Immunological Response to Dual Aβ/Tau Vaccine PRX123 Surrogate and Effects on Brain Amyloid Plaques in Rapidly Depositing Transgenic Animal Model

Clara Tourino, PhD[1], Alyssa Trumble, BS[1], LeeAnn Louie, BA[1], Robin M. Barbour, BS[1], Gene G. Kinney, PhD[1], Wagner M. Zago, PhD[1], and Brian M. Campbell, PhD[1,*]

[1]Prothena Biosciences, Inc., South San Francisco, CA, USA

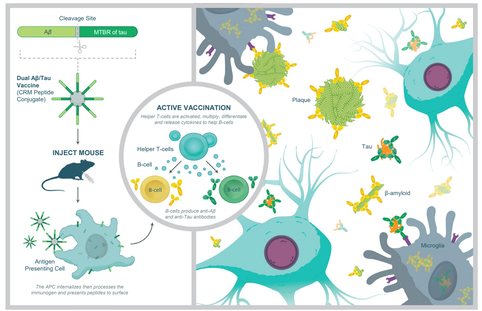
Poster 82687

BACKGROUND

- Alzheimer's disease (AD) pathology is defined by hallmark features including Aβ plaque deposits and tau tangles throughout the brain. Clinical evidence demonstrates that N-terminal anti-Aβ antibodies capable of robustly reducing Aβ plaques in the brains of AD patients can slow cognitive decline. Indeed, all anti-Aβ antibodies provisionally approved for treating AD by the FDA are based on evidence of Aβ plaque removal using PET imaging.
- In addition, the burden and regional distribution of tau pathology strongly correlates with the severity and topography of prospective brain atrophy and cognitive decline. Preclinical evidence indicates that antibodies binding MTBR-tau can prevent cell to cell transmission, potentially suppressing the pathogenic spatiotemporal spread of tau and further delaying cognitive decline in AD.
- Because Aβ and tau are involved in the etiology and progression of AD, it is hypothesized that simultaneously disrupting both pathologic processes may be synergistic in providing therapeutic benefit.
- Vaccine approaches targeting both Aβ and tau may be advantageous in treating preclinical AD by using the body's own immune system to generate long-lasting polyclonal responses that target both pathophysiological changes potentially resulting in a higher probability of delaying or preventing the clinical manifestation of AD onset.
- PRX123 is a dual Aβ/tau peptide vaccine against the N-terminus of Aβ and the MTBR of tau that generated robust immunogenic responses in preclinical studies, simultaneously producing antibodies that promote clearance of Aβ plaques and neutralize MTBR-tau in vitro[1,2].
- The following study was conducted to evaluate the in vivo efficacy of preclinical surrogate PRX123 (PRX123s) in preventing/removing Aβ pathology in a transgenic mouse model expressing human amyloid precursor protein and aggressively high Aβ plaque deposition.

BACKGROUND (CONTINUED)

Figure 1. Pioneering Design of Dual Epitope Aβ/tau Vaccine Candidate PRX123 That Elicits Dual Mechanisms of Action



- Synergistic mechanism designed for increased efficacy over single-target vaccines (Figure 1).
  - Strong evidence from preclinical models suggests that Aβ and tau may act synergistically in the development of AD.
  - Prothena's dual Aβ/tau vaccine program aims to induce optimal (quantity and quality) and balanced immune response to both targets, while avoiding cytotoxic t-cell response.

