Development of a Dual Aβ/Tau Vaccine for the Prevention and Treatment of Alzheimer's Disease

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INTRODUCTION

- Alzheimer's disease (AD) is characterized by two main pathological hallmarks, amyloid beta (Aβ) plaques and neurofibrillary tangles composed of tau proteins[1]
- Multiple therapeutics targeting Aβ are being developed and aducanumab, currently under FDA priority review, showed signs of reducing clinical decline in late-stage clinical development[2]
- Several therapeutics targeting various tau epitopes that aim to inhibit the cell-to-cell transmission and spread of pathological tau are in clinical development[3,4]
- The majority of vaccines and passive immunotherapies target only one of the pathological AD features; however, there is strong evidence from preclinical models that Aβ and tau may act synergistically in the development of AD[5]
- Therefore, a vaccine concomitantly targeting Aβ and tau may be a more efficacious therapeutic for the treatment and prevention of AD

AIM

- To develop a single-agent, dual-immunogen vaccine to target both Aβ and tau, investigate its ability to induce an optimal (quantity and quality) and balanced immune response to both targets, and characterize the quality of the antibody response in functional assays

METHODS

Immunogen Design

- A linear peptide containing an Aβ N-terminal peptide and a tau microtubule-binding region (MTBR) peptide, a dendritic endopeptidase cleavage site (XX)[6] (between the two peptide sequences), and a C-terminal cysteine was conjugated to maleimide-activated cross-reactive material (CRM) 197 (Fina BioSolutions, Rockville, MD) (**Figure 1**)

Figure 1. Design of the Liner Dual Peptide



Study Design

- The in-life portion of the study was conducted at BTS Research (San Diego, CA)
- The guinea pig was selected as a proof-of-concept species because of the homology of both Aβ and tau sequence to the human sequence
- Guinea pigs were immunized with 50 µg of the immunogen described earlier and 25 µg QS21 adjuvant (Desert King, San Diego, CA) in Addavax (InVivogen, San Diego, CA) intramuscularly on week 0, 3, 7, and 11; test bleeds were taken 1 week after each injection

Titer Assay

- Guinea pig bleeds were titered by enzyme-linked immunosorbent assay (ELISA) against full-length recombinant tau (Proteos, Kalamazoo, MI) and Aβ 1-42 aggregates prepared from HFIP film (Bachem, Torrance, CA)
- Plates were coated overnight at 2 µg/mL in phosphate-buffered saline (PBS) and then blocked 1 hour with 1% bovine serum albumin (BSA) in PBS
- Bleeds were diluted in PBS/0.1% BSA/ 0/1% Tween 20 (PBS/BSA/T) starting at 1/100 and serially diluted 1:2
- Pre-bleed guinea pig serum was used as a negative control while known positive anti-serum from previous mouse studies was used as a positive control
- Plates were washed with TBS/Tween 20 and goat anti–guinea pig immunoglobulin G (IgG) (heavy + light chains) horseradish peroxidase (HRP) (IgG [H+L] HRP; Jackson, West Grove, PA) was added and incubated 1 hour at room temperature
- Plates were washed in TBS/Tween 20, and antibody binding was detected with o-phenylenediamine dihydrochloride (OPD) substrate (Thermo Fisher Scientific, Waltham, MA) following manufacturer's instructions
- Plates were read at 490 nm on a SpectraMax (Molecular Devices, San Jose, CA)

Immunohistochemistry

- Cryostat sections of fresh frozen AD brain tissue (Banner Sun Health Research Institute, Sun City, AZ) were stained with the guinea pig immune sera at two dilutions (1:300 and 1:1500)
- Binding of guinea pig antibodies was detected with a rabbit anti–guinea pig secondary antibody and a DAKO DAB Detection Kit (Agilent Technologies, Santa Clara, CA) as per the manufacturer's instructions
- The staining was processed using an automated Leica Biosystems Bond Stainer (Buffalo Grove, IL)

Blocking of Soluble Aβ Aggregates Binding to Neurons

- Blocking of soluble Aβ aggregates binding to primary rat hippocampal neurons was performed as previously described[7]
  - — Briefly, soluble biotinylated Aβ aggregates were pre-incubated with various dilutions of guinea pig serum for 30 minutes at 37 degrees and then added to neurons and incubated for another 30 minutes at 37 degrees
  - — Cells were fixed, permeabilized, and then incubated overnight with microtubule-associated protein 2 (MAP2; Abcam, Cambridge, MA), and NeuN primary antibodies (EMD Millipore, Burlington, MA)
  - — Cells were rinsed and secondary antibodies for MAP2, neuronal nuclei (NeuN), and streptavidin to image Aβ were incubated 1 hour
  - — Aβ binding to neurons was quantified by high-content imaging analysis

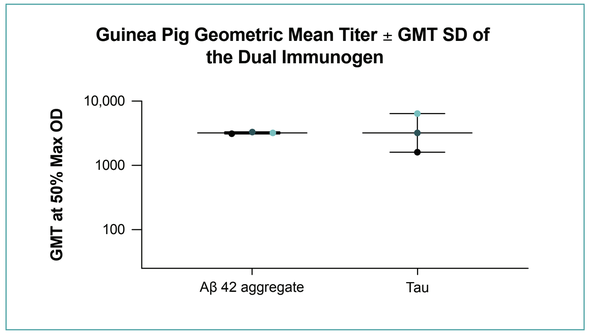
RESULTS

- The following data were derived from the terminal bleeds taken 1 week after the fourth injection

