Mutagenesis of the General Amyloid Interaction Motif (GAIM) Reveals a Structure-Activity Relationship for Misfolded Beta-amyloid and Tau Aggregates

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Introduction
The general amyloid interaction motif (GAIM) derived from the M13 phage tip protein gB2 binds a wide variety of amyloid aggregates in a conformation-dependent manner (Krishnan et al., 2014). Dimeric GAIM-gB2 fusions robustly bind and remodel Aβ42 amyloid aggregates and inhibit tau aggregation propagation in primary neurons and cells. In transgenic models of AD and tauopathy, GAIM-fusion treatment reduces Aβ plaque load, slows tau levels and improves cognition (Levinson et al., 2016). In this study, we explored the mechanism of GAIM-mediated remodeling of amyloid aggregates by mutagenesis. Using this data, we designed a next generation GAIM fusion, NPT189, which in addition to showing improved binding potency to multiple aggregates, has reduced potential for immunogenicity after removal of potential T-cell epitopes.

GAIM structure and its binding to Aß42 fibers

GAIM is a 2-domain protein that adopts an inverted horseshoe conformation. Amyloid binding is mediated by a combination of both hydrophobic and polar residues lining the inner surfaces of the horseshoe. For binding activity, GAIM domains must separately and adopt a more open conformation, which leads to the exposure of the active site. Opening and closing of the GAIM domains is controlled by cis-trans proline isomerisation and H-bonds in the flexible linker region (green).

H/D exchange studies show that GAIM binds to the central core of Aß42 fibers. This results in robust inhibition of amyloid assembly and efficient remodeling of fibers into amorphous aggregates (Krishnan et al., 2014).

Screening mutants: Aß42 amyloid binding

GAIM-ß fusion variants were screened for improved binding to Aß42 fibers and counter-screened for binding to soluble collagen aggregates. Top candidates with high Aß42 fiber binding and low collagen binding were then screened to binding to other amyloids like Tau-K18, fibril aggregates, and variant exhibiting potent binding in both primary and secondary screens were assessed for non-specific binding to unfolded or hydrophobic sequences using a larger set of proteins like ßA4, scrambled ßA, and galactin.

We identified over 15 mutants that show improved Aß42 fiber binding (EC50<50 nM) and 20 with improved tau-K18 binding (EC50<35nM). Candidates that bind to both amyloids were used in the remodeling assay.

Screening mutants: Remodeling amyloids

GAIM-fusions engage multiple regions on an amyloid fiber and binding often leads to structural rearrangement or remodeling of amyloid fibers. This was verified using fiber diffraction, TEM and cellulose-aceate filter-trap assays (Krishnan et al., 2014). We observed Aß42 fibers remodeled by GAIM-ß fusions dissolve much faster in a denaturant like urea than fibers alone. We used sub stoichiometric amounts of GAIM-ß fusions (25 Aß42 monomers to 1 GAIM-ß) to calculate remodeling efficiencies of the new variants. Variants that show improved binding to Aß42 fibers also showed improved remodeling activities. This remodeling is both time- and concentration-dependent for variants like NPT189. This unique activity might facilitate micellar clearance of amyloid-GAIM fusion complexes.

Mutagenesis Strategy

In this study 2 separate mutagenesis screens were employed to improve the therapeutic properties of the drug candidate. The first screen involved mutating and selecting potential T-cell epitopes on GAIM-ß fusions. Five potential sequences were identified and variants were sequentially removed without affecting the binding properties of GAIM.

The second screen aimed at improving the potency, specificity, stability and pharmacokinetics of the fusion proteins. Mutations that alter protein expression levels were studied and variants that poorly express were eliminated. Variants from both screens were combined to create over 20 next generation therapeutic candidates. All the variants retain their ability to bind a diverse range of amyloid aggregates (consisting of ßA, ßA and ß-amyloid and ß-light chain)

NPT189 engages a wide variety of LC aggregates

Immunoalbumin light chain (LC) aggregates are the most diverse group of amyloids. Patients carry multiple mutations and there is generally no correlation between specific mutations and the nature of mutations in the variable region. To gain insights into binding specificity to amyloids we studied the interaction of GAIM fusions with various LC aggregates. NPT189 engages a very broad range of aggregates derived from patients and localizes to LC deposits in transgenic mice.

Next Generation Ig-GAIM NPT189

• NPT189 shows potent binding to Aß42 fibers (K≈ 2.6 nM). It remodels Aß42 fibers potently at sub stoichiometric concentrations (EC50<100 nM in a 2.5μM reaction).

• NPT189 blocks seeded and unseeded assembly of amyloid aggregates. Efficiency of inhibition depends on stoichiometry of aggregation and the seeding efficiency. NPT189 (100 nM) decreased Tau-K18 (25μM) effective seeding concentration by 900-fold.

• NPT189 blocks cell-to-cell transmission of α-synuclein aggregates in a microfluidic triple chamber system. α-synuclein pre-formed fibrils (PPF) were added in chamber 1 and NPT189 was added in chambers 2 and 3. Aggregates were reduced in both chambers 2 and 3, demonstrating inhibition of propagation.

• NPT189 reduces incidence of motor deficits and neuroinflammation in propagation model mice intrastriatally injected with a-synuclein preformed fibrils.

Conclusions
• Studies of genetic variants has elucidated a GAIM structure activity relationship for interactions with amyloid fibrils.
• GAIM binding and amyloid fiber remodeling activities correlate well.
• NPT189 is a next generation fusion with both higher potency amyloid targeting and lower potential for immunogenicity in humans.
• NPT189 blocks cell to cell transmission of α-synuclein aggregates in primary neuronal cultures and reduces motor deficits and neuroinflammation in a mouse model for an- synuclein aggregate propagation.

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