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Dose-response Evaluation of 9P801, an Engineered AAV Capsid with High BBB Penetration and CNS Transduction in Non-human Primates

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SUMMARY

- TRACER-NHP™ screening platform identified AAV9 variant 9P801 as a lead capsid for primate BBB crossing and CNS transduction following intravenous dosing
- Laboratory-grade 9P801 showed highly promising performance (>2-log increase vs AAV9) in a single-dose study (2e13 VG/kg)
- We applied scalable HEK-293 manufacturing to 9P801 for a descending dose study in NHP
- Transduction properties were similar or better than capsids produced by lab-grade manufacturing, showing widespread distribution in various brain regions with a strong neuronal tropism
- 9P801 showed very widespread CNS transduction at high- and medium-doses, and easily detectable transduction at low dose (≤ 2e12 VG/kg)
- Doses of 2e12 VG/kg and higher were sufficient to achieve supra-physiological mRNA expression of a model transgene

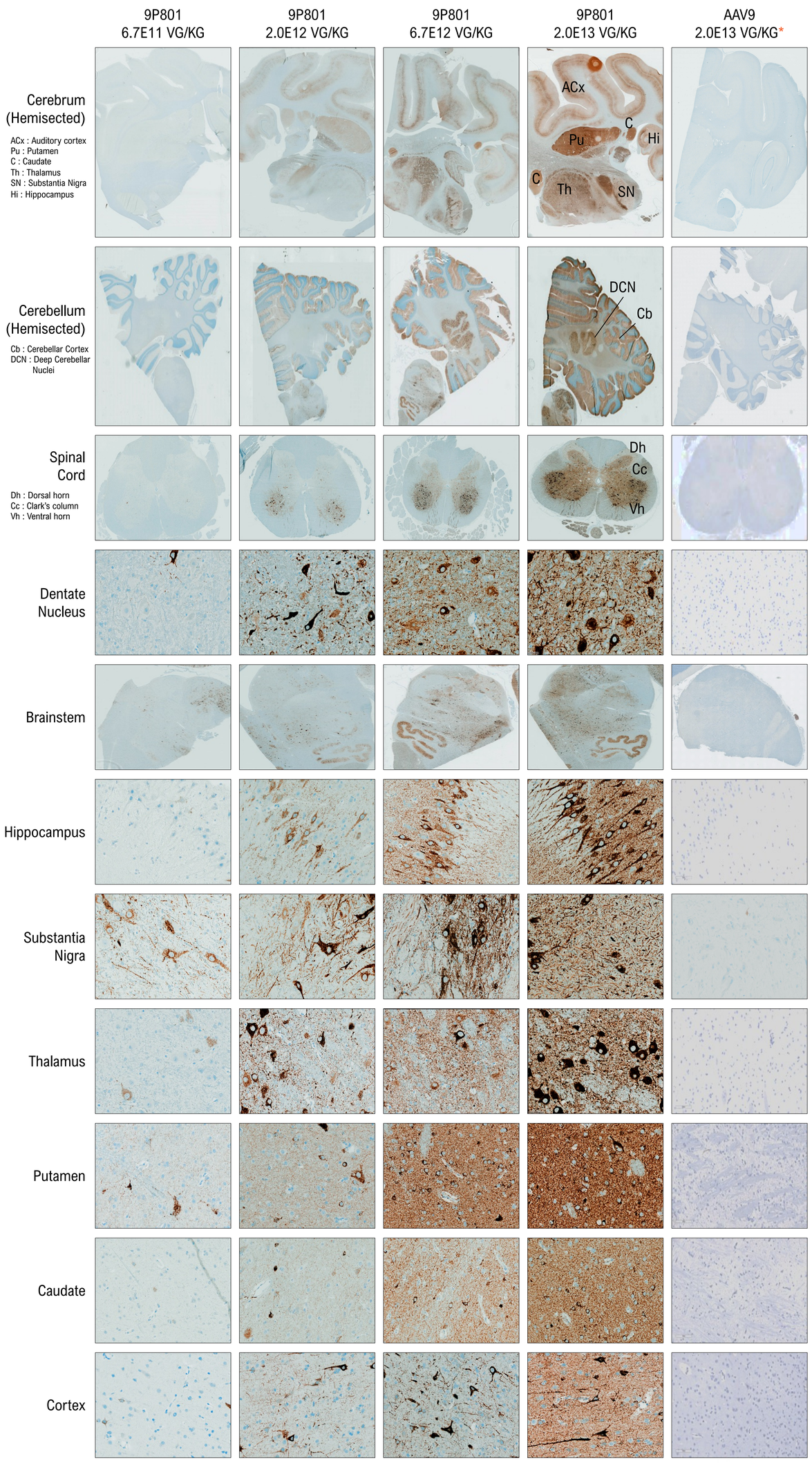
ABSTRACT

Engineering of gene therapy vectors has emerged as an exciting strategy to enable clinically relevant expression levels of a therapeutic gene at vector doses low enough to avoid undesirable events. Using TRACER™ RNA-driven directed evolution of Adeno-Associated Virus (AAV) capsids, we have recently generated 9P801, an AAV capsid variant with an unprecedented capacity for CNS transduction in adult non-human primates (NHPs) following intravenous delivery. Here we attempted to determine the minimal dose of 9P801 vector sufficient for near-physiological expression of a therapeutic payload in the CNS of adult macaques via systemic delivery. A 9P801 vector containing a hemagglutinin-tagged NHP protein under a ubiquitous promoter was injected to male NHPs at various doses spanning a 30-fold range for a duration of 28 days. Widespread transgene protein expression was detected in the spinal cord and the brain of high- and medium-dose animals, especially in the putamen, thalamus, globus pallidus and brainstem. Viral DNA and mRNA were readily detectable in all animals and showed a consistent dose response. Strikingly, the lowest dose of 9P801 allowed higher mRNA and protein expression than a 30-fold higher dose of AAV9. Comparison of transgene mRNA with the matching endogenous transcript indicated that a dose of 2e12VG/kg was sufficient to achieve supraphysiological levels in the CNS, while showing low transduction in the liver and the dorsal root ganglia (DRG). Together, our data suggest that engineered AAV vectors have the potential to achieve a large improvement of their therapeutic index by retaining strong efficacy at low dose.

METHODS

- Cynomolgus macaques (n=3 per dosing group) were dosed intravenously with 9P801 capsid containing a single-stranded primate transgene fused to an HA tag under a CBA promoter.
- At day 28 post-injection, tissues were harvested and split for IHC analysis or bioanalytical readouts.
- IHC was performed with an anti-HA antibody on brain coronal slices and spinal cord transversal sections.
- Vector DNA levels and transgene mRNA expression were analyzed by ddPCR and RT-qPCR, respectively. mRNA was measured with 1) a transgene-specific probe or 2) a CDS probe detecting both transgenic and endogenous mRNA. DNA and mRNA were normalized to housekeeping genes.

