Intravenous Administration of BBB-penetrant AAV Containing Primary Artificial MicroRNA Targeting Tau Reduces Tau Broadly and Robustly in hTau Mouse Brain

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INTRODUCTION

Objectives

Progression and spread of tau pathology in the brain correlates very well with cognitive decline in Alzheimer's disease (AD). Reduction of tau is being pursued as a promising therapeutic approach. Lowering tau with anti-sense oligonucleotides (ASOs) has been shown to reduce tau and tau pathology in preclinical and clinical studies; however, this approach requires repeated administration of the ASO either intra-ventricularly or intrathecally. To assess the potential of intravenous (IV) administration of AAV to provide therapeutically relevant levels of tau knockdown broadly throughout the brain with one-time administration, we have evaluated the use of a blood-brain barrier (BBB)-penetrant capsid containing a primary artificial microRNA (pri-amiRNA) specifically targeting tau mRNA.

Methods

1. Candidate siRNA sequences were screened *in silico* and *in vitro* in multiple cell lines to identify siRNAs with predicted selectivity for tau and that lower tau mRNA for subsequent cloning into proprietary pri-amiRNA scaffolds.
2. A pri-amiRNA containing TAU2023, a potent Tau siRNA, was vectorized with a proprietary, BBB-penetrant Voyager capsid (C9P39P) and tested using IV administration in hTau transgenic mice, with subsequent quantitation of vector genome, tau mRNA and tau protein levels in CNS tissue samples as described in the Procedures section below. Small RNAseq was also performed on cortex samples.

Results

At 4 weeks post-IV dosing with this AAV9P39.tau pri-amiRNA in hTau mice, we observed a dose-dependent increase in vector genome levels, concomitant with a dose-dependent decrease in tau mRNA and protein levels, in multiple brain regions. Significant tau mRNA and protein reductions ranged from 54% to 90% and 46% to 74%, respectively, relative to the vehicle control group. Small RNAseq on cortex samples showed no significant impact on the endogenous miRNA transcriptome.

Conclusions

These studies demonstrate that robust lowering of tau in hTau mice was achieved by treatment of mice with an AAV pri-amiRNA specifically targeting tau mRNA, with no change in the endogenous miRNA transcriptome. These encouraging results suggest that the combination of a potent pri-amiRNA targeting tau mRNA and a BBB-penetrant capsid, administered intravenously, could be a useful one-time treatment for AD and other tauopathies.

PROCEDURE

Quantitation of Vector Genome Levels by ddPCR: VG (vector genome) levels were determined following the method described in Wei et al (2021). The number of VG copies in CNS tissue samples was measured with the digital droplet polymerase chain reaction (ddPCR) assay after DNA extraction. Values with ≤5 positive droplets per reaction were defined as below the limit of detection (LOD). The lower limit of quantification (LLOQ) of the assay was 0.01 VG copies/diploid genome.

Measurement of MAPT (Tau) mRNA by qPCR:

- ***In vitro*:** HEK293T cells were seeded in 24-well plates, followed by transfection of pri-amiRNA containing TAU2023, as well as mock, positive (Tau-ASO) and negative controls. 48 hours of post transfection, cell cultures were harvested for mRNA extraction. Levels of human Tau and human XPNPEP1 mRNA were measured by RT-qPCR. Human MAPT mRNA levels were normalized to human XPNPEP1 mRNA levels, and then further normalized to the vehicle control.
- ***In vivo*:** Levels of human MAPT and mouse XPNPEP1 (or GAPDH) mRNA in CNS samples were measured by RT-qPCR. Human MAPT mRNA levels were normalized to mouse XPNPEP1 (or GAPDH) mRNA levels, and then further normalized to the vehicle control group.

Quantitation of Total Tau Protein by Immunoassay: Immunoassay (AlphaLISA (Revvity)) was used to measure total tau protein in soluble fractions isolated from CNS tissue samples based on the manufacturer's protocol.

