Apertura GENE THERAPY

#1531

Intravenous Delivery of a CNS-Penetrant AAV Gene Therapy Provides Survival Benefit in a TSC1 Disease Model

Christopher Davis PhD[1], Sisi Chen PhD[1], Ellen Horste PhD[1], Tina Hu-Seliger PhD[1], Von Wiltman BS[1], Jorge Santiago-Ortiz PhD[1], Andrew Steinsapir MBA[1,2]

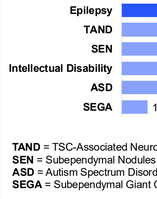
Affiliations
1. Apertura Gene Therapy, New York, NY
2. Deerfield Management Company, New York, NY

Acknowledgments
We are grateful for support from the TSC Alliance Preclinical Research Consortium and the entire team at Apertura. We thank the people living with TSC, parents, and caregivers for their time speaking with us.

References
1. