Widespread CNS Tau Knockdown for the Potential Treatment of Alzheimer's Disease and Other Tauopathies

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Introduction

- The neuronal accumulation of misfolded, neurotoxic species of Microtubule Associated Protein Tau (*MAPT*) closely tracks with neurodegeneration, disease progression, and clinical symptoms in Alzheimer's disease (AD), Progressive Supranuclear Palsy (PSP) and over a dozen other tauopathy disorders.
- Reducing *MAPT* expression is well-tolerated and efficacious in tauopathy animal models. We are developing an IV-administered, epigenetic regulation approach to reduce tau expression using an engineered zinc finger repressor (ZFR).
- Here, we report the development of a lead ZFR that targets the human *MAPT* locus with high potency and specificity across several in vitro and in vivo models.
- We also show widespread central nervous system (CNS) ZFR expression and *MAPT* knockdown in adult nonhuman primates (NHPs) following a single IV administration of STAC-BBB, an AAV capsid that we have engineered to cross the blood-brain barrier (BBB).

A ZFR genomic medicine for the potential treatment of AD and other tauopathies

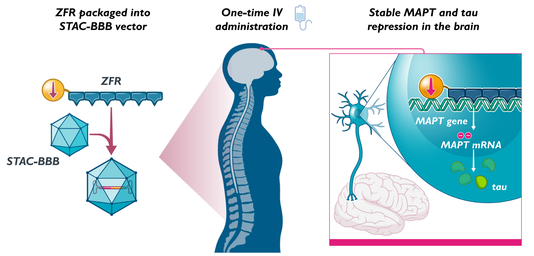
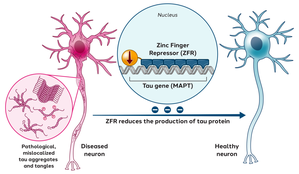


Figure 1. AAV ZFR for tauopathies

A ZFR targeting the tau gene is packaged into STAC-BBB, which crosses the BBB with a one-time IV administration. The ZFR stably represses *MAPT* transcription in neurons, resulting in specific reduction of tau protein. Lowering neuronal tau allows for clearance of all aberrant tau protein forms, improved neuron function and survival, and reduced susceptibility to tau mediated toxicity.



Conclusions and next steps

- We characterized a candidate ZFR that represses human *MAPT* by >95% with no off targets.
- Potent ZFR-mediated *MAPT* repression was demonstrated at the bulk and single-cell level in the mouse and NHP CNS. ZFR treatment reduced total tau and p-tau in htau mice.
- ZFR treatment was well tolerated in all models, doses, and time points tested.
- This work supports the initiation of an IND-enabling GLP toxicology study for a STAC-BBB delivered ZFR for the potential treatment of tauopathies, including AD and PSP.

Highly potent and specific ZFRs targeting *MAPT*

Figure 2. Initial screen for ZFRs targeting human *MAPT*

384 ZFRs were designed to target human *MAPT* and screened by transient transfection in SK-N-MC cells. ~29% of ZFRs achieved at least 50% repression of the *MAPT* transcript.

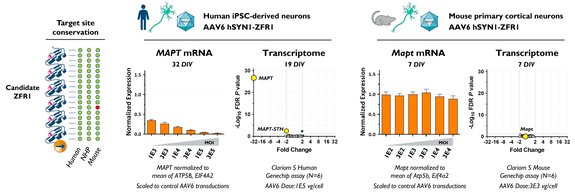


Figure 3. Characterization of a potent, specific ZFR candidate targeting human *MAPT*

ZFR1 reduced *MAPT* in human iPSC-derived neurons by >95% with no off-targets. In mouse primary cortical neurons, ZFR1 was highly specific, but showed no repression of mouse *Mapt* due to a single mismatch in the mouse target site.

ZFR1 stably lowers tau mRNA and protein by >90% in vivo after hippocampal delivery to htau mice

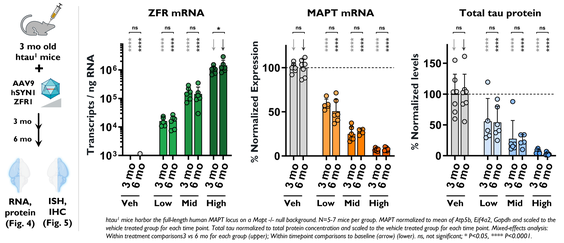


Figure 4. Reduction of human *MAPT* in the hippocampus of htau transgenic mice

htau[1] mice received dual, bilateral intrahippocampal injections of AAV9 hSYN1-ZFR1 at 3E8, 3E9, or 3E10 VG per hemisphere. Cohorts of mice were euthanized at 3 mo or 6 mo post injection. A clear dose response for ZFR expression, *MAPT* repression, and total tau protein knockdown was observed, ranging from ~50 to >90% at both the 3 mo and 6 mo timepoints. The treatment was well tolerated and there were no test-article related histopathology findings.