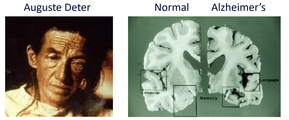
Evaluation of Total Tau Protein by Western Blot: For each cortical sample, Western blots were performed to measure Tau and β-actin protein levels. Western blot images were captured using the LI-COR imaging system and analyzed with LI-COR Image Studio.

Characterization of the Endogenous miRNA Transcriptome by Small RNAseq: RNAseq was performed by Fornax Bio. Briefly, purification of total RNA from tissue samples was performed with TRIzol Reagent followed by RNA integrity confirmation with the Bioanalyzer RNA 6000 Nano assay and quantification with the Qubit RNA HS assay. Next, the small RNA-seq library was prepared and quantified with the Qubit dsDNA HS assay, analyzed with the Fragment Analyzer HS NGS assay, and multiplexed according to the requested ratio. NextSeq Sequencing was performed with the 500/550 High-output v2.5 kit (75 cycles) single end 75 bp (SE75) sequencing which yielded ~400 million PF clusters/reads including PhiX (if applied) and followed by bioinformatic analysis.

Figure 1. Alzheimer's Disease (AD) – A Global Pandemic with Huge Need for Effective Therapies

AD is a Progressive Fatal Neurodegenerative Disease[(1)]:

- 6.2 million AD patients in the US today
- Number of patients expected to grow rapidly as population of 65 and older continues to grow; >1 in 9 Americans 65 and older has AD
- In 2020 $257 billion spent by families for out-of-pocket AD care in the US
- >50 million patients globally; expected to double by 2050
- Pathology: amyloid plaques/neurofibrillary tangles with tau aggregates in the brain, neuronal loss, synaptic loss, brain atrophy, and inflammation
- Lowering of Tau provides therapeutic benefit in animal models, appears well-tolerated and is showing evidence of activity in the clinic



Source: (1) 2021 Alzheimer's Disease Facts and Figures

Lowering of Tau provides therapeutic benefit in animal models, appears well-tolerated and is showing evidence of activity in the clinic

Figure 2. Screening Funnel Identifies Top 5 siRNAs Targeting Tau for Vectorization

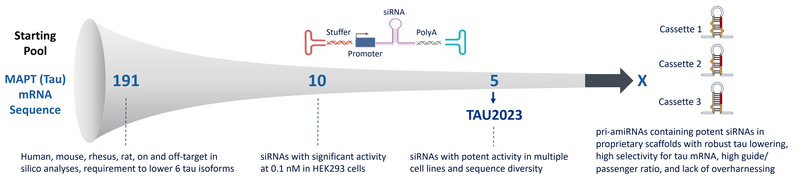
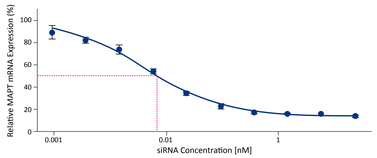
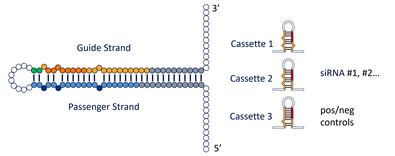


Figure 3. Top siRNA Candidate TAU2023 Selected for Vectorization Based on Predicted Selectivity for Tau and Highly Potent Tau Lowering in Multiple Cell Lines

A Highly Potent siRNA TAU2023 Identified in BT-474 Cell Line



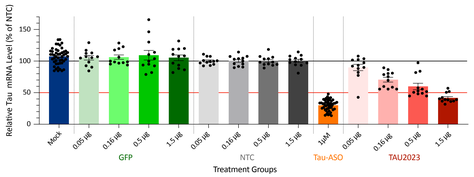
B Vectorization of TAU2023 in VYGR Pri-amiRNA Scaffolds



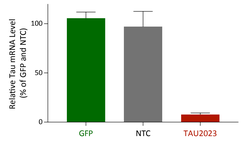
(**A**) A ten-point dose-response experiment was conducted with siRNA TAU2023 in the BT-474 cell line. The results demonstrated dose-dependent lowering of Tau mRNA with an impressive IC_{50} of 7 pM and maximal reduction by 90%. Tau mRNA levels were normalized to GAPDH mRNA levels and then further normalized to the mock control. Comparable outcomes were also observed in the HEK 293T and LNCap cell lines. (**B**) TAU2023 was vectorized into proprietary pri-amiRNA scaffolds to optimize pri-amiRNA processing.

