Targeting of Aβ toxic oligomers via a designed peptide reduces plaque formation in an AD mouse model

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Background

The recent approval of Alzheimer's Disease (AD) therapies targeting amyloid-β (Aβ) pathology, including Lecanemab and Donanemab, has renewed enthusiasm for this target and paved the way for new advances targeting even earlier in disease progression.

While AD is associated with the eventual accumulation of large, insoluble Aβ plaques in the brain, earlier low molecular weight soluble oligomers are considered to be the toxic element, causing disruption of synaptic transmission, neuroinflammation, and tau phosphorylation (1). These oligomers form a nonstandard protein structure, termed α-sheet, which is a defining feature of these toxic oligomers (2). We have designed peptides that selectively target α-sheet structure for diagnostic and therapeutic purposes.

Here we describe the specificity of our lead compound, Soluble Oligomer Binding INhibitor (SOBIN), for α-sheet oligomers over other forms of Aβ. We next explored SOBIN's effects on phagocytosis by microglia *in vitro*. We then measured the *in vivo* effects of SOBIN on learning and memory and on plaque burden in the Tg2576 AD mouse model. It has been reported in the literature that the Tg2576 mouse model has deficits in long-term potentiation and cognition starting as early as 4 months of age (3). We initially tested whether SOBIN had positive effects with pre-cognitive impairment treatment. Finally, given than many patients are likely to begin treatment after symptoms begin, we expanded our efficacy studies to determine whether beginning SOBIN treatment after the onset of cognitive impairment was effective.

Methods

SOBIN was produced using conventional solid-phase peptide synthesis using previously described methods (4). α-sheet enriched Aβ oligomers were generated as described previously (2).

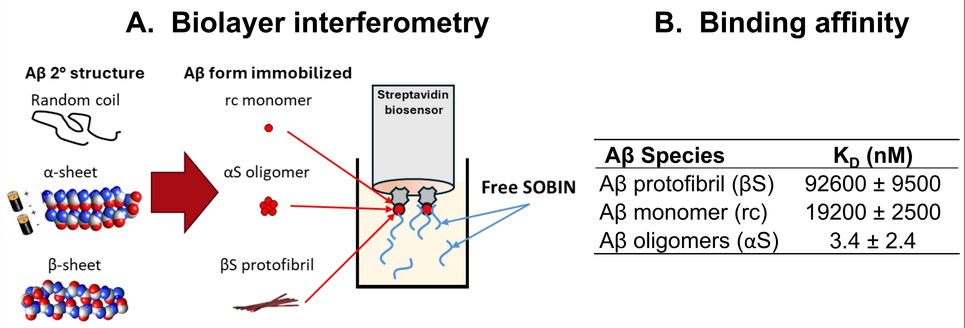
The affinity of SOBIN to random coil monomers, α-sheet oligomers, and β-sheet protofibrils was measured using biolayer interferometry (BLI).

Microglial uptake of Aβ was investigated using EOC-20 microglial cells with or without SOBIN and 0.5μM α-sheet enriched Aβ oligomers tagged with either AlexaFluor 647 (for imaging) or tagged with AlexaFluor 555 (for florescence quantification) for 24h.

In vivo efficacy of SOBIN for pre-MCI mice was evaluated in ~3-month-old Tg2576 AD mice and WT controls treated for 12 months intranasally with vehicle or SOBIN dosed 3x/week. MCI efficacy was evaluated in ~6-month-old Tg2576 AD mice and controls treated for 9 weeks. Mice were subjected to Morris water maze (MWM) behavioral testing at ~9-months-old (asymptomatic) and ~8.5-months-old (impaired). Brains were collected for Aβ plaque (6E10) or synaptophysin staining at 15-months-old (asymptomatic).

Results

SOBIN binds Aβ oligomers 27,000-fold stronger than protofibrils



Aβ Species	K_D (nM)
Aβ protofibril (βS)	92600 ± 9500
Aβ monomer (rc)	19200 ± 2500
Aβ oligomers (αS)	3.4 ± 2.4

Figure 1. A) Schematic diagram of BLI experimental setup. **B**) Binding affinities for SOBIN dissociation from different Aβ species.

