Small Interfering RNA Targeting Amyloid-Beta Precursor Protein Reduces Alzheimer's Disease Pathology in 5xFAD Mice and Abrogates Behavior Changes in Early Intervention Paradigm

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Effect of APP-lowering siRNA on Alzheimer's Disease (AD) Pathology Assessed in 5xFAD Mouse Model of AD: Experimental Design and Rationale

Figure 1: Mechanism of action of APP-Lowering siRNA

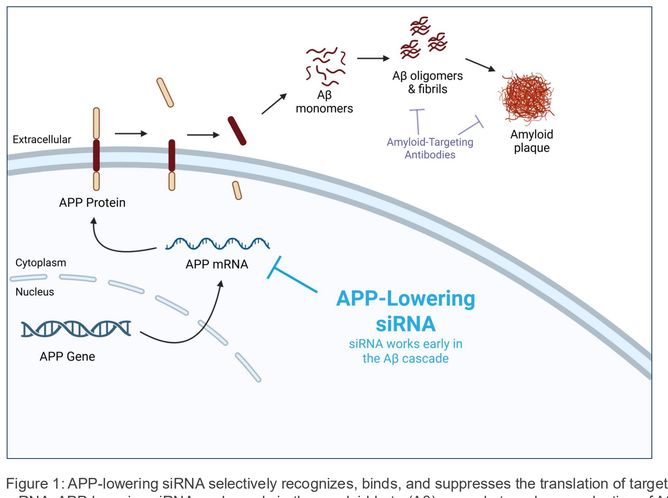

Figure 1: APP-lowering siRNA selectively recognizes, binds, and suppresses the translation of target mRNA. APP-lowering siRNA works early in the amyloid-beta (Aβ) cascade to reduce production of Aβ precursor protein (APP). This targeting strategy works upstream of interventions like anti-Aβ antibodies.

Study Objective:

To examine the impact of APP-lowering siRNA on AD pathology in the 5xFAD mouse model when administered at early or late disease stages

- APP-lowering siRNA has potential to impact AD pathology by targeting APP mRNA, upstream of Aβ production and aggregation (**Figure 1**).
- Impact of early and late intervention paradigms with APP-lowering siRNA were investigated in the 5xFAD mouse model, which overexpress human APP and PS1 harboring known AD associated mutations, causing them to rapidly develop AD pathology.
- **Methods:**
  - 5xFAD mice and NonTg controls were dosed via intracerebroventricular (ICV) injection at indicated low and high doses and timepoints (**Figure 2**).
  - The impact of APP-lowering siRNA intervention on amyloid burden, neuroinflammation, neurofilament light chain (NfL), and disease-related behavioral outcomes were assessed in life or in terminal tissue.
  - Studies were terminated when the animals reached 12 months of age.

Figure 2: Experimental design

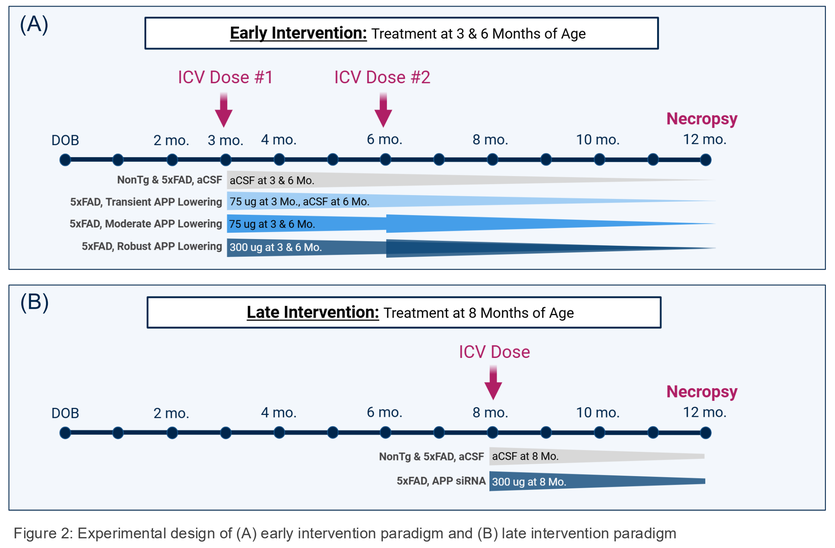

Figure 2: Experimental design of (A) early intervention paradigm and (B) late intervention paradigm

APP-Lowering siRNA Reduces AD Pathology for Both Tissue and Plasma Biomarkers in 5xFAD Mice

Figure 3. Early Intervention: Dose-dependent reduction of cortical amyloid burden