Figure 4. *In vitro* Proof of Concept with Vectorized pri-amiRNA Containing TAU2023

A Dose Dependent Reduction of MAPT mRNA in HEK293T Cells Transfected with a pri-amiRNA Containing TAU2023



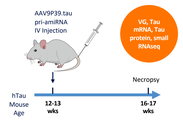
B Robust Reduction of Tau mRNA in SH-SY5Y Cells with Vectorized pri-amiRNA Containing TAU2023 Delivered by AAV9



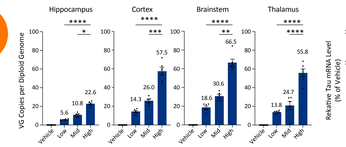
Vectorized pri-amiRNA containing TAU2023 demonstrated > 50% reduction of MAPT mRNA in vitro. Green fluorescent protein (GFP) plasmid was used as a transfection control. NTC plasmid expressing pri-amiRNA containing a scrambled siRNA was used as the negative control. (**A**) A dose-dependent decrease in Tau mRNA was observed in HEK 293T cells following transfection with a pri-amiRNA containing TAU2023. Varying concentrations of plasmid (1.5, 0.5, 0.16, and 0.05 µg plasmid per well) were transfected using FuGENE. Tau antisense oligo-nucleotide (Tau-ASO) that selectively reduces human tau was used as a comparator. Tau mRNA levels were normalized to X-prolyl aminopeptidase 1 (XPNPEP-1) mRNA levels and then further normalized to the NTC control. (**B**) Robust 92% reduction of MAPT mRNA was observed in SH-SY5Y cells with vectorized pri-amiRNA containing TAU2023 delivered with AAV9 (1E5 MOI). Tau mRNA levels were normalized to TATA-box binding protein (TBP) mRNA levels and then further normalized to the GFP and NTC controls.

Figure 5. IV Administration of AAV9P39.tau pri-amiRNA in hTau Mice Robustly Reduces Tau mRNA in Key Brain Regions

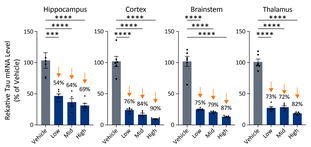
A Study Design



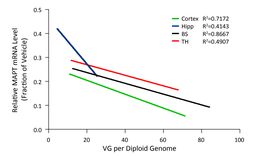
B Dose-Dependent VG Levels



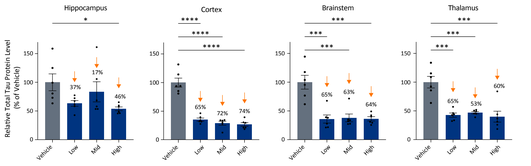
C Dose-Dependent Tau mRNA Lowering



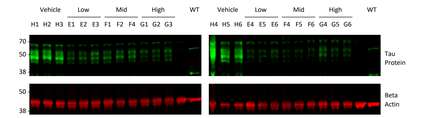
D Inverse Correlation Between Tau mRNA and VG



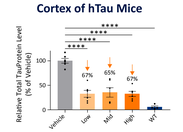
E Robust Reduction of Human Tau Protein in hTau Mice at Multiple Doses



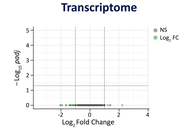
F Reduction of Human Tau Protein in Cortex of hTau Mice by Western Blot Analysis



