Systemic Gene Therapy with Engineered AAV Demonstrates Preclinical Efficacy and Safety Supporting a Disease-Modifying Treatment for STXBP1 Developmental and Epileptic Encephalopathy

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Introduction

Syntaxin-binding protein 1 (STXBP1) is a synaptic protein that regulates SNARE complex formation and synaptic vesicle release. *De novo* heterozygous (Het) disruptive mutations in *STXBP1* cause *STXBP1* Developmental and Epileptic Encephalopathy, a rare, devastating neurodevelopmental disorder and genetic epilepsy that affects an estimated 1:26,000 newborns globally (López-Rivera et al., 2020). *STXBP1* encephalopathy is characterized by impaired neuronal communication, epilepsy, severe intellectual disability, movement disorders, and sudden unexpected death in epilepsy. Successful disease-modifying treatments are expected to require supplementation of STXBP1 protein levels in neurons brain-wide. Using a mouse model of *STXBP1* encephalopathy, we have demonstrated that a gene supplementation strategy using a surrogate capsid (CAP.B10) that crosses the blood-brain barrier (BBB) after intravenous (IV) administration in mice can achieve dose-dependent and long-lasting correction of core disease phenotypes.

Using our high-throughput, non-human primate (NHP)-based AAV engineering platform, Capsida identified a novel AAV capsid that effectively crosses the BBB in NHPs after IV administration, delivering therapeutic cargos in up to 70% of neurons brain-wide. Capsida has characterized a human BBB receptor that binds this engineered capsid, which has complete homology between humans and macaques in the predicted binding pocket. When the CAP-002 drug product (*i.e.*, novel AAV capsid carrying the hSTXBP1 cargo) was delivered IV in NHPs, Capsida observed dose-dependent brain-wide DNA and RNA biodistribution at levels comparable to those that achieved phenotypic correction in the mouse model. Capsida's STXBP1 development candidate (CAP-002) is 20x liver detargeted compared to wildtype (WT) AAV9 and is safe and well-tolerated, including no dorsal root ganglion (DRG) toxicity. This breakthrough profile of brain transduction and liver detargeting allows CAP-002 to be delivered at lower doses than historically used with systemically delivered gene therapies, further mitigating potential toxicities. Using human induced pluripotent stem cells (iPSCs) that are WT or knockout (KO) for *STXBP1*, we demonstrated that CAP-002 restores STXBP1 protein expression and corrects synchronous network activity. Capsida's STXBP1 program is in IND-enabling studies and expected to enter the clinical in the first half (1H) of 2025, with the potential to be best in class and offer prospect of direct benefit (PDB) for patients with *STXBP1* encephalopathy.

Methods and Materials

Stxbp1 mouse model: *Stxbp1* heterozygous (*Stxbp1*$^{+/-}$) male and female mice (Chen et al., 2020) received a retroorbital IV injection of CAP.B10-hSTXBP1 at 8 weeks of age and were monitored for behavioral and seizure phenotypes. After 6 months, cargo DNA and RNA levels were assessed using PCR. A surrogate capsid (CAP.B10) was used to deliver the therapeutic cargo (hSTXBP1) to model phenotypic correction achieved by a capsid that crosses the BBB in mice.

In vitro human neuron model: Capsida partnered with Neurospector, a contract research organization established by Matthijs Verhage's laboratory at Vrije University Medical Center Amsterdam, to examine STXBP1 protein expression and functional correction of neuronal activity in human *STXBP1* KO neurons. Human iPSC-derived WT and KO neurons were transduced with vehicle (VEH) or CAP-002. Twenty days post-transduction, live calcium imaging was performed to monitor synchronous network activity, which is known to depend on synaptic transmission. Human neurons were then fixed and stained for STXBP1, MAP2, and SV2A to assess protein expression.

NHPs: The STXBP1 development candidate (CAP-002) was administered IV to WT male and female cynomolgus macaques at ~30 months of age, and animals were monitored for in-life health outcomes. After 6 weeks, cargo DNA and RNA levels were assessed using PCR. Separate NHPs received an IV administration of the novel AAV capsid carrying an HA-tagged surrogate cargo. After 6 weeks, immunofluorescence (IF) staining for HA and neuronal markers (NeuN; SMI32) were used to quantify the percentage of neurons expressing cargo protein.

