VY1706, A BBB-Penetrant, IV-delivered AAV Gene Therapy Provides Broad and Robust CNS tau Lowering in Tauopathy Mouse Models and Non-Human Primate

Wencheng Liu, Hechen Bao, Maneesha A. Paranjpe, Cansu Colpan, Ching-Lien Fang, Jeffrey Thompson, Yasuhiro Ito, Nilesh Pande, Anupriya Kulkarni, Munachiso Egbuchulam, Joydip Kundu, Su Jing Chan, Bridget Shaughnessy, Shiron J. Lee, Joe Clement, Camila Arce, Rebecca Spellman, Barbara S. Bueno, Wenya Yang, Rong Zhao, Amy Bruce, Jiachen Liu, Manonmani Kumar, Elisabeth R. Knoll, Brian Ezell, Gretchen Rivera, Tyler Moyer, Dillon Kavanagh, Jordan Routhier, Alex Kubiak, Jack Studer, Dhiral Shah, Kyle Grant, Matteo Placidi, Netsanet Gebremedhin, Bernardino Ghetti, Weitong Chen, Johnny Yao, Mathieu Nonnenmacher, Dinah W.Y. Sah, Todd Carter, Rajeev Sivasankaran

Voyager Therapeutics Inc., Lexington, MA, USA

INTRODUCTION

Objectives

MAPT mRNA lowering in the central nervous system (CNS) by an anti-sense oligonucleotide (ASO) has recently shown favorable biomarker changes and clinical outcome trends in an Alzheimer's disease (AD) clinical trial. However, CNS-directed ASOs require repeated administration via intra-ventricular or intrathecal administration. In contrast, a one-time, intravenous (IV) administration of a blood-brain barrier (BBB)-penetrant, self-complementary adeno-associated virus (scAAV) gene therapy durably and robustly reduces CNS levels of human tau in a tauopathy mouse model, as we reported previously.

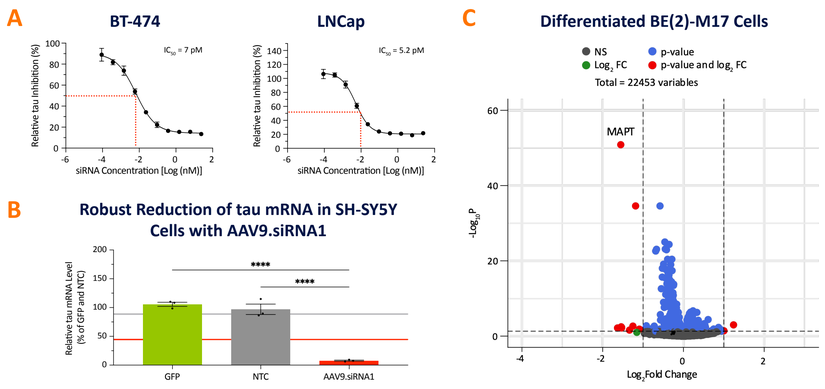
Here, we employed a BBB-penetrant, cross-species active, TRACER™ AAV capsid to deliver a primary artificial microRNA (pri-amiRNA) carrying a potent tau siRNA (VY1706) in hTau and P301S mice, and non-human primates (NHP). The resulting vector genomes (VG) delivery and tau mRNA and protein levels as well as profiling of the endogenous miRNA transcriptome in the CNS of htau mice and NHP are presented.

Observations

We observed dose-dependent increases in vector genome (VG) levels across multiple brain regions, accompanied by a corresponding reduction in tau mRNA and protein levels, in two rodent mouse models, hTau and P301S. Notably, in the P301S tauopathy model, tau pathology was robustly and dose-dependently reduced. In NHPs, we achieved broad VG distribution throughout the brain, in key AD-related regions including the entorhinal cortex, hippocampus, temporal cortex, and frontal cortex. Pharmacological activity in these regions was consistent with that observed in rodent studies, further supporting the translational potential of this approach. Importantly, VY1706 treatment was well-tolerated, with no significant treatment-related changes in body weight or adverse cage-side observations up to 11 weeks post-administration in NHPs. Additionally, VY1706 had minimal effects on the global miRNA transcriptome in AD-related brain regions and exhibited favorable miRNA processing profiles, in two tauopathy models, hTau and P301S, and NHP, reinforcing its specificity and safety. These findings demonstrate that a potent MAPT-targeting pri-amiRNA combined with a BBB-penetrant capsid represents a promising, one-time IV treatment option for AD and other tauopathies.