Figure 2. Design Attributes of Aβ/tau Dual Epitope Vaccines

		Design Strategy	Desirable Output
1 Quantity	**RESPONSE** Antibody levels, balanced Aβ/tau, persistency	• Linear peptides, proprietary cleavable linkers • Optimal carriers, immunization schedule, adjuvant	✓ Optimal antigen presentation with persistent immune response ✓ Overcomes immunodominance and immunosenescence
2 Quality	**EFFICACY** Isotypes, binding, Aβ clearance, tau neutralization	• Optimal Aβ and tau epitopes • Elements for induction of mature TH response	✓ Antibodies bind the right epitopes on pathogenic proteins ✓ IgG switch and affinity maturation
3 Safety	**SAFETY** Cytotoxic T-cell avoidance, target specificity	• Short Aβ/tau epitopes not recognizable by cytotoxic T-cells • No off-target binding risk based on peptide sequences	✓ No cytotoxic T-cell responses ✓ Specific antibodies

- PRX123 produces balanced Aβ/tau titers with a mature Th1/Th2 response in multiple species in vivo, including primates.
- In vitro assays showed antibodies produced induced Aβ plaque phagocytosis and blocked binding of soluble aggregates (protofibrils, oligomers) to cortical neurons[1,2].
- In vitro assays showed antibodies blocked binding of tau to putative neuronal receptor analog (HSPG, heparin)[1,2].

METHODS

Study Design

- APP/PS1 (Thy1-hAPP*V717I/Thy1-hPS1*A246E) transgenic mice were vaccinated with QS21 adjuvanted PRX123s, 25 µg each, beginning at 3.5 months old for 6 months. A mouse N-terminal targeted anti-Aβ antibody (m3D6, synthesized from publicly available sequences, 20 mg/kg) was administered beginning at 6.5 months old for 3 months as a positive control.

Titer Assays

- Titer analyses were performed by enzyme-linked immunosorbent assays (ELISAs) against $Aβ_{1–28}$ peptide (AnaSpec).
- Plates were coated overnight at 1 µg/mL ($Aβ_{1–28}$ peptide) in phosphate-buffered saline (PBS) and then blocked for 1 h with 1% bovine serum albumin (BSA) in PBS.
- Sera was diluted in PBS/0.1% BSA/0.1% Tween 20 starting at a 1:100 dilution and then serially diluted at 1:2.
- Antibody binding was detected with *o*-phenylenediamine dihydrochloride substrate (Thermo Fisher Scientific) following the manufacturer's instructions.
- The plates were read at 490 nm on a SpectraMax microplate reader.

Immunohistochemistry

- Binding of serum from immunized mice to Aβ and tau pathology was evaluated in cryostat sections of fresh-frozen AD brain tissue (Banner Sun Health Research Institute) stained with immune serum diluted at 1:300.
- Binding of immune serum was detected with a biotinylated species-specific secondary antibody, DAB (DAKO), and the ABC detection kit (Vector Laboratories) per the manufacturers' instructions.
- Aβ cortical plaque density was detected in brain tissue of immunized APP/PS1 mice and quantified with Halo image analysis software (Indica labs).

RESULTS

Table 1. Sera from PRX123s Immunized APP/PS1 Mice Bind Aβ Plaques in Human AD Tissue at Expected CNS Concentrations (0.3%)

Mouse Sera ID	Aβ Titer	Aβ Plaque Staining Score
59795	1600	1
58448	4000	0
59854	2400	4
64534	51200	5
64581	175	3
65703	800	4
65827	6500	1
65736	175	4
65732	100	2
58555	700	4
58394	500	1
58410	8000	1
58306	8500	1
58482	150000	5
58515	700	3
58338	3000	4
59760	40000	4

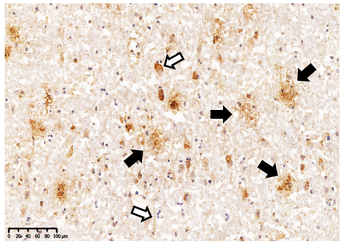
- PRX123s generated robust antibody titers capable of binding structural features indicative of Aβ plaques in post-mortem AD brain tissue at concentrations expected to be reached in the brain (0.3% of plasma concentrations).

Qualitative analysis was based on the intensity and frequency of binding to pathology in the tissue. Non-responders (Aβ titers <100) were omitted from staining. Staining was performed on two tissue sections by a rater blinded to treatment. The reported value is the average of the two sections. The rating scale ranged from 0 to 5, with 0 indicating no binding to Aβ plaques or tau pathology, and 5 indicating intense wide-spread prevalence of pathology.