SOBIN enhances uptake of Aβ toxic oligomers by microglia

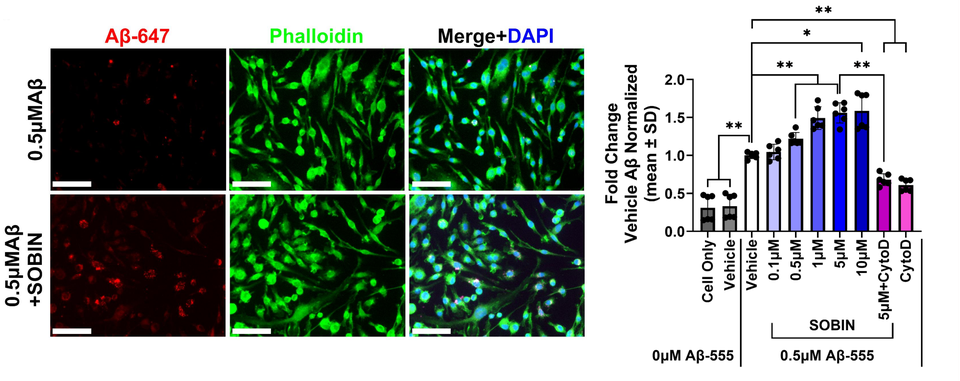


Figure 2. A) Representative images of cells exposed to toxic Aβ oligomers (red, Aβ with AlexaFluor 647 dye) alone (top row) or Aβ oligomers + 5μM SOBIN (bottom row). Cell bodies (phalloidin) shown in green (middle column). Merged Aβ-647 and phalloidin signals with nuclear DAPI staining (blue) shown in the right column. Scale bar, 50 μm. **B**) Fluorescence intensity quantification of SOBIN-facilitated uptake of oligomers in microglia cells. Cytochalasin D (CytoD) was used to confirm SOBIN's effect on phagocytosis. *n*=6. * p<0.05, ** *p*<0.01.

References

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Results

SOBIN treatment improves cognition in cognitively impaired AD mice

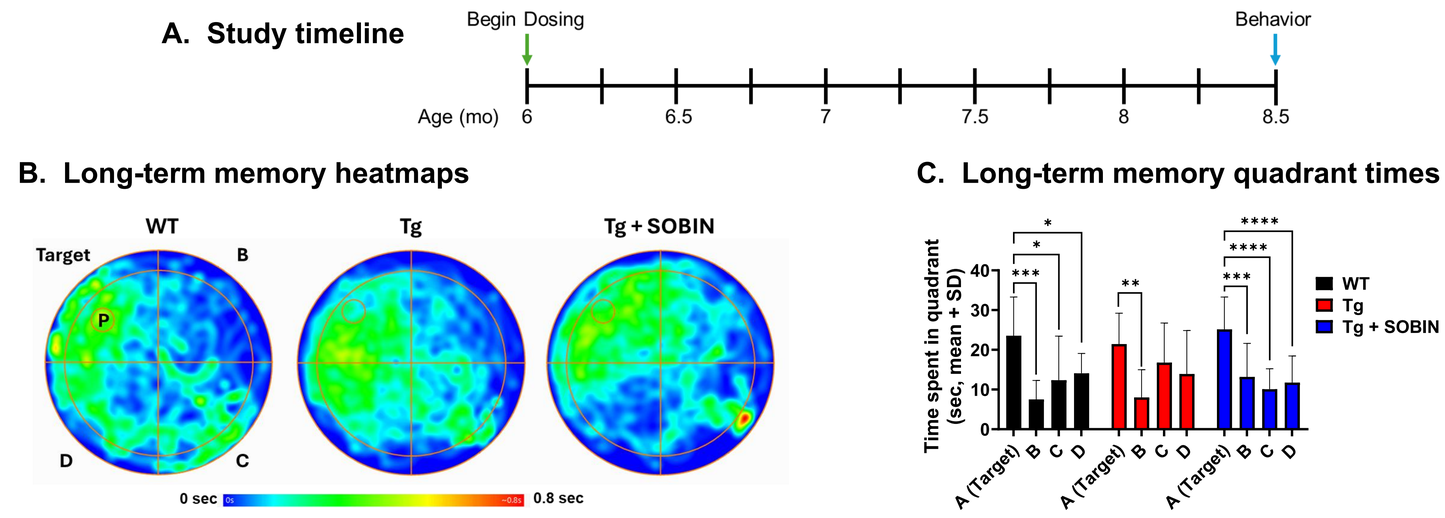

Figure 3. A) Experimental timeline of Tg2576 AD mouse (Tg) efficacy study. 6-month-old mice were dosed 3x/week intranasally with either Vehicle or 100 μg SOBIN for 9 weeks. **B**) Group average heatmaps of long-term memory probe trial in the Morris water maze performed after 9 weeks of dosing. The submerged platform (P) was in the upper left target quadrant. Mice were initially placed in quadrant C. **C**) Quantification of time spent in each quandrant during the short-term memory probe. WT *n*=9; Tg *n*=12; Tg + SOBIN *n*=13. * *p*<0.05, ** *p*<0.01, *** p<0.001, **** *p*<0.0001. WT, wild type. Tg, transgenic.

