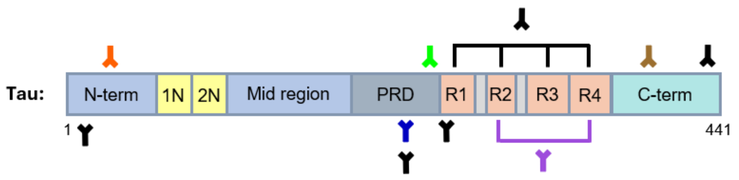


Figure 1: Schematic representation of Tau protein domains mapped to mAbs. Domain organization of the longest human Tau isoform, 2N4R Tau (441 amino acids), showing the N-terminal region, N inserts, mid-region, PRD, microtubule-binding repeats (MTBR), and C-terminal region. The approximate epitope locations of the different mouse (black) [1] and human (color) Tau antibodies evaluated in this study are indicated using generic epitope-based labels. Antibody positions and epitope boundaries are schematic and are not drawn to scale.

Methods

- Rat primary cultures (RPC) were exposed at day in vitro (DIV) 8 to AD-Tau seeds (AD brain-derived Tau aggregates, prepared as previously described [2]).
- Tau mAbs targeting epitopes across the N-terminal, PRD, MTBR, and C-terminal regions were pre-incubated with AD-Tau seeds for 1 hour before addition to the neuronal cultures. Seed–antibody mixtures remained in culture for 7 days (Figure 2).
- At DIV 15, neurons were lyzed and rodent Tau aggregation levels and rodent alpha-synuclein levels (normalization assay) were assessed using specific sandwich MSD assays.
- Antibody effects were evaluated relative to the appropriate negative control antibody. Statistical analysis was performed by fitting a linear mixed-effects model.

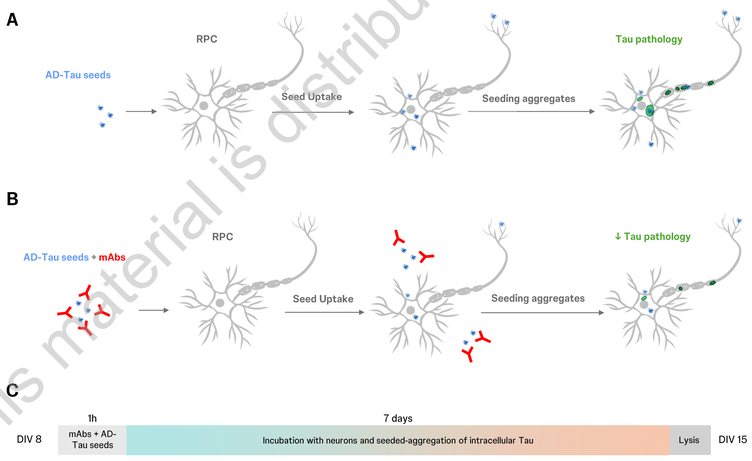


Figure 2: Schematic representation of the neuronal assay. A) When AD-Tau seeds are added to primary rat cortical neurons, these seeds are taken up by neurons and induce aggregation of endogenous rodent Tau. **B)** AD-Tau seeds are incubated with Tau mAbs before addition to the neuronal cultures. Antibody binding to AD-Tau seeds may reduce seed uptake, resulting in a lower downstream Tau-aggregation readout. **C)** Timeline of the neuronal model.

Results

Mouse mAbs targeting PRD, MTBR, and C-terminal epitopes reduced Tau-seeded aggregation of endogenous rodent Tau. Mouse mAbs targeting epitopes across different Tau domains were assessed for binding to AD brain-derived Tau seeds (Figure 3A) and for inhibition of seeded aggregation in primary rat cortical neurons (Figure 3B). Antibodies bind AD-Tau seed preparation independent of epitope location, although binding affinity and maximum binding level differed. The N-terminal antibody showed a strong binding response but did not significantly reduce the seeded-aggregation at the tested concentrations. In contrast, several mAbs targeting non-N-terminal epitopes significantly reduced seeded aggregation relative to the matched negative-control antibody. These findings indicate that binding to the AD-Tau seed preparation alone was not sufficient to identify functionally inhibitory antibodies in the neuronal Tau seeding model.

