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Development of a Dual Aβ-Tau Vaccine for the Prevention of Alzheimer Disease

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INTRODUCTION

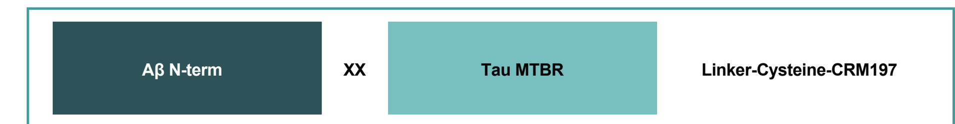
- Alzheimer disease (AD) is characterized by 2 main pathological hallmarks, amyloid-beta (Aβ) plaques and tau-enriched neurofibrillary tangles[1]
- The FDA recently approved aducanumab as the first Aβ-targeting immunotherapeutic to treat patients with AD, and several other anti-Aβ therapeutics are in late-stage development[2]
- First generation tau immunotherapies are entering later-stage development; several next generation therapeutics targeting different epitopes of the protein are aimed at inhibiting cell-to-cell transmission of pathological tau[3,4]
- Advances in the development of vaccines for AD are also progressing. However, the majority of vaccines in development target only one of these pathological features of AD, but there is strong evidence from preclinical models that Aβ and tau act synergistically in the progression of disease
- A vaccine that concomitantly targets Aβ and tau might be more efficacious than single-target vaccines for the treatment and prevention of AD. Prothena Biosciences has developed three proprietary dual Aβ-tau vaccine constructs, which aim to target both pathways effectively
- In this poster, we show partial results from 3 diverse vaccines. Constructs 2 and 3 are currently lead candidates. Construct 1, while discontinued due to suboptimal properties, served as validator of our novel technology to generate appropriate quantity and quality of anti-Aβ and MTBR-tau antibodies in non-human primates

METHODS

Immunogen Designs

- Proprietary linear peptides containing the Aβ N-terminal region, a dendritic endopeptidase site, and 3 different microtubule binding region (MTBR) were designed as constructs 1, 2, and 3 (**Figure 1**)[5]
- In addition, the peptides included a C-terminal spacer and a cysteine conjugated to maleimide-activated CRM197 (Fina BioSolutions)
- All the peptides included identical Aβ peptide sequences, whereas the tau MTBR peptide used was different in each of the 3 constructs

Figure 1. Design of the Linear Dual Peptide



Aβ, amyloid beta; MTBR, microtubule binding region; N-term, N-terminal peptide.

Study Design

- Immunogens were tested in several animal species, including mice, guinea pigs, and cynomolgus monkeys
- Immunogen amounts ranged from 25 to 50 µg per injection, while coinjected QS21 adjuvant (Desert King) was used at 25 µg in mice and 50 µg in guinea pigs and cynomolgus monkeys
- Mice were injected subcutaneously, and guinea pigs and cynomolgus monkeys were injected intramuscularly
- Various injection schedules in each species were studied

Titer Assays

- Titer analyses were performed by enzyme-linked immunosorbent assays (ELISAs) against full-length recombinant tau (Proteos) and Aβ 1-28 peptide (AnaSpec)
- Plates were coated overnight at 1 µg/mL (Aβ 1-28 peptide) and 2 µg/mL (tau) in phosphate-buffered saline (PBS) and then blocked for 1 h with 1% bovine serum albumin (BSA) in PBS
- Sera or cerebrospinal fluid (CSF) was diluted in PBS/0.1% BSA/0.1% Tween 20 (PBS/BSA/T) starting at a 1:100 dilution (CSF started dilution at 1:2) and then serially diluted at 1:2
- Normal mouse serum or preimmunization sera for guinea pigs and cynomolgus monkey serum (or day 7 CSF collection) were used as negative controls, and known positive antisera from previous mouse studies were used as positive controls
- Samples and controls were incubated on the plate for 1.5 h at room temperature (RT)
- Plates were washed with Tris-buffered saline (TBS)/Tween 20 and incubated for 1 h at RT with species-specific secondary antibody horseradish peroxidase (HRP) (Jackson ImmunoResearch or Thermo Fisher Scientific)
- Plates were then washed in TBS/Tween 20, and antibody binding was detected with o-phenylenediamine dihydrochloride (OPD) substrate (Thermo Fisher Scientific) following the manufacturer's instructions
- The plates were read at 490 nm on a SpectraMax microplate reader (Molecular Devices)

