# Silencing of *MAPT* via AVB-406 in human iPSC-derived neurons: A translational framework for neurodegenerative diseases

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AVIADOBIO

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## OBJECTIVE

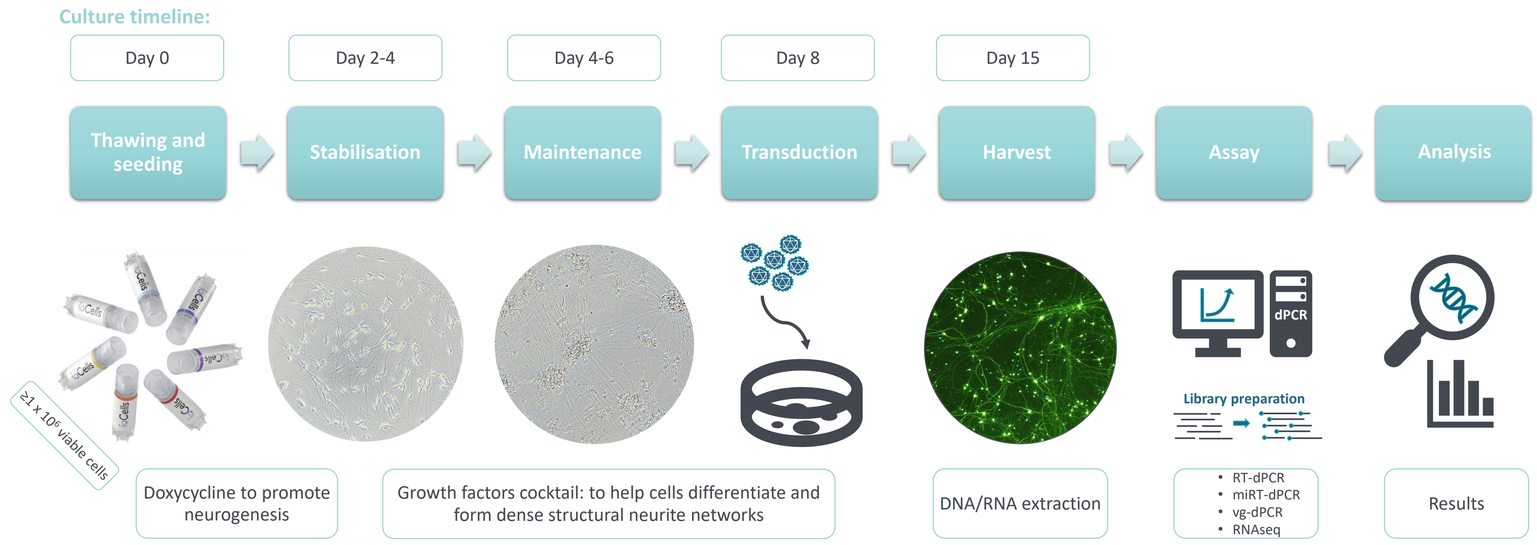
To develop and assess **AVB-406** in a translationally relevant model through effective knockdown of ***MAPT*** transcripts and evaluation of off-target effects in human induced pluripotent stem cell (hiPSC)-derived neurons.

## INTRODUCTION

- **Tau pathology**, arising from the misfolding, aggregation and aberrant post-translational modification of the microtubule-associated protein Tau, encoded by ***MAPT***, represents a defining feature of several neurodegenerative disorders, including Alzheimer's disease and MAPT-associated frontotemporal lobar degeneration[1].
- Developing durable and well-tolerated gene silencing strategies, therefore, represents a critical priority in the pursuit of transformative treatments for tauopathies, with the potential to slow or alter disease progression.
- By integrating recombinant adeno-associated virus (rAAV) delivery technology with our **vMiX™** (viral miRNA expression) platform, a novel RNA interference system, we aimed to assess **AVB-406** in human iPSC-derived neurons and demonstrate effective and precise ***MAPT*** silencing[2,3,4].

## METHODS

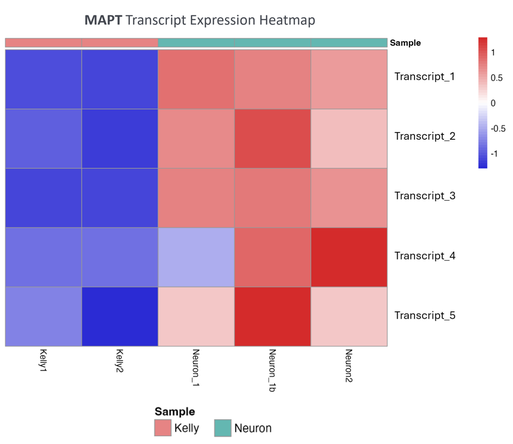
**Figure 1:** Experimental workflow of iPSC-derived glutamatergic neurons used for AVB-406 development and off-target analysis




**Day 0:** The iPSC-derived glutamatergic neurons are revived and seeded at required density using the recommended medium supplemented with doxycycline for sustained induction. **Day 2-6:** Media change every 48 hrs. **Day 8:** Cells are transduced at various MOIs. **Day 9-14:** Cell maintenance and monitoring. **Day 15:** Cells are harvested and both DNA and RNA are extracted from the same samples for downstream applications.