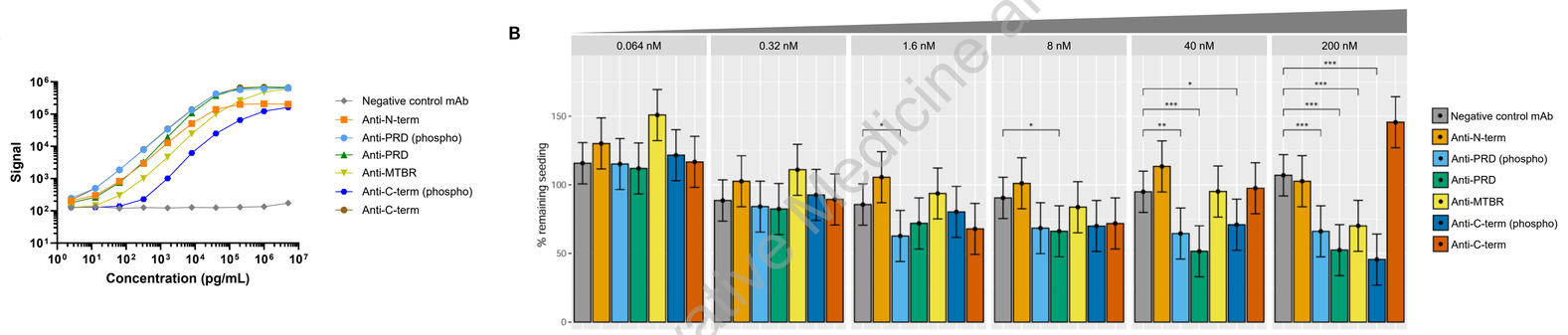


Figure 3: Binding and functional activity of mouse Tau monoclonal antibodies. A) Concentration-dependent binding of mouse Tau monoclonal antibodies targeting the N-terminal, PRD, MTBR, and C-terminal regions to Tau species within the AD brain-derived seed preparation. **B)** Effect of the same antibodies on AD-Tau-seeded aggregation of endogenous rodent Tau in primary rat cortical neurons. AD-Tau seeds were pre-incubated with the antibodies for 1 hour before addition to the neuronal cultures. After 7 days, seeded aggregation of endogenous rodent Tau was quantified using a rodent-specific aggregate Tau sandwich MSD assay and normalized to rodent alpha-synuclein. Antibody effects versus the matched negative-control antibody were estimated at each concentration using linear contrasts from the mixed-effects model. To account for multiplicity, Sidak's correction was used (n=6 independent experiments; *p-value ≤ 0.05; **p-value ≤ 0.01; ***p-value ≤ 0.001).

Human mAbs targeting PRD, MTBR, and C-terminal epitopes reduced Tau-seeded aggregation of endogenous rodent Tau. Human Tau mAbs showed concentration-related binding to Tau species within the AD-Tau seed preparation, with differences in binding affinity and maximum binding level among antibodies (Figure 4A). mAb anti-N-terminal showed a strong binding response, demonstrating accessibility of its targeted N-terminal epitope in the preparation. In the neuronal assay, mAbs targeting the PRD, MTBR, and C-terminal epitopes significantly reduced seeded aggregation at multiple tested concentrations (Figure 4B). In contrast, the N-term mAb did not significantly reduce seeded aggregation despite binding to the AD-Tau seed preparation. Taken together, these findings show that binding to the AD-Tau seed preparation alone was not sufficient to predict functional activity of the mAbs in the neuronal Tau seeding model.