Figure 1. **IHC Detection of Transgene Protein in Cynomolgus Macaque**



*Historical data - 2 weeks post-dosing

Figure 2. **Detection of Viral Genomes and Transgene mRNA**

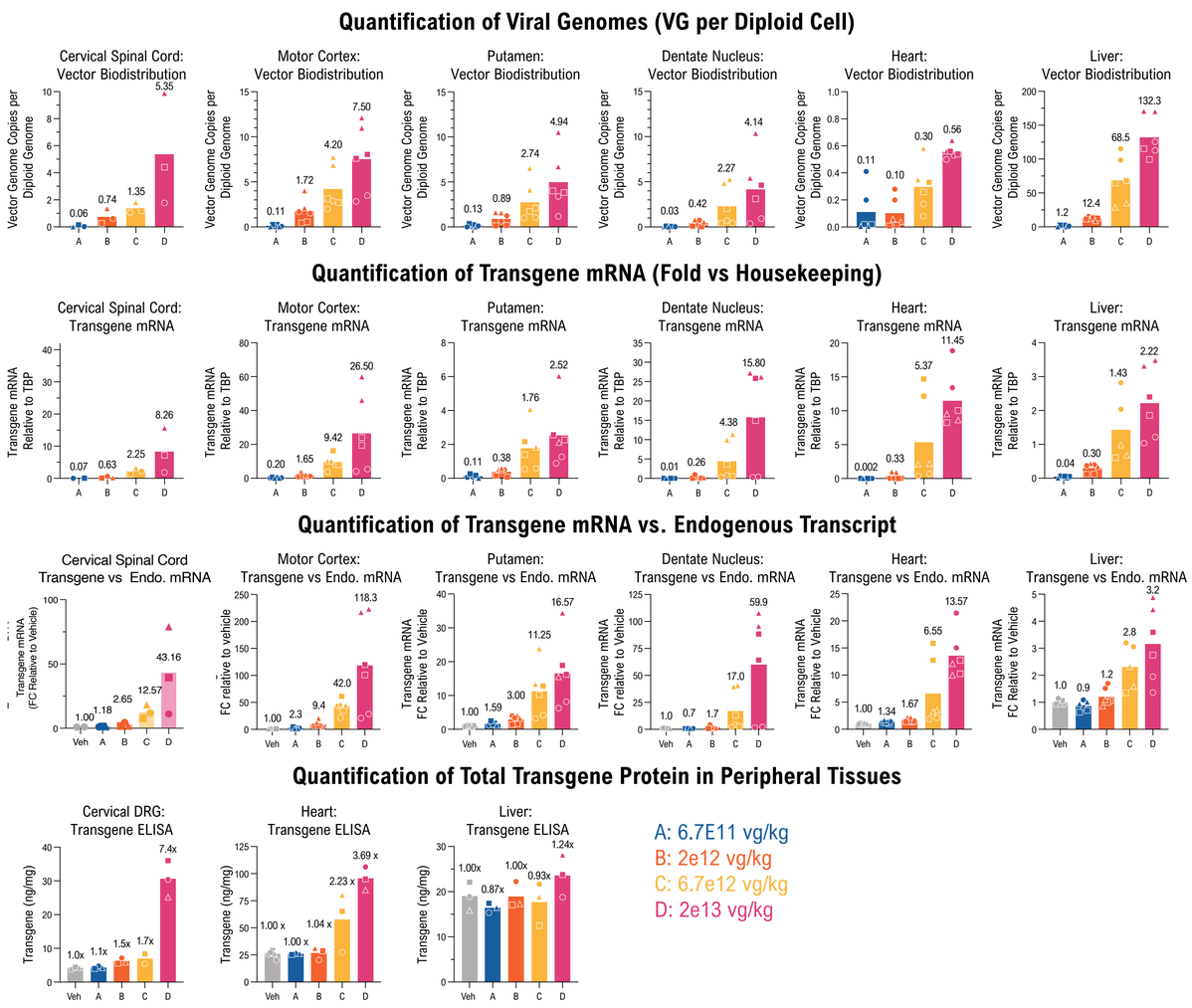
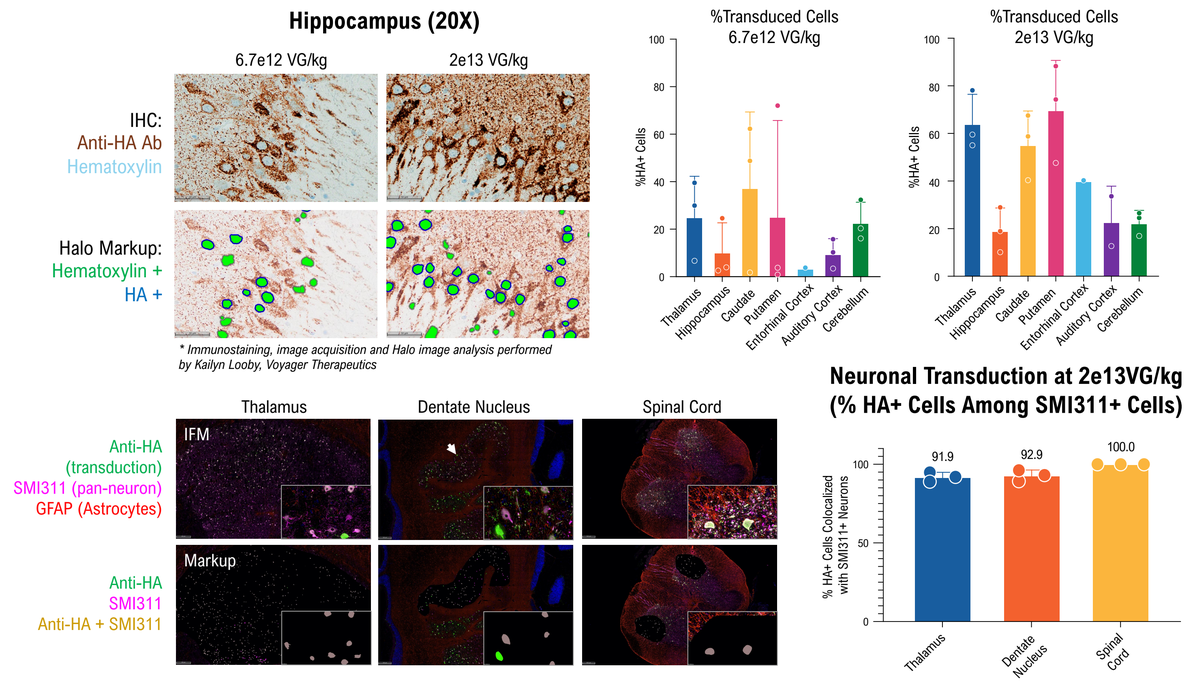


Figure 3. **Quantification* and Characterization of Transduced Cells in NHP**



* *Immunostaining, image acquisition and Halo image analysis performed by Kailyn Looby, Voyager Therapeutics*

CONCLUSIONS

- The engineered NHP BBB-penetrant capsid 9P801 is compatible with scalable manufacturing.
- Intravenous delivery of 9P801 achieved widespread CNS transduction in adult NHPs at doses of 2e12VG per kg and above.
- Supraphysiological levels of transgene mRNA were achieved at doses of 2e12 VG per kg and above.
- A dose of 2e13 VG/kg was sufficient to transduce **>40% of total cells** in highly permissive brain regions (thalamus, caudate, putamen) and >20% total cells in less permissive regions (entorhinal cortex, auditory cortex, hippocampus).
- A dose of 2e13 VG/kg achieved transduction of **>90% SMI311-positive neurons** in the thalamus, dentate and spinal cord.