CAP-002: Systemic AAV Gene Therapy with Next Generation Capsids for Treatment of *STXBP1* Encephalopathy (Abstract 504)

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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 disruptive mutations in *STXBP1* cause *STXBP1* encephalopathy (also known as genetic epilepsy due to *STXBP1* mutations), a rare, devastating neurodevelopmental disorder and genetic epilepsy that affects approximately 1:30,000 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 require supplementation of STXBP1 protein levels in neurons brain-wide. Using a mouse (MUR) 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 (Chen et al., 2024, ASGCT Abstract #38).

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. When the CAP-002 drug product (*i.e.*, novel AAV capsid carrying the hSTXBP1 cargo) was delivered IV in NHPs, Capsida observed brain-wide DNA and RNA biodistribution and brain-wide STXBP1 expression at levels comparable to those that achieved phenotypic correction in the MUR model. Capsida's STXBP1 development candidate (CAP-002) is >5x liver de-targeted and is safe and well-tolerated, including no DRG toxicity. This breakthrough profile of brain transduction and liver de-targeting allows CAP-002 to be delivered at lower doses than historically used with systemically delivered gene therapies, further mitigating potential toxicities. Capsida's STXBP1 program has initiated IND-enabling studies, and the efficacy profile of this first in class therapy is expected to provide a disease-modifying solution with 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 (Het; *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, and total STXBP1 protein levels were assessed using western blot. 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.

NHPs: The STXBP1 development candidate (CAP-002) was administered IV to wild-type (WT) male and female cynomolgus macaques at ~15 months of age, and animals were monitored for in-life health outcomes. After 6 weeks, cargo DNA and RNA levels were assessed using PCR, and total protein levels were assessed using ELISA. Separate NHPs received an IV administration of the novel AAV capsid carrying an HA-tagged surrogate cargo. After 6 weeks, immunofluorescence staining for HA and a neuronal marker (NeuN) was used to quantify the percentage of neurons expressing cargo protein.

NHP-Driven Capsid Engineering Platform

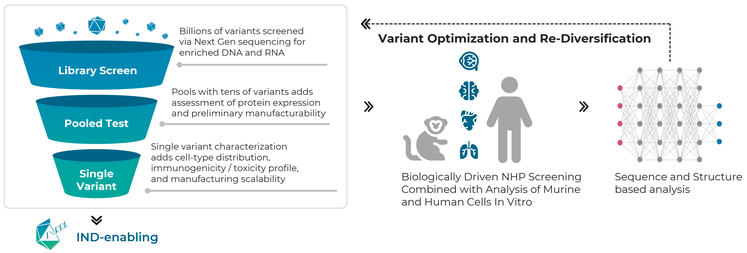
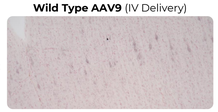
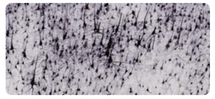


Figure 1. NHP-Driven Capsid Engineering Platform

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)	**Capsida Engineered Capsid** (IV Delivery)
	NHP Cortex 	NHP Cortex 
Neuronal Transduction	Limited ability to cross biological barriers, esp. BBB – < **1% transduction** with wild type AAV9 IV	Capsida engineered capsids cross BBB with high levels of neuronal transduction – **up to 70% neurons**
Safety Concerns	Safety concerns / **liver toxicity**	Enabling lower dosing and **~4000x difference** in CNS to liver targeting vs wild type AAV9
Patient Populations	Traditional gene therapies **primarily for rare diseases**	**Access to more** common diseases across all ages
Risks	Direct injection into the brain or CSF is invasive with **significant risks**	Targeted IV administration **increases effectiveness** and **reduces risks**

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

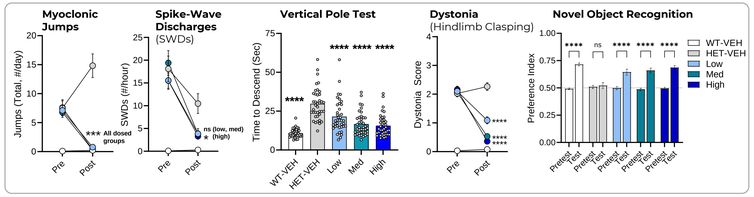


Figure 2. CAP.B10-hSTXBP1 Corrects Seizures, Motor, and Cognitive Behaviors in *Stxbp1*$^{+/-}$ Mice. IV administration of CAP.B10-hSTXBP1 produces dose-dependent correction of seizure, motor, and cognitive behaviors. Treatment significantly reduces myoclonic jumps and SWD seizures, the time required 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; see Chen et al., 2024, ASGCT Oral Abstract #38)

CAP-002 RNA Biodistribution is >97x higher than AAV9 in CNS and >5x De-Targeted from Liver

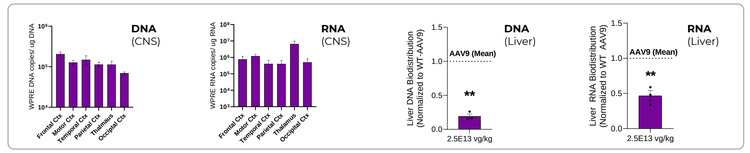


Figure 3. CAP-002 biodistribution in the NHP (representative data). IV administration of CAP-002 at 2.5E13 vg/kg produces DNA transduction and RNA expression in the CNS at levels expected to provide benefit, with significantly decreased liver DNA (>5x) and RNA (>2x) levels compared to WT-AAV9 at the same dose. (One-sample t-test vs. normalized AAV9; **p<0.01)

Results

CAP-002 Achieves Protein Levels in NHPs Similar to Low and Med Dose of CAP.B10-hSTXBP1 in MUR

***Stxbp1*$^{+/-}$ Mice dosed with CAP.B10-hSTXBP1** (Western Blot)

WT NHPs dosed with CAP-002 (ELISA)

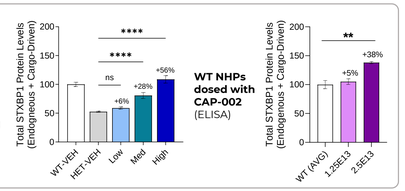


Figure 4. STXBP1 protein expression in Stxbp1$^{+/-}$ mice and WT NHPs.

