Introduction

Alzheimer’s disease (AD) is characterized by both amyloid plaques and intracellular neurofibrillary tangles accompanied by progressive cognitive decline. We have shown that NPT001 (filamentous bacteriophage M13) does dose-dependently clear Aβ plaques and restores normal cognitive performance in aged Tg2576 mice (Poster P4-287). Recently, we isolated the protein motif from NPT001 responsible for the amyloid-interacting activity, which we call the general amyloid interacting motif or GAIM (Poster P2-050). The present study was conducted to examine whether fusion proteins baited for GAIM could be made to clear Aβ aggregates in transgenic Alzheimer’s mouse models.

Intrahippocampal injection of either NPT001 (human IgG1–Fc–GAIM) or NPT003 (human IgG2–Fc–GAIM) into aged Tg2576 mice resulted in significantly lower Aβ plaques in hippocampus and surrounding necrosis. The decrease in Aβ was accompanied by significant increases in Iba-1 and synaptophysin. Similar to observations in aged Tg2576 mice, intrahippocampal injection of NPT008 into aged 3xTg mice resulted in significant decreases in Aβ in hippocampus and Aβ in the hippocampus. The timing and magnitude of the effects of NPT008 and NPT003 on Aβ in 3xTg mice and Tg2576 mice suggest systemic administration of NPT008 to wild-type mice produce brain penetration. Moreover, chronic systemic administration of NPT008 to aged Tg2576 mice ameliorated stereotypy behavioral phenotypes, cognitive deficits and significantly reduced Aβ plaques within 9 weeks of dosing. NPT008 and NPT014 reduced Aβ plaque and increased synaptic density within 7 days of direct-to-brain administration. Moreover, all Aβ plaque was reduced after 9 weeks of systemic dosing in transgenic mouse models. These data support the use of GAIM-containing molecules as a novel therapeutic approach for reducing Aβ plaque.

NPT002 is native filamentous bacteriophage M13. Circular single-stranded DNA phage

- Does not cause cell lysis
- Only infects E.coli plus (found in mammalian gut)
- Naturally occurring, not recombinant
- Mammals are chronically exposed with no reported adverse effects

NPT001 and NPT003 are naturally variants of M13 that are functionally identical in vitro and in vivo and used interchangeably

To determine whether bivalent GAIM fusion compounds were able to reduce Aβ plaque, aged (17–19 mo) male Tg2576 mice were intrahippocampally injected with 2µL of NPT008 (8.2 µg), 7 days after injection mice were euthanized and brains were explanted and fixed. 50µm sections were cut and Aβ plaque load was determined using Thioflavin S staining and a monoclonal antibody for Aβ (82E1). Significant, dose-dependent decreases in Aβ plaque were observed in hippocampus. N=12 mice/group. Values are mean±SEM, p<0.001, p<0.01, all comparisons to Tg PBS (Dunnnett’s test).

To determine whether bivalent GAIM fusion compounds were able to reduce Aβ plaque, aged (19–20 mo) 3xTg mice were intrahippocampally injected with 2µL of NPT008 (0.072 µg - 0.72 µg). 7 days after injection mice were euthanized, brains were explanted and fixed, and 50 µm sections were cut. Levels of phospho-Tau were determined using a monoclonal antibody for paired helical filaments (PHF1) and Aβ plaque load was determined using a monoclonal antibody for Aβ (82E1). No significant decreases in phospo-Tau were observed in hippocampus. Significant reductions in Aβ plaque, similar in magnitude to reductions observed in aged Tg2576 mice, were also observed. No pathology was observed in necrotic areas of aged 3xTg mice. N=7-9 mice/group. Values are mean±SEM, p<0.001, p<0.01, all comparisons to Human-Fc IgG control (Dunnnett’s test).

To determine if chronic, systemic administration of NPT008 could ameliorate cognitive deficits in aged Tg2576 mice, spontaneous alternation in the y-maze was assessed.

Spontaneous Alternation: To assess spatial working memory, testing was conducted over 10 min in a Y-maze was monitored 1 day after dose #12. Mice treated with NPT008 exhibited significantly more spontaneous alternation relative to mice treated with PBS. No significant differences were observed in the number of arm entries (not chosen) or the time spent in each arm.

Values are mean±SEM. N=6-8 mice/group. * p < 0.05, t-Test.

Figures:

**Fig 1.** NPT002, a novel biologic for neurodegenerative diseases

**Fig 2.** From Filamentous Phage to Bivalent GAIM Fusion Proteins

**Fig 3.** NPT002 decreased hippocampal Aβ plaque in aged male Tg2576 mice

**Fig 4.** Bivalent GAIM fusion compounds decrease Aβ plaque in aged Tg2576 mice

**Fig 5.** Bivalent GAIM fusion compounds increase Iba-1 and synaptophysin

**Fig 6.** Bivalent GAIM fusion compounds do not affect GFAP or induce microglia/macrophage

**Fig 7.** NPT008 reduces Aβ plaque and phospho-Tau in aged 3xTg mice

**Fig 8.** Systemic NPT008 ameliorates behavioral phenotypes in aged Tg2576 mice

**Fig 9.** Systemic NPT008 ameliorates cognitive deficits in aged Tg2576 mice

**Fig 10.** Systemic administration of NPT008 reduces Aβ plaque in aged Tg2576 mice