Reduction of β-Amyloid and phospho-Tau in transgenic mice by a novel fusion protein bivalent for a General Amyloid Interaction Motif (GAIM)

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Introduction
Alzheimer’s disease (AD) is characterized by both amyloid β (Aβ) plaque depositions and intraneuronal neurofibrillary tau tangles accompanied by progressive cognitive decline. We have shown that NPT002 (filamentous bacteriophage M13) dose-dependently clears Aβ plaques, reduces phospho-Tau and restores normal cognitive performance in β-A42 mouse models. Recently, we isolated the protein motif from NPT002 responsible for the amyloid-interacting activity, which we call the general amyloid interaction motif or GAIM. The present studies were conducted to evaluate whether NPT088 (IgG1-Fc-GAIM) was also able to alter cognitive, biochemical and neuropathology endpoints in transgenic Aβ and Tau Alzheimer’s mouse models when administered systemically.

Methods
Mice: All mice were housed under LD 12:12 and provided access to food and water ad libitum. 2xTg-AD mice were obtained from Taconic and individually housed. Mice were 16.5-18 weeks old at study start. A colony was established from C57BL/6J and Tg671,1Swedish mice obtained from JaxMice. Three days prior to study start, mice were individually housed. Mice were 3-3.5 months at study start. All studies included an IgG dose of anti-C14 24h prior to the first dose of NPT088 to tolerate mice.

Behavior: Blind Observations. Mice were placed into an open field for 5 min and scored for spontaneous behaviors (displacements, corner jumps, spontaneous ambulations). Mice were placed into a 50cm-L x 50cm-W x 40cm-H open field arena for 10 min. Spontaneous Alternation. Mice were placed into a 30cm-diameter, 40cm-high, 40cm-deep circular arena. Mice were allowed to explore the arena for 5 min. Mice were exposed to 2 identical objects for 15 min during the sample phase. After a 4 min intertrial interval, mice were re-exposed to 1 familiar and 1 novel object for 10 min during the test phase.

Biochemistry: Brain tissue was homogenized in RIPA (1% (w/v) protease and phosphatase inhibitors), pipetted on ice and spun at 100,000× g (30 min at 4°C). The supernatant was collected as the soluble fraction. The remaining pellet was homogenized in high salt and spun at 45,000 rpm (30 min at 4°C). The supernatant was collected as the insoluble fraction. Human Aβ38 levels were measured with an Enzyme Immunoassay (ELISA).

Neuropathology: Coronal sections (40 µm) from immersion fixed (4% PFA) brains were cut on a sliding microtome. Floating sections were incubated with antibodies (1:1000) against Aβ (AT270), Tau (AT8), pSer422 (P2H/422), and AT270. Immunolabeling was visualized with ECL and quantified in a BioRad UVP Western Workstation.

Results & Conclusions:
Systemic administration of NPT088 to aged 2xTgAD mice resulted in significant:
- Reduction of stereotyped behavioral abnormalities.
- Increases in spatial working memory.
- Increases in short-term mnemonic memory.
- Reduction of AT8 and pSer422 in cortex and hippocampus.
- Reduction of Aβ plaque in hippocampus & cortex within 14 doses.
- No adverse events were observed.

Systemic administration of NPT088 to aged tTG4510 mouse resulted in significant:
- Neutralization of hyperactivity.
- Increases in spatial working memory.
- Increases in mnemonic memory.
- Reduction of AT8 and pSer422 in cortex.
- Reduction of Tau-associated neuropathology in hippocampus & cortex.
- No adverse events observed.

These findings indicate that systematically administered NPT088 is well tolerated, significantly reduces neuropathology and improves cognitive function in Aβ and Tau transgenic mouse models. Collectively, these data support the use of NPT088 as a novel therapeutic for reducing Aβ plaque and phospho-Tau.

Dose-dependent effects of systemic NPT088 in aged Tg2576 mice

Phage to Bivalent GAIM Fusions

Reduction of Stereotyped Motor Behaviors (Week 13)

Reversal of Cognitive Deficits

Reduction of Insoluble & Soluble Aβ42

Reduction of Aβ Plaque

Reduction of Tau Neuropathology

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