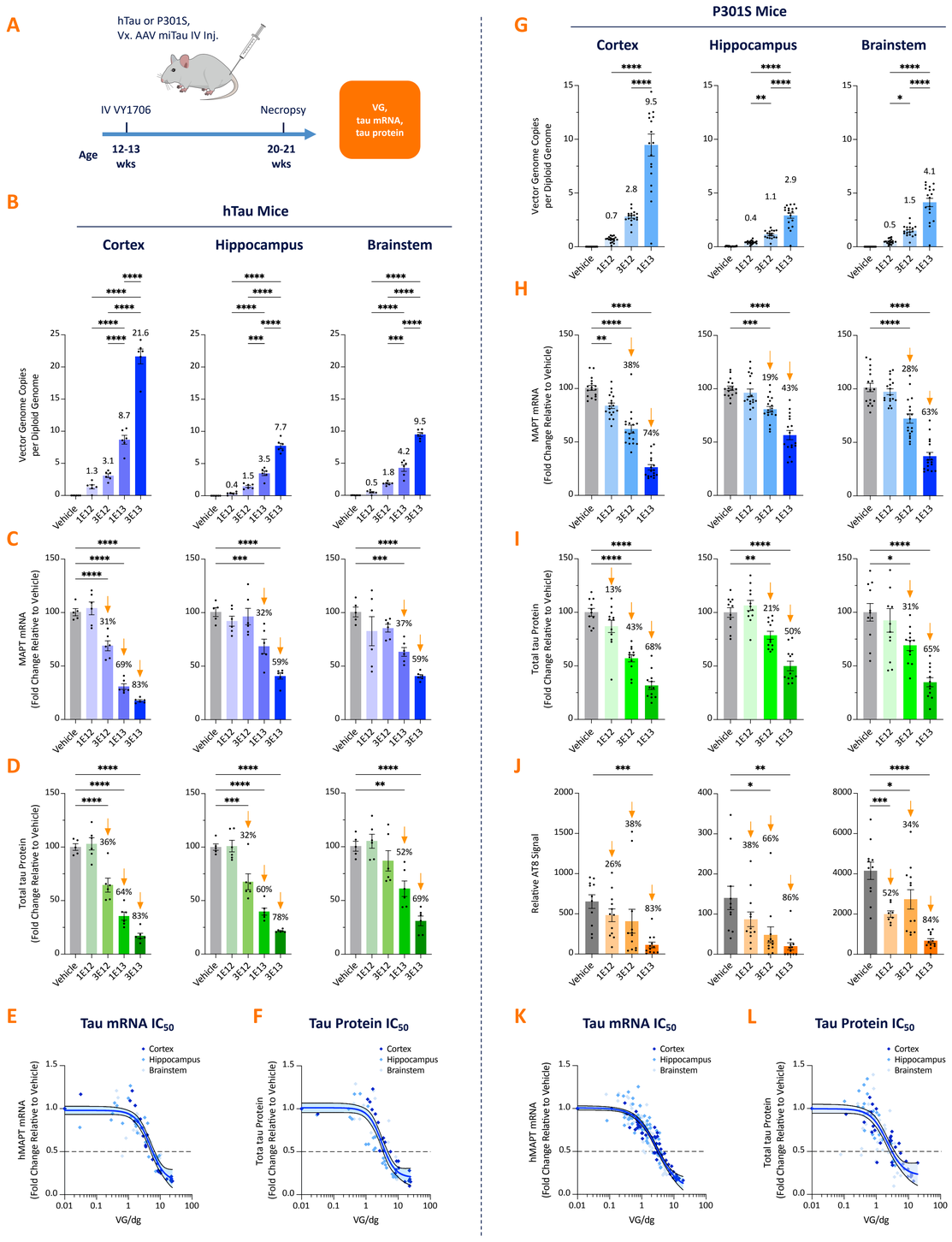
RESULTS

Figure 1. On-target Potency, *in vitro* Activity and Off-target Assessment of siRNA1