SOBIN treatment improves cognition and reduces Aβ plaque and synaptophysin loss in asymptomatic AD mice

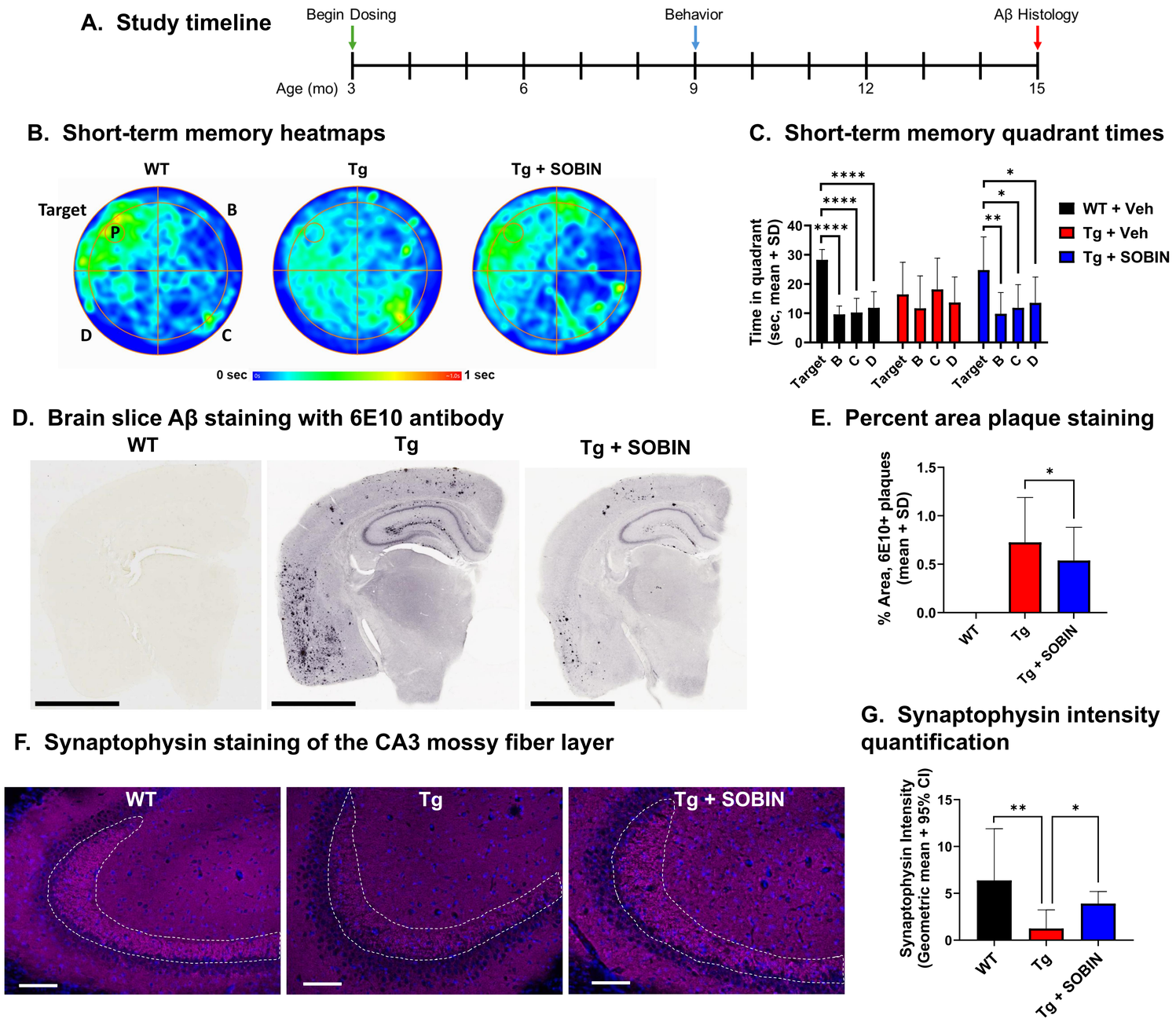

Figure 4. A) Experimental timeline of Tg2576 AD mouse (Tg) efficacy study. Mice were dosed 3x/week intranasally with either Vehicle or 100 μg SOBIN for 12 months. **B**) Group average heatmaps of short-term memory probe trial in the Morris water maze performed after 6 months of dosing. The submerged platform (P) was in the upper left target quadrant. Mice were initially placed in quadrant C. **C**) Quantification of time spent in each quandrant during the short-term memory probe. WT *n*=8; Tg *n*=16; Tg + SOBIN *n*=9. **D**) Representative brain slice images with Aβ staining (6E10 antibody). Scale bar, 5mm. **E**) Quantification of the percent area covered by 6E10 plaque staining of 10 sections from 7 animals for a total of *n*=70 sections per group. **F**) Representative images of the mossy fiber layer (dotted white outline) of the CA3 region in the hippocampus stained for synaptophysin (magenta) and nuclei (blue). Scale bar, 100μm. **G**) Quantification of the synaptophysin staining intensity of the mossy fiber layer. *n*=6-7 animals per group. * *p*<0.05, ** *p*<0.01, **** *p*<0.0001. WT, wild type. Tg, transgenic.

Conclusions

- SOBIN is highly selective for toxic α-sheet Aβ oligomers over other species of Aβ
- SOBIN enhances phagocytosis of toxic α-sheet Aβ oligomers by microglial cells
- SOBIN treatment provides benefits to cognition when started post-cognitive impairment onset
- SOBIN treatment prior to onset of cognitive impairment in Tg2576 mice improves cognition
- SOBIN treatment reduces Aβ plaque deposition and mossy fiber synaptophysin expression