Discovery of a Novel C3-Targeting and CNS Active siRNA as a Potential Therapeutic for Alzheimer's Disease

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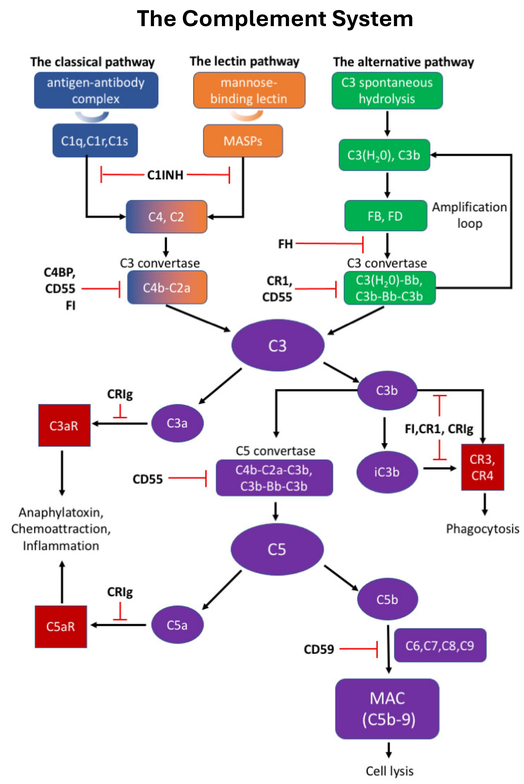
Background: Complement proteins are key modulators of innate immunity involved in defense against pathogens. The central component, C3, is an attractive therapeutic target for Alzheimer's disease (AD), due to its location at the nexus of the 3 known activation pathways, and key roles in neuroinflammation and synaptic pruning. C3 mRNA is elevated in post-mortem AD brains and correlates with Braak staging, C3 protein and its breakdown products are elevated in AD patient CSF, and C3 gene knockdown in ageing, amyloid or tauopathy mouse models is synaptoprotective and attenuates measures of cognitive decline.

Small interfering (si)RNAs drive post-transcriptional silencing of a gene target through a process known as RNA-interference (RNAi), harnessing the intrinsic machinery of target cells in a catalytic manner, therefore delivery of a small amount of drug can yield a potent pharmacodynamic effect. Thus, siRNAs represent an attractive modality for targeting abundant disease targets, such as C3. Increasing the hydrophobicity of siRNAs has been found to enhance distribution through CNS following direct-to-CSF routes of administration, enabling siRNA delivery to CNS tissues. Here, we present the discovery of a novel siRNA for selective silencing of C3 in CNS.



Chen, Ying, *et al*. "The complement system in the central nervous system: from neurodevelopment to neurodegeneration." *Biomolecules* 12.2 (2022): 337.

Figure 1: *In vitro* Pharmacology. Dose response activity curves of C3-targeting siRNAs *in vitro*. A) Screening funnel to ID lead siRNA. B) A library of siRNAs with different sequences and modification patterns were assessed for activity in a transfection-based study in HepG2 cells (example of lead siRNA + 4 additional compounds shown). C) A subset of potent siRNAs from the HepG2 assay were synthesized with a GalNAc conjugate and assessed for activity in a free uptake study in primary human hepatocytes. D) Primary human astrocytes were treated with TNF-α to stimulate C3 gene expression, then siRNAs were transfected into cells and potency was evaluated.

Figure 2: Immune-Stimulatory Potential. Heat map data of the immune-stimulatory potential of a lead siRNA in a human PBMC transfection assay. The lead siRNA was assessed for its ability to stimulate expression of A) IP-10 and B) MIP-1α in hPBMCs from 3 donors. Positive and negative controls were used to verify functionality of the assay and ensure normal reactivity of the donor cells, respectively.