## *MAPT* isoform expression profile

**Figure 2:** Expression profile of *MAPT* in iPSC-derived neurons *vs* a neuroblastoma cell line

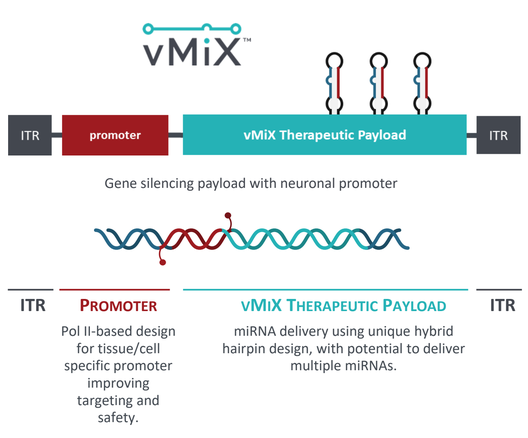



- Next Generation Sequencing (NGS) technologies were employed to assess the transcriptomic profile of ***MAPT*** in glutamatergic neurons *versus* undifferentiated Kelly cells.
- The workflow is as follows: *cutadapt* was used to remove library adapters and low-quality bases; *kallisto* was used for isoform counts, differential transcript analysis was performed using sleuth in R, and the heatmap was generated using pheatmap in R. Plotted transcripts=FDR < 0.05.

- **Higher expression of all transcript isoforms of *MAPT* was observed in iPSC-derived glutamatergic neurons compared to Kelly cells.**

## The vMiX™ system

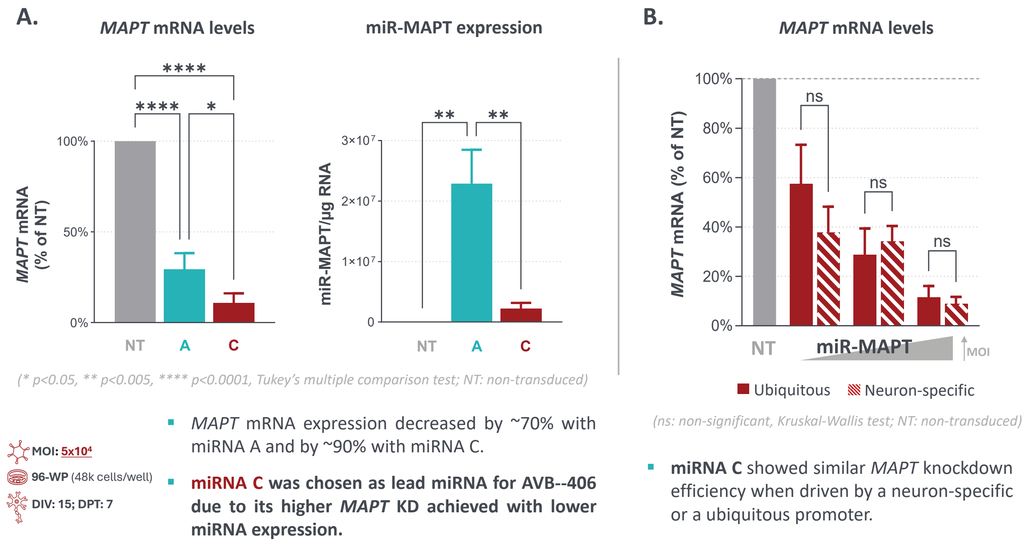
**Figure 3:** Schematic representation of the vMiX™ platform




- **vMiX™ is a novel rAAV-based RNAi platform designed for selective gene silencing.**

## Lead miRNA and promoter selection

**Figure 4:** Identification of lead miR-MAPT and promoter comparison




*(* p<0.05, ** p<0.005, **** p<0.0001, Tukey's multiple comparison test; NT: non-transduced)*