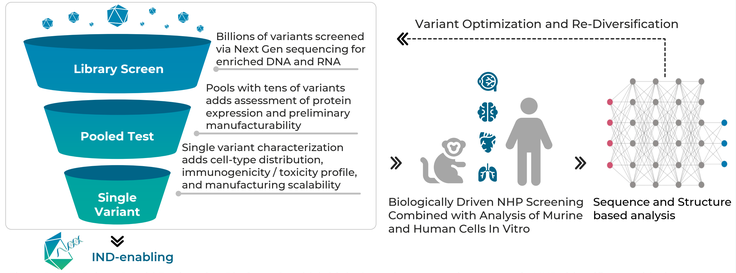
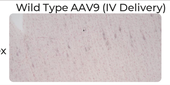
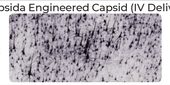


Figure 1. NHP-Driven Capsid Engineering Platform. Capsida's high-throughput screening process in NHPs identifies capsids that target desired tissues and cell types while de-targeting undesired tissues.

Results

	CNS Challenges with WT AAV9	Capsida Solutions with Engineered Capsids
	Wild Type AAV9 (IV Delivery) NHP Cortex 	Capsida Engineered Capsid (IV Delivery) NHP Cortex 
Neuronal Transduction	Limited ability to cross biological barriers, especially BBB – < 1% transduction with WT AAV9 IV	Capsida's engineered capsids bind to an identified human BBB receptor and show high levels of neuronal transduction - up to 70% of neurons
Safety Concerns	Liver and dorsal root ganglia (DRG) toxicity	At least 16x liver and 50x DRG detargeting
Patient Populations	Narrow therapeutic index limits to ultra-rare/rare diseases	Broader therapeutic index enables more common diseases across all ages
Risks	Direct injection to brain or CSF causes significant risks and inconsistent expression IV delivery increases risk of off-target effects (especially liver) and triggering immune response	IV reduces risks and allows consistent expression No clinical pathology, adverse histopathology, or immune-related adverse events

CAP-002 Capsid Carrying a Surrogate Cargo Shows Brain-wide Neuronal Transduction

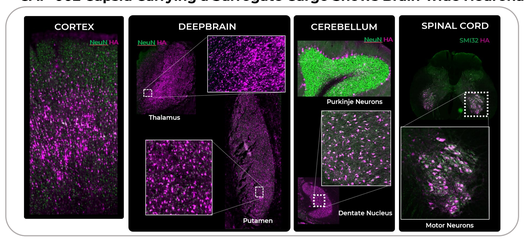


Figure 2. Neuronal transduction in WT NHPs achieved by the capsid in the CAP-002 drug product delivering an HA-tagged surrogate cargo.

After IV delivery, the CAP-002 capsid achieves levels of neuronal transduction in cortex, deepbrain, cerebellum, and spinal cord that are expected to provide a potential for therapeutic benefit in a first-in-human clinical trial.

Gene Supplementation with CAP-002 in Human iPSC-Derived *STXBP1* KO Neurons Restores STXBP1 Protein Expression and Neuronal Network Activity

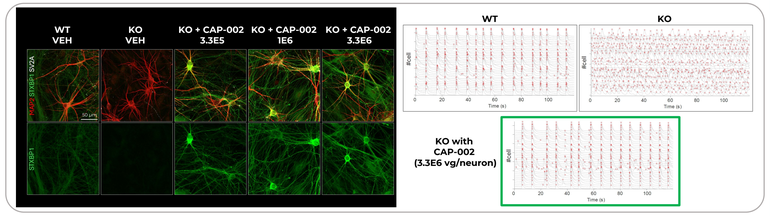


Figure 3. CAP-002 Efficiently Transduces Human Neurons, Restores STXBP1 Protein Expression, and Restores Normal Activity in KO Neurons. Confocal images of *STXBP1* WT and KO iPSC-derived human neurons stained for STXBP1 (green), MAP2 (red) to image cell bodies and dendrites, and SV2A (white) to image synaptic terminals. STXBP1 protein is absent in KO neurons, and transduction with CAP-002 restores STXBP1 protein expression to WT levels and achieves a WT cellular distribution pattern at all doses. Live calcium imaging demonstrates that CAP-002 restores synchronous network activity in KO neurons. Traces show the timing of activity-dependent calcium transients (red dots) in ~33 neurons per condition; spontaneous action potentials in VEH-treated KO neurons are desynchronized compared to synchronous firing of WT neurons and KO neurons treated with CAP-002. All doses are in vector genomes (vg)/neuron.

