DEVELOPMENT OF NPT088 FOR TREATMENT Of NEURODEGENERATIVE DISORDERS

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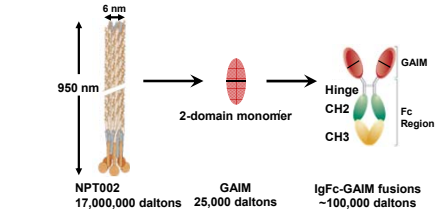
Introduction

Mis-folded protein aggregates are central to the pathobiology of neurodegenerative diseases, including HD. NeuroPhage Pharmaceuticals is developing novel therapeutics broadly targeting protein aggregation. We have shown that NPT002 (filamentous bacteriophage M13) potently dissociates and blocks assembly of a variety of amyloids.

Recently, we isolated the protein motif from NPT002 responsible for the amyloid-interacting activity, which we call the general amyloid interacting motif or GAIM. Our lead candidate, NPT088 (IgG_1-Fc-GAIM) improves cognitive and neuropathology endpoints in Alzheimer's mouse models when administered systemically.

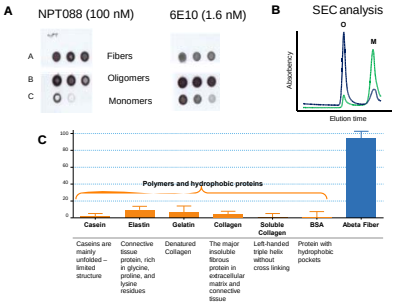
We here extend these studies to assess targeting of aggregated Huntingtin protein by GAIM.

From Filamentous Phage to Bivalent GAIM Fusion Proteins



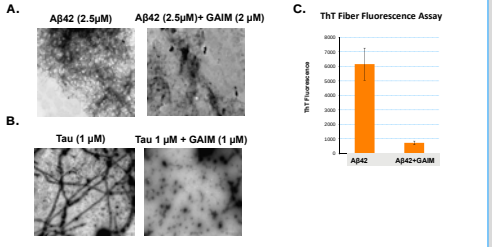
Recently we isolated the active region from NPT002, referred to as the general amyloid interacting motif ("GAIM"). NPT088 represents a bivalent GAIM fusion protein (human IgG_1-Fc-GAIM fusion).

GAIM specifically binds fibrillar and oligomeric Aβ assemblies



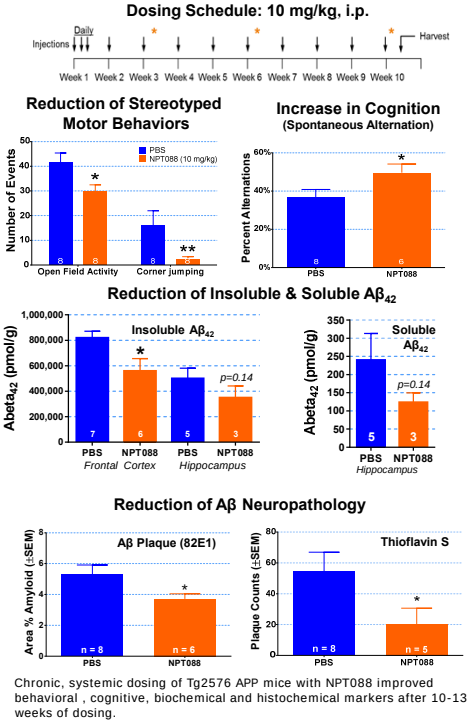
A. Aβ42 fibers were generated by incubating recombinant peptide in DMSO and HCl for 3 days at 37ºC, while oligomeric Aβ42 was created by incubating peptide in DMSO and PBS for 1 day at 25ºC and collecting the supernatant. NPT088 binds to fibers and oligomers spotted onto cellulose membranes, but not to monomers (whose presence was confirmed by probing with the anti-Aβ antibody 6E10). **B.** Size exclusion analysis of the Aβ preparations **C.** Comparision of NPT088 binding to non-amyloid proteins vis a vis Aβ42 fibers.

GAIM blocks Aβ and tau fibrillisation



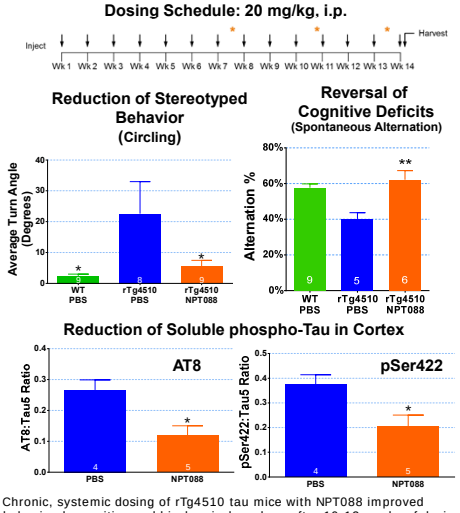
Aβ42 (2.5 μM) was incubated with or without GAIM (2 μM) for 7 days at 37ºC, and then subjected to TEM **(A)** or a Thioflavin T fluoresence assay **(C)**. Fibrillisation of Aβ42 was prevented by the presence of GAIM. Similar results were observed for tau fibril formation **(B)**.