Titers

- The geometric mean titers to Aβ and tau were similar, with titer being defined as the lowest reciprocal dilution of serum equal to 50% of the maximum optical density with both the geometric mean titers of Aβ and tau quantitating at 3200 (**Figure 2**)

Figure 2. Guinea Pigs Developed a Balance Titer to Aβ and Tau

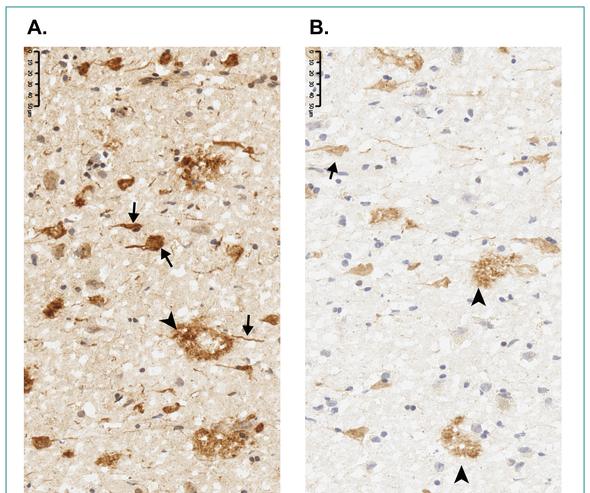


Colors indicate individual animals. GMT, geometric titer; OD, optical density; SD, standard deviation.

Immunoreaction to Aβ Plaques and Tau Tangles

- Immunohistochemistry showed that the immune sera from all three guinea pigs immunized with Aβ/tau vaccine immunoreacted to Aβ plaques, tau tangles, and dystrophic neurites
- **Figure 3** shows representative staining serum from one guinea pig at 1/300 and 1/1500
  - — The ability of a 1/1500 dilution to show strong binding to Aβ plaques and tau pathology indicates that an immune response could achieve therapeutic antibody levels in the brain at a 0.1% cerebrospinal fluid/plasma ratio, which is observed with most monoclonal antibodies delivered systemically

Figure 3. Immunohistochemistry With Guinea Pig Vaccine Serum Diluted at A. 1/300 or B. 1/1500

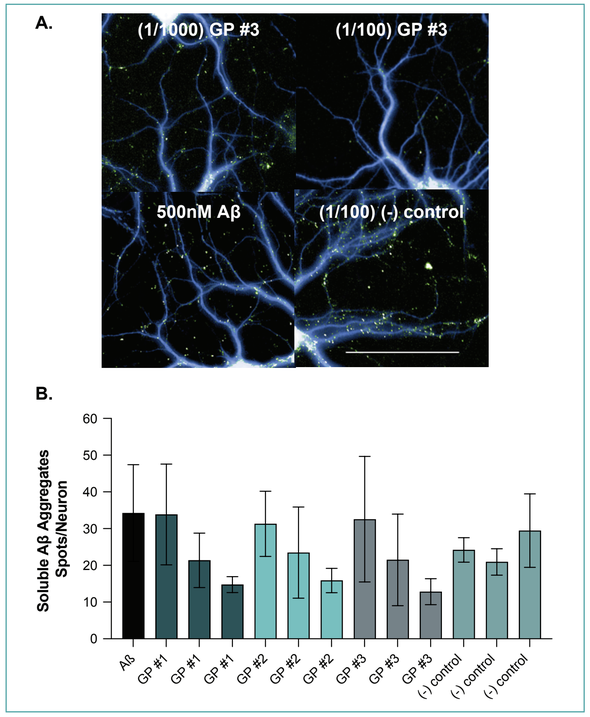


Arrows: tau pathology
Arrowheads: Aβ pathology

Blockade of Soluble Aβ Aggregate Binding to Neurons

- Guinea pig serum from all 3 guinea pigs immunized with Aβ/tau vaccine inhibited the binding of soluble Aβ aggregates to rat hippocampal neurons in a dose-dependent manner
- A representative image taken from the high-content imager is shown in **Figure 4A**, while quantification of the data is shown in **Figure 4B**

Figure 4. Guinea Pig Serum Inhibits Binding of Soluble Aβ Aggregates in a Dose-dependent Manner; A. Representative Image; B. Quantification of Binding of Soluble Aβ Aggregates to Neurons



GP, guinea pig.

CONCLUSIONS

- A dual-immunogen linear peptide vaccine construct was designed and shown to generate a balanced titer to Aβ and tau as measured by ELISA
- The immune sera reacted with Aβ and tau pathology by immunohistochemistry and blocked the binding of soluble Aβ aggregates to rat hippocampal cells
- Optimization of the construct and adjuvant are ongoing to facilitate an immunogen with optimal manufacturing characteristics and an optimal adjuvant to support the generation of balanced high titers in an elderly population
- These results support the development of a single, active immunotherapeutic agent targeting both pathological features of AD

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AUTHOR DISCLOSURES

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