Results

Gene Supplementation with CAP.B10-hSTXBP1 Induces Phenotypic Correction in *Stxbp1*$^{+/-}$ Mice

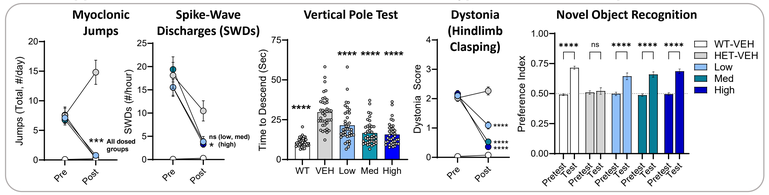


Figure 4. CAP.B10-hSTXBP1 Corrects Seizures, Motor, and Cognitive Behaviors in Stxbp1$^{+/-}$ Mice. IV administration of CAP.B10-hSTXBP1 significantly reduces myoclonic jumps and SWD seizures, the time required to descend from a vertical pole, and the magnitude of dystonia (hindlimb-clasping). Treatment significantly improves preference for a novel object, demonstrating memory of the familiar object. (Jumps / SWD / Dystonia / NOR: Repeated measure two-way ANOVA or Mixed Model; Pole Test: One-way ANOVA; All analyses multiple comparisons vs. HET-VEH; ns, not significant; *P<0.05; **p<0.01; ***P<0.001; ****P<0.0001

CAP-002 CNS RNA Biodistribution Achieves Levels Expected to Provide Benefit and CAP-002 is 20-fold Detargeted from Liver and 143-fold Detargeted from DRGs

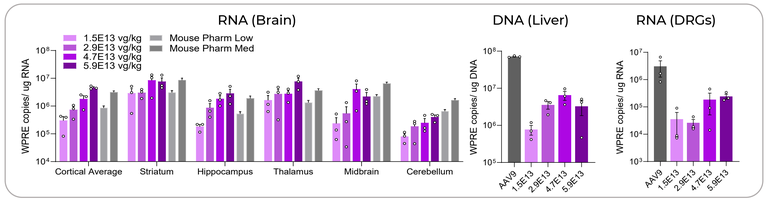


Figure 5: CAP-002 biodistribution in NHPs. IV administration of CAP-002 at 4 doses produces dose-dependent increases in DNA transduction (not shown) and RNA expression in the brain. RNA expression achieved by 2.9E13 vg/kg and by 4.7E13 and 5.9E13 is similar to, respectively, the Low and Med doses in the Mouse Pharmacology study expression, which fully corrected seizures and provided meaningful correction of motor and cognitive deficits. Compared to AAV9 at an equivalent dose to 2.9E13 vg/kg, DNA transduction in the liver is significantly decreased (20-fold) and RNA expression in the DRGs is significantly decreased (143-fold).

Conclusions

- Capsida's next-generation capsids are engineered to efficiently cross the BBB, specifically target therapeutic cell types (e.g., up to 70% of neurons in the cortex), and de-target off-target organs (e.g., 20x lower liver burden)
- The CAP-002 capsid binds a novel BBB receptor conserved across NHPs and humans and achieves brain-wide protein expression
- CAP-002 corrects firing in *STXBP1* KO neurons in vitro and, in NHPs, achieves dose-dependent brain-wide distribution and expression at levels expected to correct seizures and meaningfully improve motor and cognitive deficits for patients with *STXBP1* encephalopathy
- In NHPs, CAP-002 is also de-targeted from non-therapeutic areas, including 20x lower liver burden and 143x lower DRG expression, resulting in a well-tolerated safety profile with no adverse histopathological findings
- Capsida is planning for IND submission of CAP-002, a potential best- and first- in-class treatment of *STXBP1* Developmental and Epileptic Encephalopathy, in 1H 2025

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