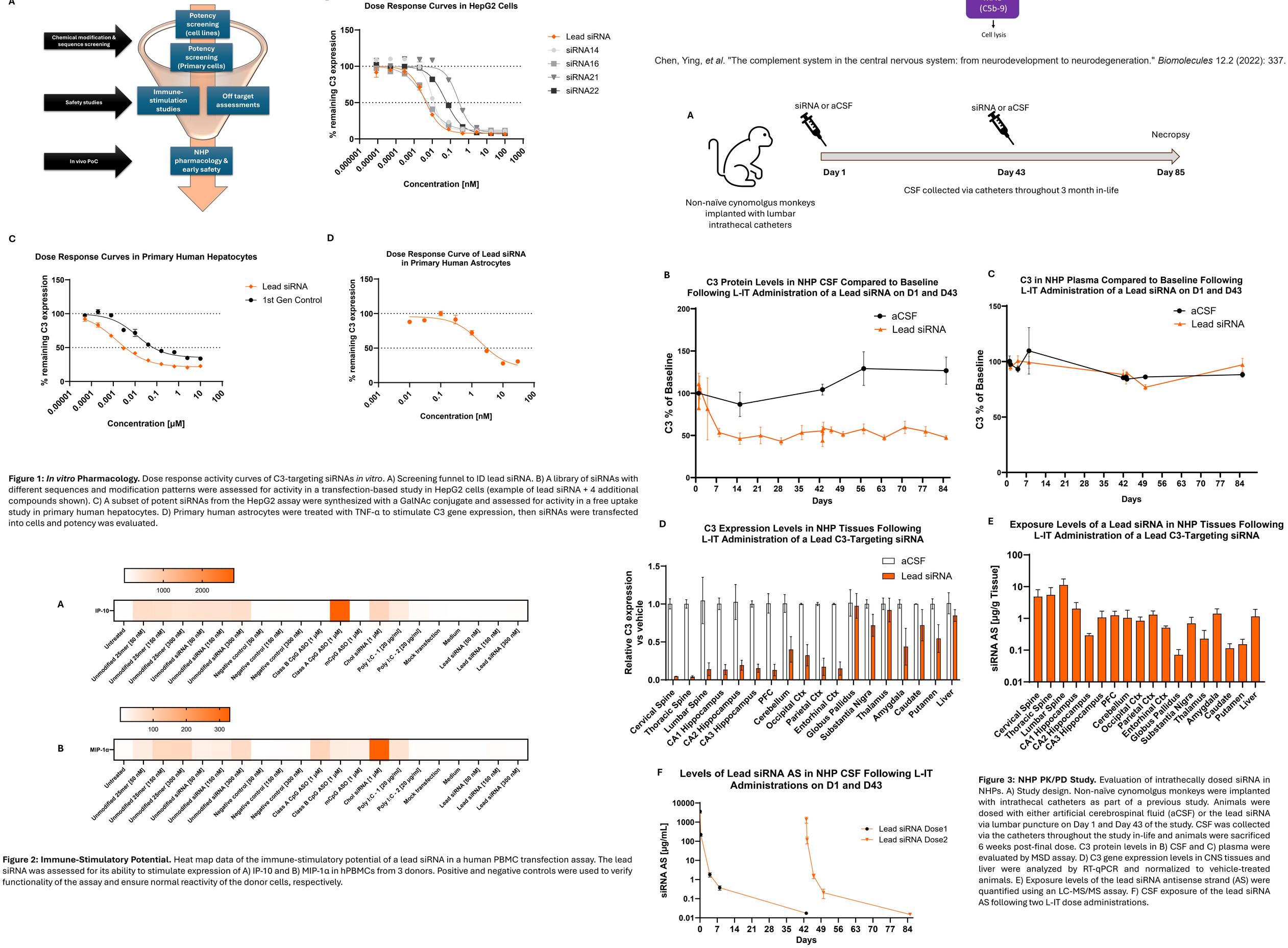


Figure 3: NHP PK/PD Study. Evaluation of intrathecally dosed siRNA in NHPs. A) Study design. Non-naïve cynomolgus monkeys were implanted with intrathecal catheters as part of a previous study. Animals were dosed with either artificial cerebrospinal fluid (aCSF) or the lead siRNA via lumbar puncture on Day 1 and Day 43 of the study. CSF was collected via the catheters throughout the study in-life and animals were sacrificed 6 weeks post-final dose. C3 protein levels in B) CSF and C) plasma were evaluated by MSD assay. D) C3 gene expression levels in CNS tissues and liver were analyzed by RT-qPCR and normalized to vehicle-treated animals. E) Exposure levels of the lead siRNA antisense strand (AS) were quantified using an LC-MS/MS assay. F) CSF exposure of the lead siRNA AS following two L-IT dose administrations.

Conclusions:

- The central complement component C3 plays a putative role in neurodegeneration and neuroinflammation in Alzheimer's Disease
- siRNAs represent a viable therapeutic strategy to target complement gene expression in the CNS
- Here we present the discovery of a potent novel C3-targeting siRNA, with silencing activity in Alzheimer's relevant brain regions in NHP
- Preliminary studies suggest this compound is non-immunostimulatory and is tolerated in NHPs