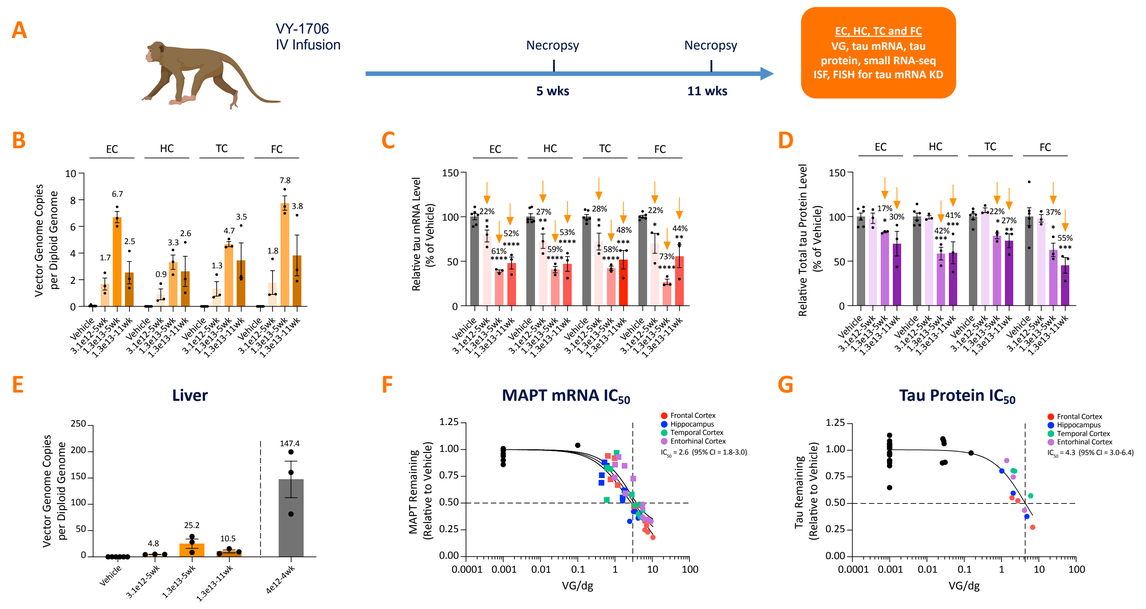
siRNA1 demonstrated robust potency in tau mRNA knockdown in various cell lines, and minimal off-target effect. (A) A ten-point dose-response experiment was conducted with siRNA1 in the BT-474 cells (left), and LNCap (right). The results demonstrated dose-dependent lowering of tau mRNA with an IC_{50} ranging from 5-7 pM and reduction by ~90%. tau mRNA levels were normalized to GAPDH mRNA levels and then further normalized to the mock control. (**B**) Robust 92% reduction of tau mRNA was observed in SH-SY5Y cells with vectorized siRNA1 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 (green fluorescence protein) and NTC (non-targeting control) controls. (**C**) No significant differences were observed in global miRNA transcriptome of the differentiated BE(2)-M17 cells transfected with the siRNA1 compared to those with the mock control. Statistical significance was evaluated with a one-way ANOVA and Tukey's multiple comparisons post-hoc test; **** indicates $p < 0.0001$. Data are shown as the group mean ± SEM. N=6 per group.