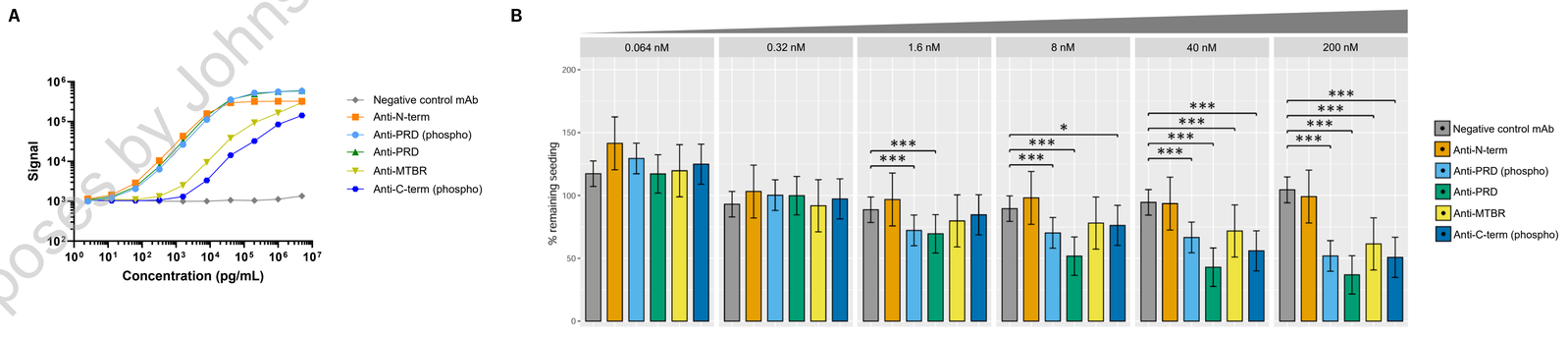


Figure 4: Binding and functional activity of human Tau monoclonal antibodies. A) Concentration-dependent binding of human Tau monoclonal antibodies targeting the N-terminal, PRD, MTBR, and C-terminal regions to Tau species within the AD brain-derived seed preparation. **B)** Effect of the same human mAbs on Tau-seeded aggregation of endogenous rodent Tau in primary rat cortical neurons. AD-Tau seeds were pre-incubated with the antibodies for 1 hour before addition to the neuronal cultures. After 7 days, seeded aggregation of endogenous rodent Tau was quantified using a rodent-specific aggregate Tau sandwich MSD assay and normalized to rodent alpha-synuclein. Antibody effects versus the matched negative-control antibody were estimated at each concentration using linear contrasts from the mixed-effects model. To account for multiplicity, Sidak's correction was used (n≥3 independent experiments; *p-value ≤ 0.05; **p-value ≤ 0.01; ***p-value ≤ 0.001

Discussion

- The N-terminal mAbs bound Tau within the AD brain-derived seed preparation but did not inhibit neuronal seeding, indicating that binding to the preparation alone does not predict functional activity.
- AD-Tau preparations contain heterogeneous Tau species with different biochemical and seeding properties. Strong binding may therefore reflect recognition of abundant Tau species that are not functionally relevant to seeding.
- The neuronal assay complements binding measurements by identifying antibodies that interfere with AD-Tau seeding activity in neurons, providing functional differentiation to support antibody selection. However, it does not define the mechanism of inhibition or predict clinical efficacy, which may also depend on brain exposure, in vivo target engagement, Fc-dependent mechanisms, disease stage, specific clinical endpoint, and treatment duration.
- The present data do not establish a consistent functional advantage of MTBR- or C-terminal-directed antibodies over the evaluated PRD-directed antibodies. However, these data do not support formal ranking of non-N-terminal epitopes, and the clearest association was the absence of inhibition by the tested N-terminal mAbs, despite their binding to AD-Tau seeds.

References

1. Vandermeeren, M., et al., Anti-tau monoclonal antibodies derived from soluble and filamentous tau show diverse functional properties in vitro and in vivo. J Alzheimers Dis, 2018. 65(1): p. 265-281.
2. Guo, J.L., et al., Unique pathological tau conformers from Alzheimer's brains transmit tau pathology in nontransgenic mice. J Exp Med, 2016. 213(12): p. 2635-2654.

Acknowledgements

The authors would like to acknowledge Sofie Versweyveld and Greet Meulders for preparing the primary cortical neuron cultures that supported this work. We would also like to acknowledge the J&J Clinical Development Team for their support.

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