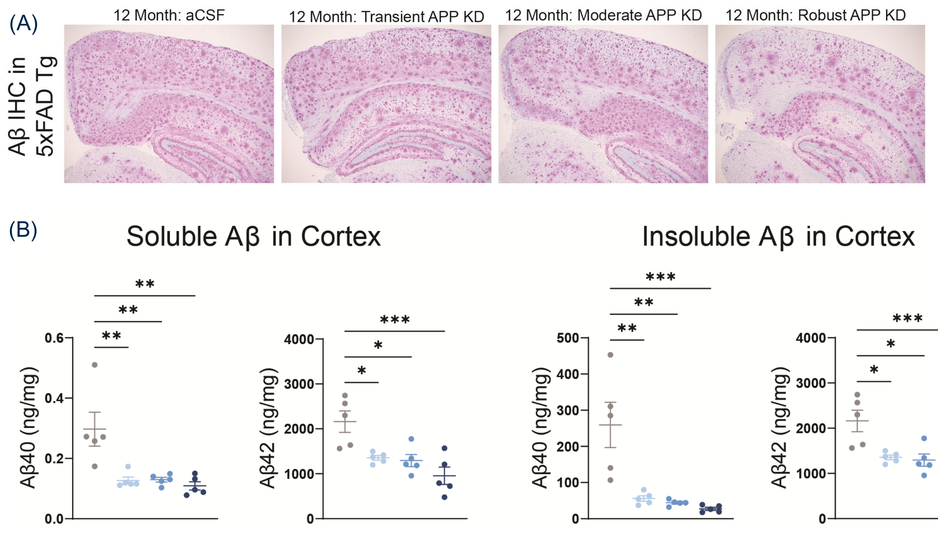

Figure 3: (A) Immunohistochemical stain of Aβ in coronal brain sections, and (B) biochemical analysis of amyloid burden using MSD in cortex samples (Ordinary one-way ANOVA; Soluble Aβ42 p = 0.001; Insoluble Aβ42 p = 0.0027; Tukey Multiple comparisons; *, p < 0.05; **, p < 0.01, ***, p < 0.001).

Figure 4. Early Intervention: Dose-dependent reduction in plasma NfL and cortical GFAP

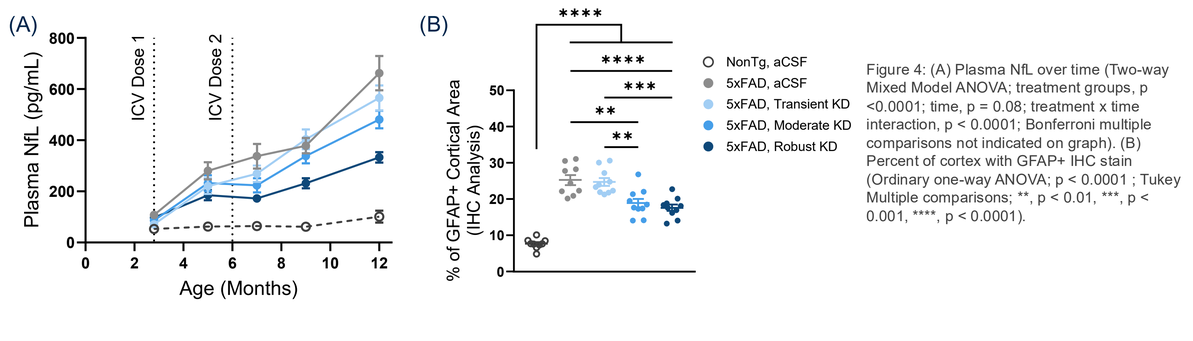

Figure 4: (A) Plasma NfL over time (Two-way Mixed Model ANOVA; treatment groups, p <0.0001; time, p = 0.08; treatment x time interaction, p < 0.0001; Bonferroni multiple comparisons not indicated on graph). (B) Percent of cortex with GFAP+ IHC stain (Ordinary one-way ANOVA; p < 0.0001 ; Tukey Multiple comparisons; **, p < 0.01, ***, p < 0.001, ****, p < 0.0001).