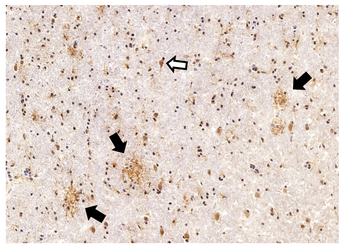
RESULTS (CONTINUED)

Figure 3. Sera from PRX123s (0.3%) Immunized APP/PS1 Mice Bind Aβ Plaques and Tau Pathology in Human AD Tissue

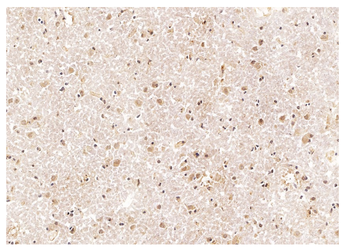
AD Brain Tissue
Animal sera: 58482
Aβ Plaque staining score: **5**



AD Brain Tissue
Animal sera: 64581
Aβ Plaque staining score: **3**



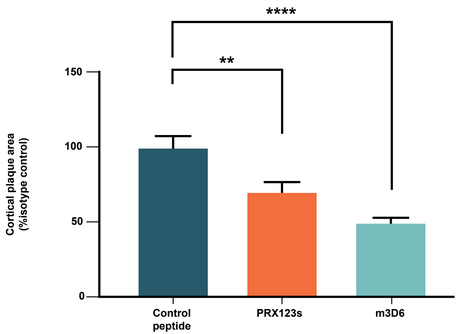
Non-AD Brain Tissue
Animal sera: 58482
Aβ Plaque staining score: **0**



Sera from immunized mice demonstrate binding to features indicative of hallmark pathology in post-mortem human AD brain tissue including Aβ plaques (solid arrows) and tau neurofibrillary tangles (open arrows; cell bodies and neurites). Staining in control, non-AD brain tissue demonstrated no observable specific binding. Images represent tissue staining by serum from mouse 58482 (Aβ score = 5) and 64581 (Aβ score = 3) at 20x magnification, scale bar = 100 µm.

RESULTS (CONTINUED)

Figure 4. PRX123s Induced Clearance of Aβ Plaques From the Brains of APP/PS1 Transgenic Mice



- Immunohistochemical analysis of brain tissue from vaccinated APP/PS1 mice demonstrated significant reduction in cortical Aβ plaques compared to a peptide control group. Clearance was similar to treatment with a plaque-clearing N-terminal monoclonal antibody (20 mg/kg) used as positive control. **p<0.01; ****p<0.0001 compared to isotype control.

CONCLUSIONS

- PRX123s elicited immunogenic responses to Aβ and tau in a transgenic mouse model of Aβ pathology that recognized both Aβ plaques and tau neurofibrillary tangles in human AD brain tissue.
- Treatment of APP/PS1 transgenic mice with PRX123s elicited a significant reduction/prevention in cortical Aβ plaque levels.
- **Together these data demonstrate that PRX123 may be capable of generating high-quality antibody responses to clear Aβ plaques at brain exposures which may be achievable in patients and support the continued development of PRX123—a dual Aβ-tau conjugated linear peptide vaccine designed to treat and/or prevent Alzheimer's disease.**

REFERENCES

1. Barbour R et al. Poster presented at AAIC; July 26–30, 2021; Denver, CO, USA
2. Barbour R et al. Poster presented at ADI; June 9–11, 2022; London, UK

AUTHOR DISCLOSURES

All authors are employees of Prothena Biosciences Inc and shareholders of Prothena Corporation plc.

Clara Tourino was an employee of Prothena Biosciences Inc and shareholder of Prothena Corporation plc at the time of this study.

ACKNOWLEDGMENTS

This study was sponsored by Othair Prothena Limited, Dublin, Ireland, a member of the Prothena Corporation plc group. Editorial support was provided by AMICULUM USA, Princeton, NJ and funded by Prothena Biosciences Inc.