Immunohistochemistry

- Cryostat sections of fresh-frozen AD brain tissue (Banner Sun Health Research Institute) were stained with immune serum diluted at 1:300
- Binding of the immune serum was detected with a biotinylated species-specific secondary antibody, DAB (DAKO), and the ABC detection kit (Vector Laboratories) as per the manufacturers' instructions
- Staining was processed with an automated Leica bond stainer (Leica Biosystems)

ELISpot for T-Cell Epitope Analysis

- Enzyme-linked immunosorbent spot (ELISpot) analysis was conducted at Charles River Laboratories
- Peripheral blood mononuclear cells (PBMCs) were used for cellular immune response via an ELISpot assay
- Isolated PBMCs were added to the wells of the ELISpot plates containing different treatments (dimethylsulfoxide [DMSO]-negative control, tau protein, Aβ protein, CRM197 protein, or PHA-positive control)
- After a 24-h incubation, the ELISpot plates were developed following the manufacturer's instructions
- The plates were imaged using an ImmunoSpot instrument, and spots were quantified using ImmunoSpot software (Cellular Technology Limited)
- Positive ELISpot responses were determined by calculating the lower limit of detection (LLOD) for the peptide stimulant wells at the pretreatment time point (Day 7), and any normalized value above the LLOD was considered a positive response
- The LLOD was calculated using the following formula: LLOD = median of normalized value of spot-forming cells (SFCs)/10^6 (PBMCs) + 3 standard errors of the mean
- A normalized value of mean spots was calculated by subtracting the DMSO response from the peptide-specific response
- For tau protein, positive response was identified as a normalized value of >35 mean spots
- For Aβ and CRM197 proteins, positive response was identified as a normalized value of >18 and >542 mean spots, respectively

Blocking of Soluble Aβ Aggregate Binding to Neurons

- Blocking of soluble Aβ aggregate binding to primary rat hippocampal neurons was performed as previously described[6]
- Soluble biotinylated Aβ aggregates were preincubated with various dilutions of guinea pig serum for 30 min at 37ºC and then added to neurons and incubated for another 30 min at 37ºC
- Cells were fixed, permeabilized, and then incubated overnight with MAP2 (Abcam) and NeuN (EMD Millipore) primary antibodies
- Cells were rinsed and then incubated for 1 h, and Alexa Fluor secondary antibodies and streptavidin (Thermo Fisher Scientific) were used to detect Aβ soluble aggregates
- Aβ binding to neurons was quantified by high-content imaging analysis using the Operetta CLS system (Perkin Elmer)

Phagocytosis of Aβ Protofibrils After Treatment With Mouse IgG by THP1 Cells

- Synthetic protofibrils of $Aβ_{1-42}$ containing an S26C mutation were generated as described[7]; mature protofibrils were conjugated to pHrodo Red Maleimide (Thermo Fisher Scientific) before in vitro phagocytosis assays
- Ammonium sulfate precipitation of the immune serum was used to enrich immunoglobulin G (IgG) and remove interfering proteins[8]
- Desalting was performed in 3 cycles of dilution and concentration in a 0.5-mL 100-kDa-cutoff concentrator
- After final concentration, the sample was reconstituted to starting volume, and a bicinchoninic acid assay was run to determine protein concentration
- Phagocytosis assay:
  - Twenty-five microliters of 10 µg/mL pHrodo Aβ protofibril stock and 25 µL of 40 µg/mL IgG stock were premixed and then added to 50 µL of 10^5 THP1 cells/mL within a v-bottom plate and incubated for 2 h at 37ºC in 5% CO_2
  - Cells were washed 2 times in RPMI plus 10% low-Ig serum and antibiotics, incubated for 10 min at 37ºC 5% CO_2, and then washed 2 times in PBS (no Ca^{2+} or Mg^{2+}) plus 1% fetal bovine serum (FBS)
  - Cell pellets were resuspended in 100 µL of PBS plus 1% FBS and analyzed by flow cytometry