Figure 5. Late Intervention: Reduction in cortical amyloid burden compared with baseline and compared with age-matched aCSF-treated animals

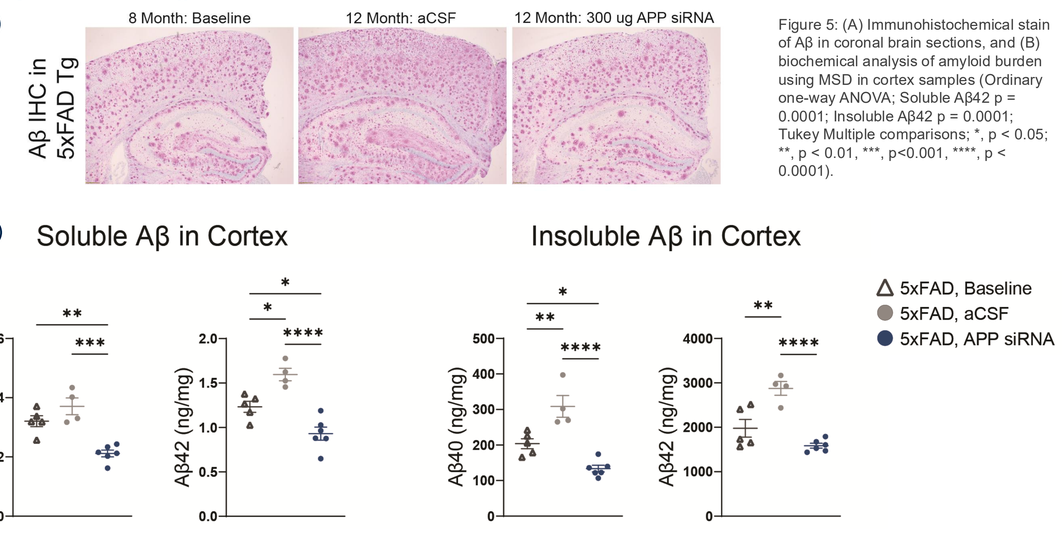

Figure 5: (A) Immunohistochemical stain of Aβ in coronal brain sections, and (B) biochemical analysis of amyloid burden using MSD in cortex samples (Ordinary one-way ANOVA; Soluble Aβ42 p = 0.0001; Insoluble Aβ42 p = 0.0001; Tukey Multiple comparisons; *, p < 0.05; **, p < 0.01, ***, p<0.001, ****, p < 0.0001).

Figure 6. Late Intervention: Reduction in plasma NfL and cortical GFAP

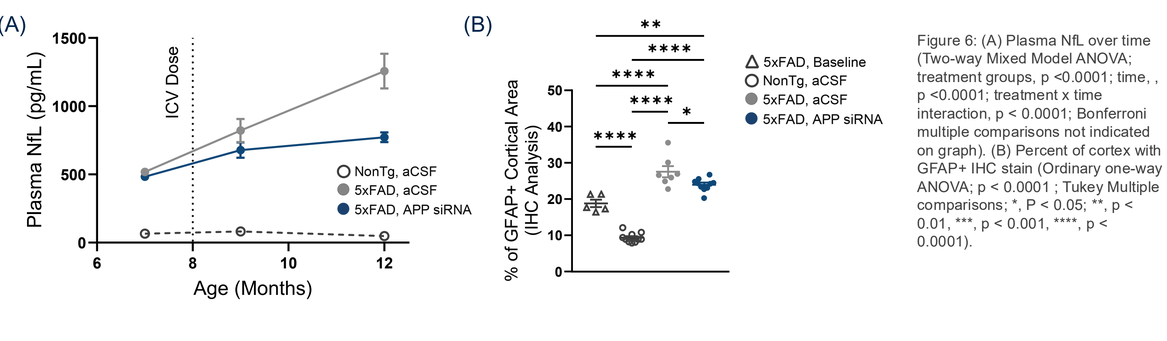

Figure 6: (A) Plasma NfL over time (Two-way Mixed Model ANOVA; treatment groups, p <0.0001; time, , p <0.0001; treatment x time interaction, p < 0.0001; Bonferroni multiple comparisons not indicated on graph). (B) Percent of cortex with GFAP+ IHC stain (Ordinary one-way ANOVA; p < 0.0001 ; Tukey Multiple comparisons; *, P < 0.05; **, p < 0.01, ***, p < 0.001, ****, p < 0.0001).

APP-Lowering siRNA Abrogates Behavior Changes in Early Intervention Paradigm

The elevated plus maze (EPM) behavioral test was used to assess behavioral abnormalities in 5xFAD mice. 5xFAD mice increase the time spent in the open arms of the EPM as disease pathology progresses. Increased time spent in the open arms is representative of abnormal behavior and decreased anxiety-like behavior.