MOI: 5x10⁴
96-WP (48k cells/well)
DIV: 15; DPT: 7

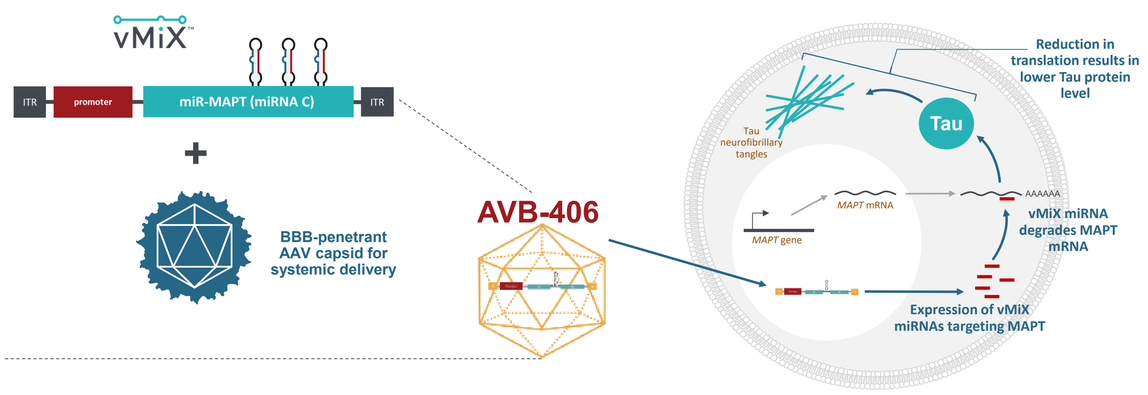
- *MAPT* mRNA expression decreased by ~70% with miRNA A and by ~90% with miRNA C.
- **miRNA C was chosen as lead miRNA for AVB--406 due to its higher *MAPT* KD achieved with lower miRNA expression.**

*(ns: non-significant, Kruskal-Wallis test; NT: non-transduced)*

- **miRNA C** showed similar *MAPT* knockdown efficiency when driven by a neuron-specific or a ubiquitous promoter.

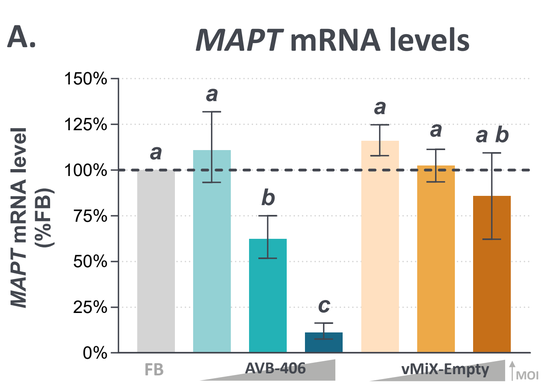
## *In vitro* assessment of AVB-406 in iPSC-derived glutamatergic neurons

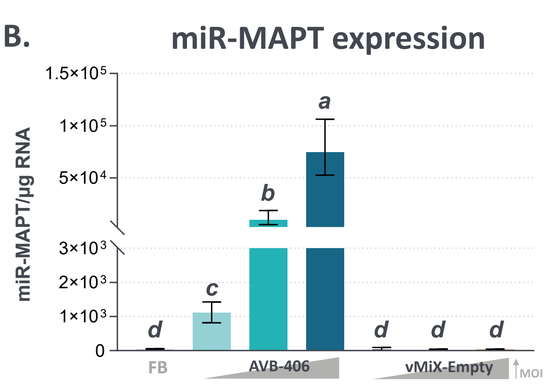
**Figure 5:** AVB-406 therapeutic approach to prevent Tau aggregation in tauopathies



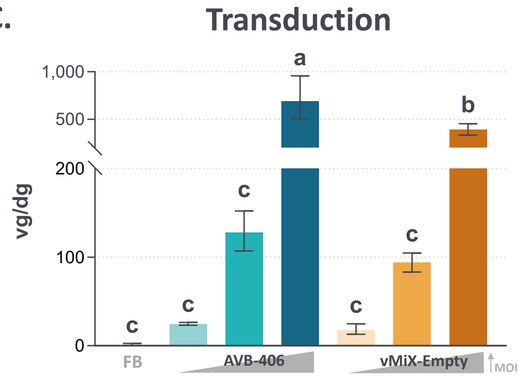

- **By targeting *MAPT* mRNA, AVB-406 aims to reduce Tau protein production, a process with the potential to limit neurofibrillary tangle formation.**