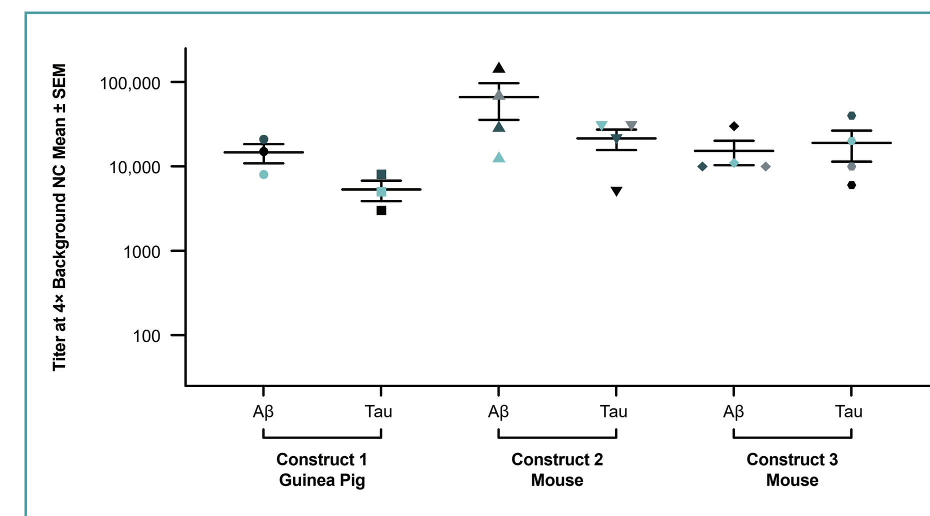
Blocking of Tau Binding to Heparin by Mouse Antiserum

- In order to assess the ability of the serum to block uptake of tau into cells, an ELISA measuring the blocking of tau binding to heparin plates was developed; recombinant tau was biotinylated in-house
- Heparin-coated plates (bioWORLD) were blocked with 2% BSA/PBS for 1 h
- In a separate deep-well polypropylene 96-well plate, serum was diluted from 1:25 to 1:400 in 2% BSA/PBS (60 µL total volume)
- To this dilution, 60 µL of 200 ng/mL biotinylated tau in 2% BSA/PBS was added for final serum and tau concentrations of 1:50 to 1:800 and 100 ng/mL, respectively
- The mixture of serum and tau was incubated for 2 h, and then 100 µL/well was transferred to the blocked heparin plates and incubated for 1 h
- The plates were washed, and goat anti-mouse IgG (H+L) HRP (Thermo Fisher Scientific) was added at a 1:5000 dilution and incubated for 1 h at RT
- The plates were washed in TBS/Tween 20, and 100 µL of TMB substrate (Thermo Fisher Scientific) was added and incubated for 8 min
- The reaction was stopped with H_2SO_4, and the plates were read at 450 nm in a SpectraMax microplate reader (Molecular Devices)

RESULTS

- The data shown were generated with antisera derived from blood taken 1 to 2 weeks after the final injection was administered to mice, guinea pigs, or cynomolgus monkeys; the data for all time points are shown only with antisera from cynomolgus monkeys

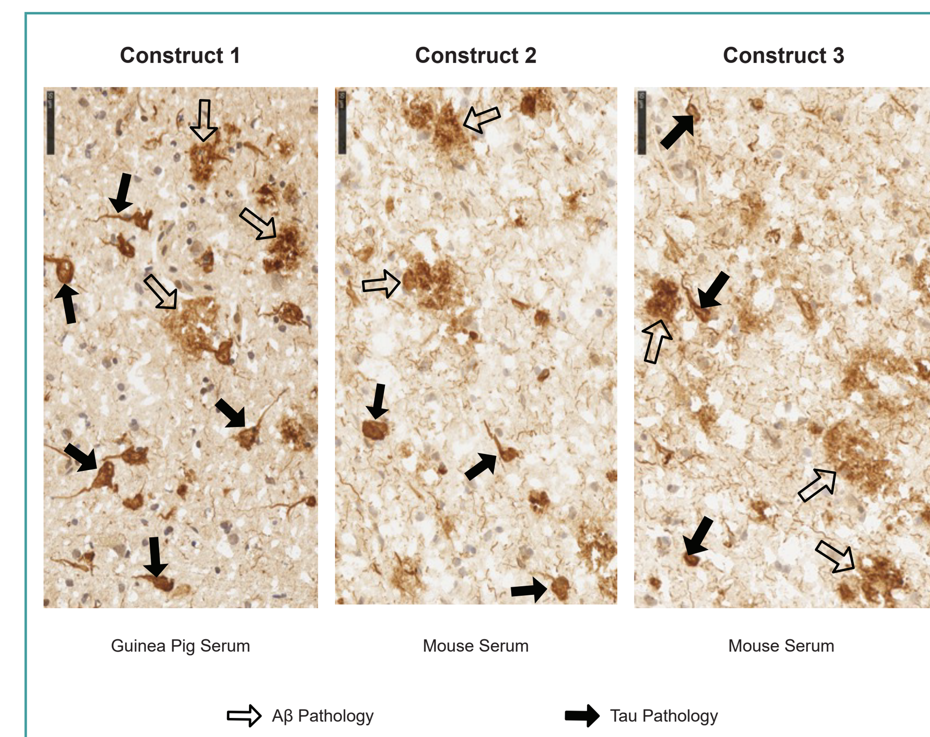
Figure 2. Screening Titers Determined That Balanced Responses to Aβ and Tau are Raised in Guinea Pigs and Mice