Figure 7A. Early Intervention: Dose-dependent change in behavior phenotype with robust APP KD resulted in similar behavior to non-Tg animals at all timepoints

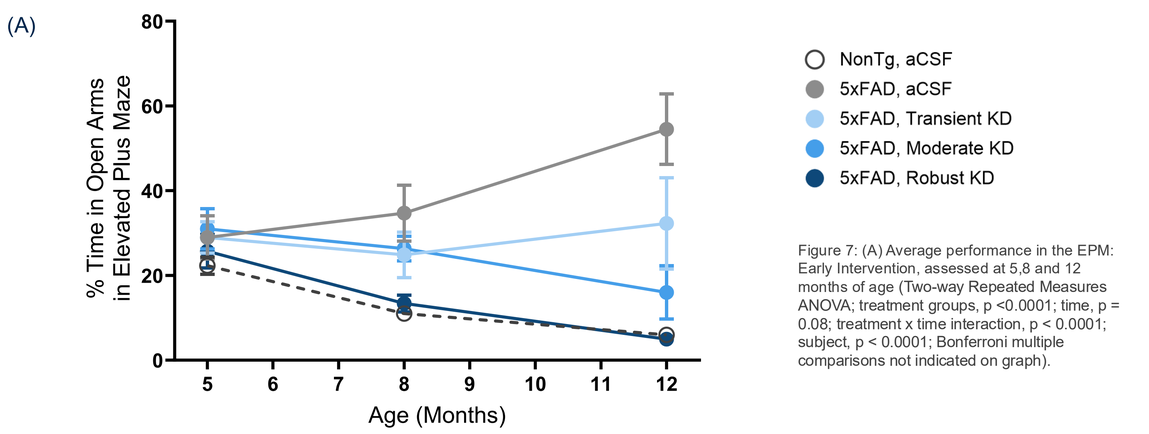

Figure 7: (A) Average performance in the EPM: Early Intervention, assessed at 5,8 and 12 months of age (Two-way Repeated Measures ANOVA; treatment groups, p <0.0001; time, p = 0.08; treatment x time interaction, p < 0.0001; subject, p < 0.0001; Bonferroni multiple comparisons not indicated on graph).

Figure 7B. Late Intervention: No significant change in behavior phenotype at 12 months of age in siRNA-treated compared with age-matched aCSF-treated animals

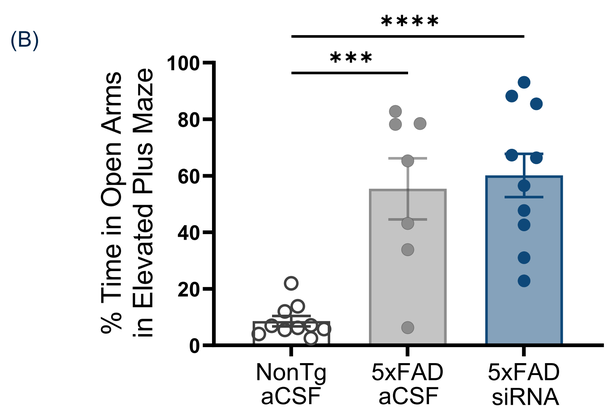

Figure 7: (B) Average performance in the EPM : Late Intervention, assessed at 12 months (Ordinary one-way ANOVA; p < 0.0001; Tukey Multiple comparisons; ***, p < 0.001, ****, p < 0.0001)

Conclusions

- **Treatment with APP-lowering siRNA reduced Alzheimer's disease pathology in the 5xFAD mouse model when administered in both late or early disease stages.**
- **Early intervention lowered amyloid burden in tissue, reduced markers of glial inflammation, reduced plasma NfL, and decreased abnormal behavior in the elevated plus maze, all in a dose-dependent manner.**
  - **Robust lowering of APP with APP-lowering siRNA completely prevented the emergence of disease associated-abnormal behavior.**
- **Late intervention treatment lowered cortical amyloid burden to levels below the 8-month baseline and reduced elevations in GFAP and NfL compared with aCSF-treated animals.**
- **These results support the continued development of mivelsiran, an investigational, first-in-class APP-lowering RNAi therapeutic, in patients with AD (NCT05231785).**

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Abbreviations: Aβ (amyloid-beta), AD (Alzheimer's Disease), ANOVA (analysis of variance), APP (amyloid-beta precursor protein), aCSF (artificial cerebrospinal fluid), EPM (Elevated Plus Maze), GFAP (glial fibrillary acidic protein), IHC (immunohistochemistry), KD (knockdown), MSD (Meso Scale Discovery Immunoassay), NfL (Neurofilament Light Chain), NonTg (nontransgenic, healthy control), PS1 (presenilin-1 protein), RM ANOVA (Repeated Measures ANOVA), RNAi (RNA interference), SIMOA (Single Molecule Array) siRNA (small interfering RNA)