Quantitation of Western Blots of Human Tau Protein in Cortex of hTau Mice



G No Significant Impact on the Endogenous microRNA Transcriptome



Vectorized pri-amiRNA containing TAU2023 demonstrated a robust and significant VG-dependent reduction of MAPT mRNA in hippocampus, cortex, brainstem, and thalamus of hTau mice. (**A**) Study design. htau mice, aged 12-13 weeks, were injected intravenously (IV) with vectorized pri-amiRNA containing TAU2023 encapsulated in AAV C9P39 capsids. After 4 weeks, the hippocampus (Hipp), cortex, brainstem (BS), and thalamus (TH) were harvested to quantify vector genome, Tau mRNA and Tau protein levels. Small RNAseq was also performed on cortex samples. (**B**) A dose-dependent increase in vector genome (VG) levels was observed in all CNS regions. The specific VG levels for each dose group are indicated. (**C**) A dose-dependent decrease in Tau mRNA was evident in all CNS regions. Tau mRNA levels were normalized to mouse XPNPEP-1 mRNA levels and then further normalized to the vehicle control. (**B**) and (**C**) Statistical significance was evaluated with a one-way ANOVA with Tukey's multiple comparisons post-hoc test; *, **, ***, and **** indicate p < 0.05, 0.005, 0.0005 and 0.0001, respectively. (**D**) There was an inverse correlation between Tau mRNA and VG levels in all CNS regions. (**E**) Robust, significant reduction of total tau protein levels was observed across hippocampus, cortex, brainstem and thalamus of hTau mice dosed with vectorized pri-amiRNA containing TAU2023. Total human tau levels were quantified using AlphaLISA and normalized to the vehicle control. Percentage reductions are indicated above each bar. Statistical significance was evaluated with a one-way ANOVA with Tukey's multiple comparisons post-hoc test; *, ***, and **** indicate p < 0.05, 0.0005 and 0.0001, respectively. (**F**) Total tau protein levels in cortex were also assessed by Western blot analysis. Western blot images were captured using the LI-COR imaging system, with human tau shown in green and β-actin in red. Quantification of human tau and β-actin signals was performed using LICOR Image Studio. The tau protein signals were normalized to the β-actin signals, then further normalized to the vehicle group. A significant reduction in total tau levels was observed in all treatment groups compared to the vehicle control. Statistical significance was evaluated with a one-way ANOVA with Tukey's multiple comparisons post-hoc test; **** p < 0.0001. (**G**) There were no significant changes in the endogenous miRNA expression between vehicle and low dose AAV9P39.tau pri-amiRNA groups, based on the miRNA transcriptome profile. The volcano plot shows the −log10 BH-p adjusted value on the y axis and the log2 Fold change on the y axis. The y-value indicates statistical significance, or the False Discovery Rate. The x-value indicates the difference between the dosing groups. The dotted line indicates the p = 0.05, and +/- 2-fold changes on the y and x axis, respectively. No changes achieve significance using DESeq2 analysis. Data are shown as the group mean ± SEM. N=6 per group except N=3 for (G). WT: Wild type mouse.

CONCLUSIONS

- Following IV dosing of the vectorized pri-amiRNA containing TAU2023 using a novel BBB-penetrant capsid, a dose-dependent increase in vector genomes and concomitant MAPT mRNA and protein knockdown were observed in key brain regions of treated hTau mice, four weeks post-injection.
- Significant MAPT mRNA reductions were observed, ranging from 54% to 90% relative to the vehicle-treated hTau mice. Total tau protein levels were also significantly reduced, with average reductions ranging from 46% to 74%, in multiple brain regions.
- Small RNAseq analysis in the cortex of hTau mice treated with vectorized pri-amiRNA containing TAU2023 indicates no significant impact on the endogenous microRNA transcriptome at a dose that results in 65% lowering of cortical tau protein.
- Taken together, these results show that a combination of a potent and well-tolerated MAPT targeting pri-amiRNA and a BBB-penetrant capsid could represent a promising one-time, IV treatment option for AD and other tauopathies.