Aβ, amyloid beta; NC, negative control; SEM, standard error of the mean.

- The antisera from all animals immunized with constructs 1, 2, and 3 produced balanced Aβ and tau titers (**Figure 2**)

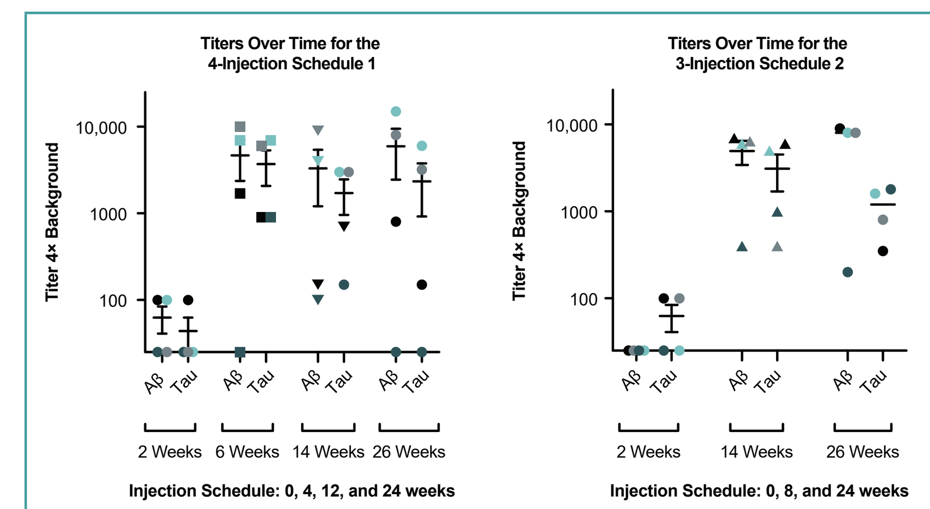
Figure 3. Sera From All 3 Constructs Strongly Recognized Pathological Aβ and Tau in Human AD Brain Tissue With Well-Characterized Pathology



Aβ, amyloid beta.

- All 3 constructs resulted in robust immunohistochemical staining of pathological Aβ and tau in human AD brains at a 1:300 dilution by immunohistochemistry in human tissue with well-characterized pathology (**Figure 3**)

Figure 4. Cynomolgus Monkeys Raised Balanced Responses to Aβ and Tau



Aβ, amyloid beta.

- Construct 1 induced balanced titers in cynomolgus monkeys (**Figure 4**)

Table 1. Cynomolgus Monkeys Produced CSF Titers With a Serum Ratio of 0.05% to 0.2%