The CAP-002 drug product achieves dose-dependent increases in STXBP1 protein above WT levels in NHPs, which is similar to levels of protein expression achieved by the low and medium dose of CAP.B10-hSTXBP1 in mice. (One-way ANOVA, multiple comparisons vs. WT, *p<0.05, **p<0.01, ****p<0.0001)

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

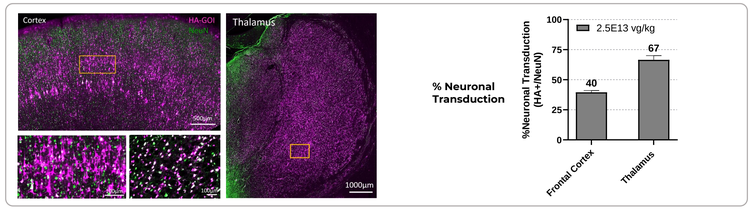


Figure 5. Neuronal transduction in WT NHPs achieved by the capsid in the CAP-002 drug product delivering an HA-tagged surrogate cargo.

After IV delivery, the CAP-002 capsid achieves levels of neuronal transduction in cortical and subcortical regions that are expected to provide a prospect of direct benefit in a first-in-human clinical trial. GOI, gene of interest.

Conclusions

- Capsida's engineering platform in NHPs has identified novel capsids that cross the BBB after IV delivery
- The STXBP1 development candidate (CAP-002) achieves brain-wide DNA transduction, RNA expression, and protein expression and has brain RNA levels >97-fold higher than AAV9 and liver DNA levels >5-fold lower than AAV9
- Brain protein levels are similar to the MUR low and medium doses, providing evidence that CAP-002 can provide prospect of direct benefit (PDB) in a clinical trial with a best in class profile
- Studies with the CAP-002 capsid delivering an HA-tagged cargo at the same dose demonstrated neuronal transduction up to 40% in cortex and ~70% in thalamus, which are levels of transduction expected to provide PDB
- Capsida has initiated IND-enabling studies with the STXBP1 development candidate (CAP-002) for first in class treatment of *STXBP1* encephalopathy

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PRESENTED ON TUESDAY
- Chen et al, AAV Gene Therapy Corrects Neurological Phenotypes with Clinically Relevant Doses in a Mouse Model of STXBP1-Related Developmental and Epileptic Encephalopathy (Oral Abs 38)

PRESENTED ON WEDNESDAY
- Shi et al, Directed Evolution of AAV9 Libraries in Non-Human Primates Identifies a Capsid Family with Enhanced Central Nervous System Tropism and Liver De-Targeting Following Systemic Delivery (Oral Abs 122)
- Morales et al, Characterization of engineered AAV capsids from different HEK293 cell culture fractions, crude lysate versus cell pellet material (Abs 529)
- Volpe et al, Alternative Plasmid Designs Including Two Plasmid Transfection Systems for Improved Production and Packaging of Engineered AAV Capsids (Abs 530)

PRESENTED ON THURSDAY
- Shi et al, Directed Evolution of AAV2 Libraries Yields Capsids with Improved Performance in the Central Nervous System and Cross-species Translatability (Abs 992)
- Gejji et al, Separation of Empty and Full Engineered Adeno-Associated Virus Capsids Using a Weak Anion Exchange (Abs 1038)

PRESENTED ON FRIDAY
- McDowell et al, Systemic AAV Gene Therapy with CNS-Targeted Engineered Capsids Achieves Significant GCase Activity Increases in the Primate Brain to Support the Potential Treatment of GBA-PD (Oral Abs 274)

References

1. López-Rivera JA, Pérez-Palma E, Symonds J, Lindy AS, McKnight DA, Leu C, Zuberi S, Brunklaus A, Møller RS, Lal D (2020) A catalogue of new incidence estimates of monogenic neurodevelopmental disorders caused by de novo variants. Brain 143:1099–1105.
2. Chen W, Cai Z-L, Chao ES, Chen H, Longley CM, Hao S, Chao H-T, Kim JH, Messier JE, Zoghbi HY, Tang J, Swann JW, Xue M (2020) Stxbp1/Munc18-1 haploinsufficiency impairs inhibition and mediates key neurological features of STXBP1 encephalopathy. Elife 9:e48705.
3. Chen W, Kim JH, Michaels A, Rivera A, Sham Y, Elwonger C, Carneiro JP, Nguyen S, Moin Z, Zou S, Jin K, Wang A, Dhar N, Park P, Vega J, Chen A, Horist B, Chen H, Lopez A, Knoll A, Flytzanis N, Xue M (2024) AAV gene therapy corrects neurological phenotypes with clinically relevant doses in a mouse model of STXBP1-related developmental and epileptic encephalopathy. ASGCT Abstract #38, Session: Neurologic Diseases I.