Figure 2. Dose Response Pharmacology and Efficacy of VY1706 in Mouse Models



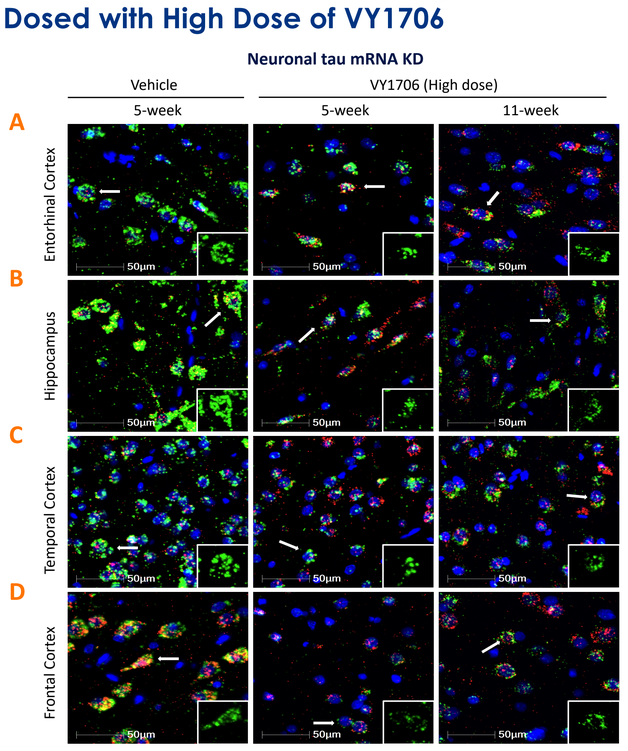
(**A**) Study design. Twelve- to thirteen-week-old hTau or P301S mice were injected intravenously (IV) with VY1706. Eight weeks later, the cortex, hippocampus, and brainstem were harvested to quantify vector genome, tau mRNA and protein levels. Dose dependent increase VG, accompanying by corresponding dose dependent reduction of tau mRNA/protein in multiple brain regions of hTau mice (**B**-**D**) or of P301S mice (**G**-**H**) were observed. Furthermore, there was a strong inverse correlation between VG levels and tau mRNA reduction with IC_{50} at 4.9 VG/dg (**E**), as well as between VG levels and Tau protein reduction with IC_{50} at 3.1 VG/dg (**F**) in all the analyzed CNS regions of hTau mice. Similar, there was a strong inverse correlation between VG levels and tau mRNA reduction with IC_{50} at 3.0 VG/dg (**K**), as well as between VG levels and tau protein reduction with IC_{50} at 2.2 VG/dg (**L**) in all the analyzed CNS regions of P301S mice. (**J**). A dose dependent, robust, and significant reduction of pathological tau protein levels was observed in cortex, hippocampus, and brainstem of P301S mice dosed with VY1706. Pathological human tau levels were quantified using AT8 ELISA and normalized to the vehicle control. Statistical significance was evaluated with a one-way ANOVA and Tukey's multiple comparisons post-hoc test; *, **, ***, and **** indicate $p < 0.05$, 0.005, 0.0005 and 0.0001, respectively. Data are shown as the group mean ± SEM. N=6 per group except N=3 for (**F**). Percentage reductions are indicated above each bar.

Figure 3. VY1706 Treatment in Non-Human Primates (NHPs)-Biodistribution, tau mRNA and Protein Knockdown



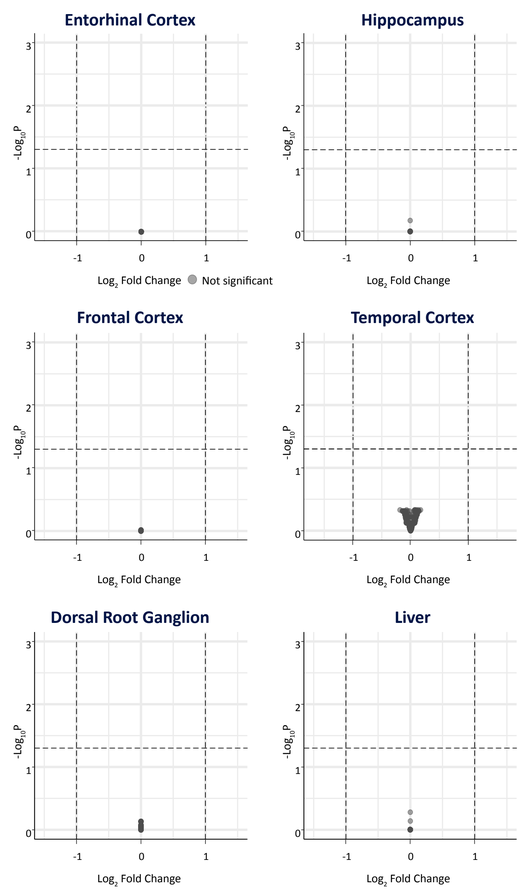
Broad biodistribution and robust tau mRNA and protein reduction in key Alzheimer's disease related brain regions of NHP. (**A**) Study design. 2–3-year-old non-human primates were injected intravenously (IV) with VY1706. Five or eleven weeks later, the hippocampus (HC), entorhinal (EC), temporal (TC) and frontal cortex (FC) were harvested to quantify vector genome, tau mRNA and protein levels. Small RNA-seq was also performed on these four CNS regions, dorsal root ganglion, and liver. (**B**) A dose-dependent increase in vector genome (VG) levels was observed in all 4 CNS regions of 5-week-, and 11-week- NHP administered with VY1706. The mean VG levels for each dose group are indicated. (**C**) A dose-dependent decrease in tau mRNA was evident in each of the four CNS regions of the VY1706-treated NHPs that were 5-week or 11-week in-life, indicating a sustained pharmacologic activity up to 11 weeks. tau mRNA levels were normalized to Cyno XPNPEP-1 mRNA levels and then further normalized to the vehicle control. (**D**) A dose-dependent decrease in tau protein was evident in each of the analyzed CNS regions of the VY1706-treated NHP that had 5-week or 11-week in-life. tau protein levels were normalized to total protein levels and then further normalized to the vehicle control. (**E**) Our 2nd generation BBB-penetrant capsid exhibited liver detargeting compared to historical WT-AAV9 data expressing an alternative transgene at a 4e12 vg/kg dose. (**F**) There was a strong inverse correlation between tau mRNA and VG levels in all the analyzed CNS regions with VG/dg > 2.6, as well as between tau protein and VG levels in all the analyzed CNS regions with VG/dg > 4.3 (**G**).