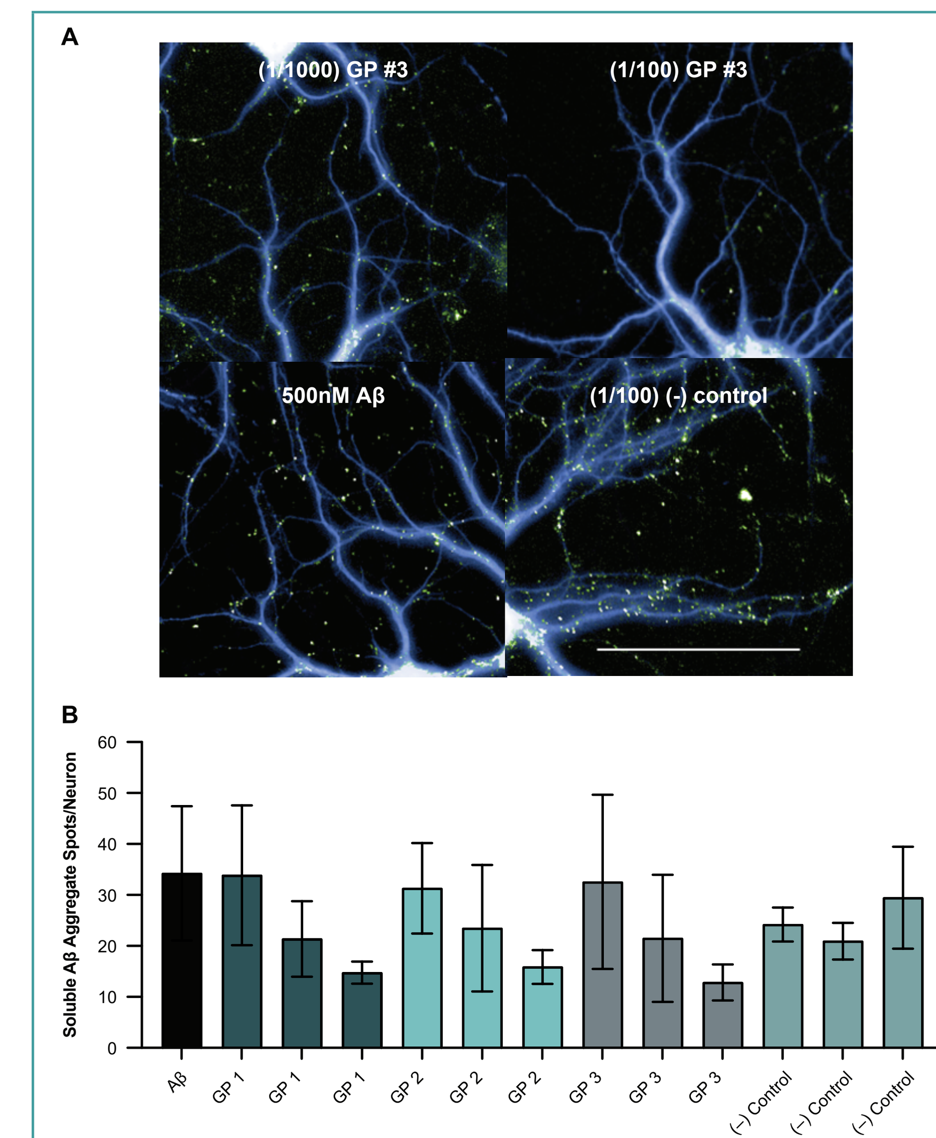
Animal No.	Aβ Titer in CSF/ Serum	Tau Titer in CSF/ Serum	CRM197 Titer in CSF/Serum
1003	0.1	0.1	0.1
1501	0.1	0.1	0.1
2102	0.1	BLQ	0.1
2501	0.1	0.1	0.2

Only monkeys with a titer of >1:2000 to Aβ, tau, and CRM197 were assayed. Aβ, amyloid beta; BLQ, below the limit of quantification.

- Construct 1 in cynomolgus monkeys resulted in CSF antibody levels of approximately 0.1% of the serum antibody titers (**Table 1**)
- Construct 1 did not generate a T-cell response to either Aβ or tau
- **The Abeta/tau vaccine was successful in avoiding T-cell response to Aβ or tau**
  - Predose samples from animals in group 1 (4 immunizations) or group 2 (3 immunizations) did not show pre-exciting T-cell responses to any of the 3 proteins (tau, Aβ, and CRM197 protein)
  - No animals assigned to either group 1 or group 2 elicited a T-cell response to either the tau or Aβ protein
  - For the CRM197 protein, 2 (animal numbers 2001 and 2501) of 4 animals in group 2 responded with mean normalized values of 633 and 1064, respectively (LLOD = 542)
  - All animals had a positive response to the positive control PHA

Characterization of the antisera derived from animals immunized with constructs 1, 2, and 3 were examined in a battery of in vitro functional assays

Figure 5. Guinea Pig Serum Inhibits Binding of Soluble Aβ Aggregates to Neurons in a Concentration-Dependent Manner: (A) Representative Image; (B) Quantification of Soluble Aβ Aggregate Binding



Aβ, amyloid beta; GP, guinea pig.