Chronic, systemic NPT088 ameliorates behavioral, biochemical and neuropathological phenotypes in aged Tg2576 AD mice



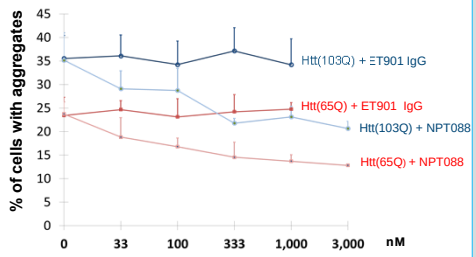
Chronic, systemic dosing of Tg2576 APP mice with NPT088 improved behavioral , cognitive, biochemical and histochemical markers after 10-13 weeks of dosing.

Chronic, systemic NPT088 amerliorates behavioral, cognitive & biochemical phenotypes in rTg4510 Tau mice



Chronic, systemic dosing of rTg4510 tau mice with NPT088 improved behavioral, cognitive and biochemical markers after 10-13 weeks of dosing.

NPT088 reduces aggregate burden in polyQ-Huntingtin expressing cells in culture



HeLa cell lines stably expressing a short (17 amino acids), GFP-tagged Htt fragment containing an expanded CAG (65 or 103 repeats) region were treated for 5 days with increasing concentrations of NPT088 from 30 nM to 3 μM. Sister wells were treated with equivalent molar concentrations of a negative control antibody, ET901 (Eureka Therapeutics). This is a fully-human IgG1 isotype control monoclonal antibody which binds specifically to a hapten molecule that does not exist in mammalian cells. PBS was also used.

Levels of GFP-fluorescent Htt aggregates in the cytoplasm of fixed cells were counted and expressed as a percentage of PBS control.

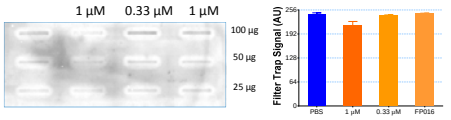
In both cell lines, NPT088 reduced the % of aggregate-positive cells in a concentration-dependent manner.

In HeLa 17aa Htt(65Q) cells, the % of aggregate-positive cells for each of the groups was: 23.7 ± 2.6% in PBS, 13.7 ± 1.4% at 1 μM NPT088 and 12.8 ± 3.2% at 3 μM M NPT088 (mean ± s.d.; p<0.0001 versus ET901).

In HeLa 17aa Htt(103Q) cells, the % of aggregate-positive cells for each of the groups was: 35.1 ± 5.8% in PBS, 23.1 ± 2.2% at 1 μM NPT088 and 20.7 ± 1.5% at 3 μM M NPT088 (mean ± s.d.; p<0.0001 versus ET901).

Data is derived from 3 independent experiments, each in triplicate.

NPT088 reduces levels of filter-trapped Htt aggregate in cultured cells



HeLa 17aa Htt(103Q) cells were treated for 6 days with 0.3 μM or 1 μM NPT088. Sister wells were treated with PBS or 1 μM FP016 (negative control human IgG_1-Fc).

Cells were pelleted, lysed in 1% Triton-X100 and denatured with 8M urea, before being loaded onto a BioRad slot blot with 0.2 μm pore cellulose acetate, blocked with 3% BSA (1h, rt) and probed overnight with anti-GFP.

Treatment with 1 μM NPT088 lowered the amount of Htt aggregate retained on the membrane.

Conclusions

- We have isolated a general amyloid interacting motif (GAIM) and fused it to a human IgG_1-Fc fragment; this development candidate is named NPT088
- GAIM/NPT088 specifically binds oligomeric and fibrillar Aβ42 while sparing monomers, and can prevent formation of Aβ42 and tau fibers. It can also disaggregate existing fibers (data not shown).
- Chronic systemic administration of NPT088 to mouse models of AD (Tg2576 and rTg4510) improves behavioral readouts and cognitive performance as well as biochemical and histopathological measures.
- We have extended these findings to mutant Htt and found that NPT088 treatment of cells over-expressing mutant Htt reduces aggregate burden as measured by fluoresence microscopy and filter trap assay.
- NPT088 is currently being tested in R6/2 mice for reduction of Htt aggregates.