[1] *Andorfer C. et al. J Neurochem. 2003;86(3):582-590.*

ZFR1 reduces *MAPT* expression at the single-neuron level over a 100-fold dose range in vivo

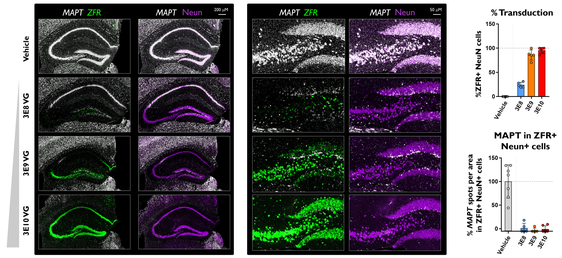


Figure 5. Single-cell analysis of ZFR+ neurons in the hippocampus of htau mice

A dose response for neuronal transduction and hippocampal coverage was observed across a 100-fold AAV dose range as assessed by multiplexed ISH/IHC (see Fig. 4 for study details). At all doses tested, individual neurons expressing ZFR1 showed >95% human *MAPT* reduction. ZFR1 therefore achieves potent single-neuron *MAPT* knockdown over a wide range of expression levels.

Widespread knockdown of human *MAPT* following IV delivery of ZFR1 to aged htau mice

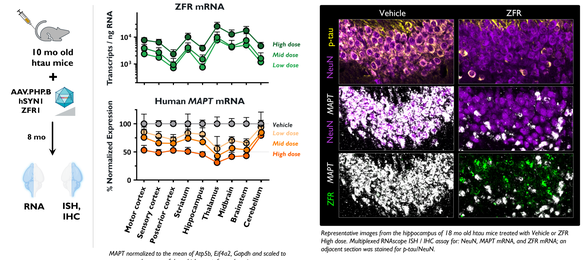


Figure 6. Reduction of human *MAPT* throughout the brain of aged htau mice

10 mo old htau mice received tail vein injections of AAV.PHP.B hSYN1-ZFR1 at 1.2E13, 3.5E13, or 1.2E14 VG/kg. Mice were euthanized 8 mo after injection. A dose response for ZFR expression and *MAPT* repression was observed in all 9 brain regions analyzed, with ~50-70% reduction at the highest dose in most regions. Single-cell analysis using multiplexed ISH/IHC revealed potent *MAPT* repression across the brain, including reduction of hyperphosphorylated p-tau in hippocampal neurons. The treatment was well tolerated and there were no test-article related histopathology findings.

STAC-BBB enables whole CNS tau knockdown in NHPs

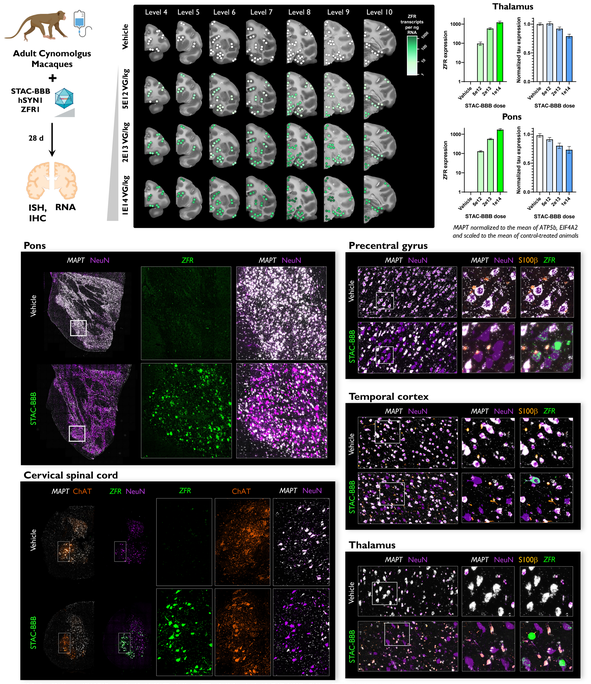


Figure 7. Bulk and single-cell ZFR expression and *MAPT* repression across the NHP CNS

ZFR1 was delivered intravenously to adult NHPs using a novel BBB-penetrant capsid, STAC-BBB. Bulk RT-qPCR analysis of 220 punches per NHP showed dose-dependent ZFR expression throughout the entire brain at all dose levels. Dose-dependent *MAPT* repression was observed in many regions including the pons and thalamus. *MAPT* expression from non-neuronal cells contributes to this bulk signal. Single-cell analysis revealed widespread ZFR expression and neuron-specific *MAPT* repression throughout the brain and all spinal cord levels. This includes regions critical for AD and PSP.

Acknowledgments and disclosures

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