- Antibodies to Aβ induced by construct 1 inhibited binding of Aβ-soluble aggregates to rat neuronal cultures in a concentration-dependent manner in an in vitro model of neutralization of Aβ-induced neuronal toxicity (**Figure 5**)
- Guinea pig serum inhibited the binding of Aβ to rat hippocampal neurons in a concentration-dependent manner, with no detectable inhibition at 1:1000 dilution of serum, 35% inhibition at 1:300, and 58% inhibition of binding at 1:100
- A representative image taken from the Operetta high-content imager (Perkin Elmer) is shown in **Figure 5A**, and quantification of the data is shown in **Figure 5B**

Figure 6. Tau–Heparin Sulfate Proteoglycan Interactions Occur Across a Broad Interface in Tau, Largely Within the MTBR Domain, and Are Believed to Be Critical for Tau Secretion and Uptake; Antibodies to MTBR Block Uptake Into Recipient Cell

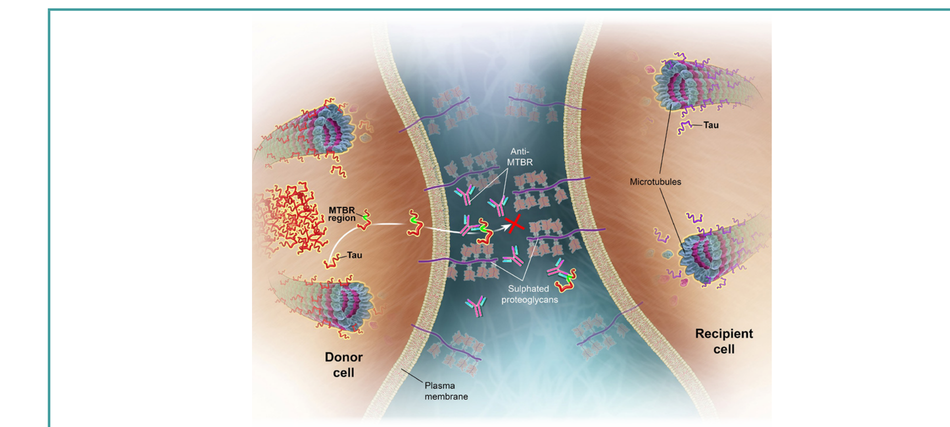
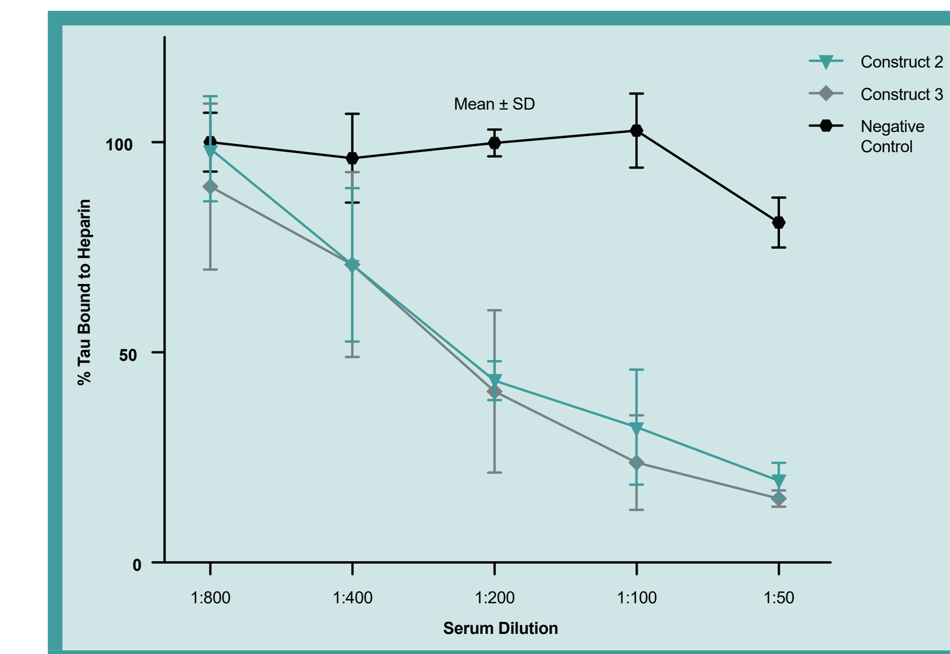
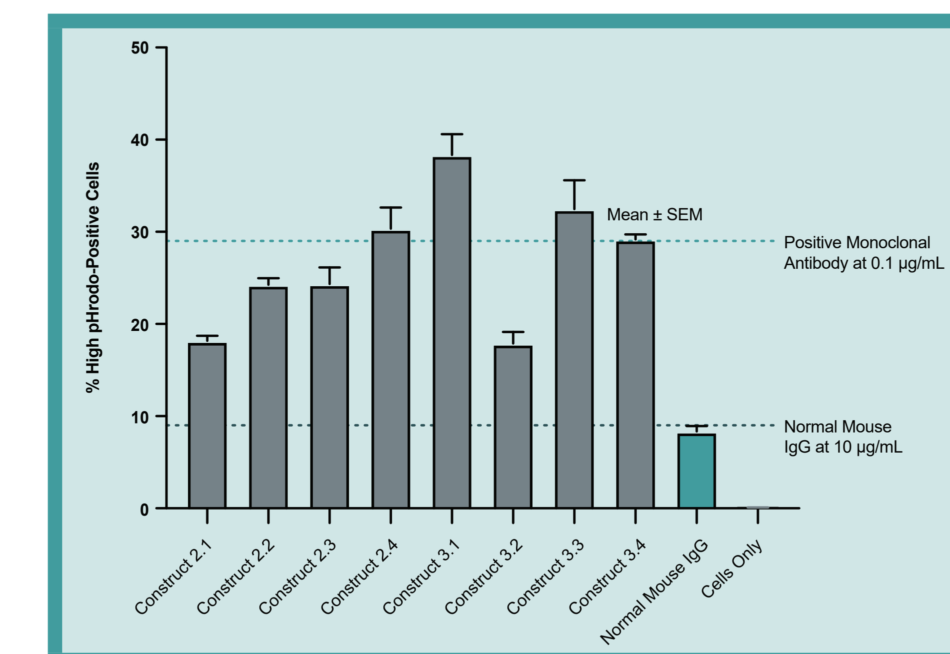