**Figure 6:** Effective modulation of *MAPT* expression via AVB-406



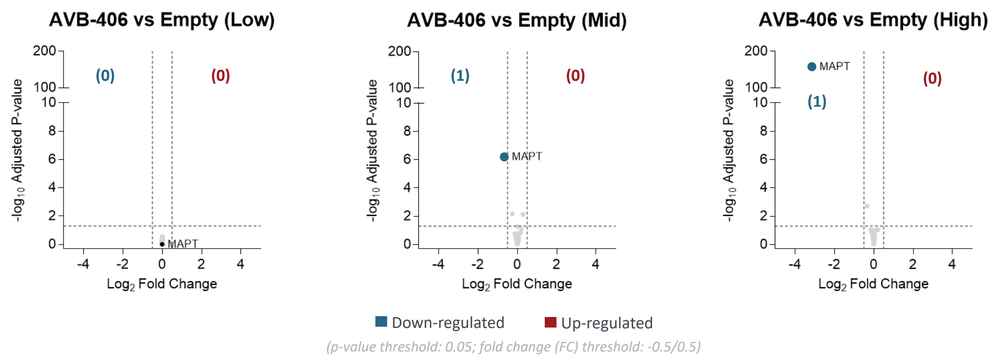

*(Groups with different letters differ significantly, Tukey's multiple comparison test; FB: formulation buffer)*




- iPSC-derived glutamatergic neurons were transduced with **AVB-406** or **rAAV-vMiX-Empty**, a negative control using the same design without expressing the miRNA and cultured for 7 days before harvesting and DNA/RNA extraction. Neuronal maturation and neurite network integrity was assessed by microscopic observation to ensure tolerability.
- *MAPT* mRNA levels were quantified by RT-dPCR (A), miR-MAPT guide expression was measured by miRT-dPCR (B) whilst vg/dg levels were quantified by dPCR (C). Datasets (N=3) are shown as mean ± SD.
- **Transduction with AVB-406 produced a consistent, dose-dependent knockdown of *MAPT*, with a pronounced effect at high MOI (~ 90% KD) and a partial effect at medium MOI (~ 38% KD).** No significant knockdown was detected at low MOI or with the control vector.
- **miR-MAPT expression as well as vg/dg reflected a clear dose-response relationship across the MOI range tested consistent with the corresponding *MAPT* KD trend.**

## AVB-406 off-target transcriptomic analysis

**Figure 7:** Off-target analysis of AVB-406 *vs* vMiX-Empty in glutamatergic neurons

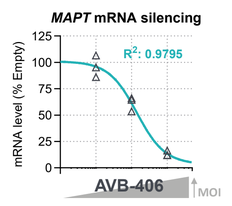



*(p-value threshold: 0.05; fold change (FC) threshold: -0.5/0.5)*

- Volcano plots from poly(A) RNA sequencing comparing AVB-406 to the MOI-matched vMiX-empty control at each MOI level revealed a clear dose-dependent KD of *MAPT*. At the lowest MOI, AVB-406_low vs Empty_low no reliable KD of *MAPT* was observed. At the medium MOI, *MAPT* reached significance with a $\log_2$FC = –0.66. At the highest MOI, *MAPT* emerged as the single significantly DEG in the entire comparison, with a $\log_2$FC = –3.15.
- ***MAPT* is significantly downregulated at mid and high MOI with no observed off-target transcriptional effects supporting effective and specific target engagement of miR-MAPT under the conditions tested.**

**Figure 8:** Comparison of AVB-406 RNA-seq *vs* RT-dPCR data

| MOI | RT-dPCR | RNAseq | |
|---|---|---|---|
| | | Normalised counts | DE analysis |
| Low | 112.0% ±18.9 | 106.0% ±6.9 | 100% |
| Mid | 63.1% ±11.5 | 60.2% ±2.1 | 63.4% |
| High | 11.7% ±4.0 | 10.7% ±2.1 | 11.3% |




- ***MAPT* knockdown assessed by RNA-seq was consistent with the RT-dPCR results**, providing independent validation of the silencing efficiency of AVB-406 in glutamatergic neurons.

## CONCLUSIONS

- **AVB-406** produced dose-dependent ***MAPT* knockdown** in hiPSC-derived glutamatergic neurons, reaching **~90% at the highest MOI** tested.
- ***MAPT*** was the **only differentially expressed gene detected** at the highest MOI, consistent with selective target engagement by miR-MAPT under the tested conditions.
- Our data establish a translationally relevant *in vitro* framework for the continued development of AVB-406 in *MAPT*-associated tauopathies.

**ABBREVIATIONS: AAV:** adeno-associated virus; **BBB:** Blood brain barrier; **DEG:** Differential expressed genes; **DIV:** Days *in vitro*; **DPT:** Days post-transduction; **FB**: formulation buffer; **FC:** fold-change; **hiPSCs**: human induced pluripotent stem cells; **hrs:** hours; **ITR:** inverted terminal repeats; **KD**: Knock-down; **mRNA:** messenger RNA; **miRNA/miR:** microRNA; **MOI:** multiplicity of infection; **ns:** non-significant; **NT:** non-transduced; **RefG:** reference gene; **RNAi:** RNA interference; **RNAseq**: RNA sequencing; **RT-dPCR:** reverse transcription-digital polymerase chain reaction; **vg/dg:** viral genome/diploid genome; **vs:** versus; **WP**: well-plate.

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