Figure 4. Robust tau mRNA Reduction in Neurons of AD-related Brain Regions of NHP Dosed with High Dose of VY1706



A fluorescence in-situ hybridization (FISH) assay was used to measure Cynomolgus tau mRNA levels in Rbfox3-positive neurons within (**A**) Entorhinal Cortex, (**B**) Hippocampus, (**C**) Temporal Cortex, and (**D**) Frontal Cortex. Rbfox3-positive neurons were labeled with Opal 570 in red, while tau mRNA was visualized with Opal690 in green (both pseudo-colored). Inset images reflect how VY1706 treatment led to a reduction in tau mRNA within Rbfox3-positive neurons at both 5- and 11- weeks post-administration compared to the vehicle-treated group. (**E**) Quantitative tau mRNA levels across different brain regions and dosing/duration groups are shown in (**E**) Red is Rbfox3 and green tau mRNA.

Figure 5. miRNA Transcriptome Evaluation



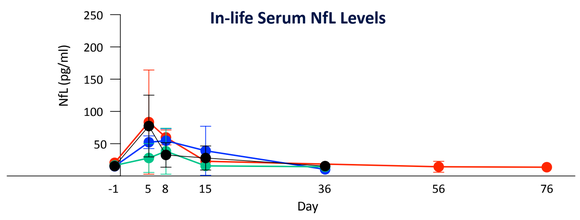
There were no significant changes in the endogenous miRNA transcriptome in the entorhinal cortex, hippocampus, temporal cortex, frontal cortex, dorsal root ganglion and liver between vehicle and high dose VY1706-treatment groups, based on the miRNA transcriptome profile. The volcano plot shows the $-\log_{10}$ BH-p adjusted value on the y axis and the $\log_2$ 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.

Table 1. Small RNA-seq Processing Profiles

siRNA1 miRNA profile	VY-1706 % relative to endogenous miRNA (less than 5%)		VY-1706 Guide/Passenger ratio (>10)		VY-1706 5' end processing precision (% N) (>85%)	
Dose	3.10E+12	1.30E+13	3.10E+12	1.30E+13	3.10E+12	1.30E+13
Entorhinal CTX	0.2	1.1	50.5	33.1	96.5	94.3
Hippocampus	0.1	0.9	73.2	47.6	96.4	94.8
Frontal CTX	0.2	1.4	49.5	40.6	92.8	90
Temporal CTX	0.2	1.1	50.6	42.8	92.9	91
DRG	0	0.1	14	17.9	93.8	90
Liver	0.1	0.5	70.8	65	92.8	90

Favorable small RNA-seq profiles were observed in the entorhinal cortex, hippocampus, temporal cortex and frontal cortex. DRG, and liver of NHP dosed with VY1706.

Figure 6. Serum-NfL as a Sensitive Fluid Biomarker Surrogate for Neurotoxicity



NfL levels in serum from VY1706 treated- or vehicle groups show transient elevation that resolves to baseline by day 30. The transient NfL increase observed at day 5 in the vehicle group is consistent with published observations in NHPs with other AAV constructs, suggesting that this early fluctuation is not test article related.

CONCLUSIONS

- VY-1706 demonstrated dose dependent tau knockdown, broad biodistribution, and sustained pharmacological activity in key AD-relevant brain regions of NHPs out to 3 months post-dosing.
- VY1706 demonstrated robust neuronal tau mRNA reduction in key AD-relevant brain regions of NHP.
- VY1706 treatment in NHP and rodent is safe and well tolerated at 5 weeks and 11 weeks, and 8 weeks, respectively.
- Dose dependent increases in VG delivery and concomitant decreases of tau mRNA/protein were observed in key AD-relevant brain regions of hTau and P301S mice treated with VY1706
- The combination of a potent and well-tolerated pri-amiRNA targeting tau and a cross-species BBB-penetrant capsid could represent a promising one-time, IV treatment option for AD and other tauopathies.
- We are advancing VY1706 to IND-enabling studies.