Figure 7. Constructs 2 and 3 Inhibit the Tau–Heparin Interaction in a Concentration-Dependent Manner



- Antibodies to tau induced by constructs 2 and 3 blocked the binding of tau to heparin in a concentration-dependent manner in an in vitro model of cellular uptake of tau (**Figure 7**)

Figure 8. Sera From Mice Immunized With Constructs 2 and 3 Induced Phagocytic Uptake of Aβ to an Extent Similar to That of Control Monoclonal Anti-Aβ Antibody



Light-green dotted line, binding level of the positive antibody; dark-green dotted line, binding level of nonimmune mouse IgG. Each bar represents an individual animal. IgG, immunoglobulin G.

- Constructs 2 and 3 induced phagocytosis of pHrodo-labeled Aβ aggregates in an in vitro model of plaque clearance (**Figure 8**)

Table 2. Summary of Construct Characteristics

Animal No.	Construct 1	Construct 2	Construct 3
Induced balanced titers	✓	✓	✓
Stained pathological Aβ and tau	✓	✓	✓
Induced response in NHPs	✓	TBD	TBD
Resulted in expected CSF/serum ratio	✓	TBD	TBD
Did not induce T-cell response	✓	TBD	TBD
Blocked Aβ-soluble aggregate binding to rat neurons	✓	TBD	TBD
Induced Aβ protofibril phagocytosis	TBD	✓	✓
Blocked tau binding to heparin	TBD	✓	✓

Aβ, amyloid beta; CSF, cerebrospinal fluid; NHP; nonhuman primate; TBD, to be determined.

- A summary of all data can be found in **Table 2**

CONCLUSIONS

- **Prothena's proprietary dual-vaccine constructs were shown to simultaneously induce balanced antibody titers to Aβ and tau in multiple animal experiments**
- **All 3 vaccine constructs presented here generated antibodies that strongly reacted with Aβ and tau pathology in human AD brain tissue**
- **Construct 1 produced an adequate antibody response profile in cynomolgus monkeys without eliciting a T-cell response to either Aβ or tau**
- **Central nervous system exposures of tau and Aβ antibodies were within 0.1% to 0.2% CSF/serum ratio, as expected**
- **Constructs 2 and 3 generated adequate antibody responses that induced phagocytosis of Aβ fibrils and blocked the binding of tau to an analog of heparin sulfate proteoglycan, a putative neuronal receptor of tau**
- **These results support the continued development of an active immunotherapeutic agent that simultaneously targets the two main pathological features of AD**

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AUTHOR DISCLOSURES

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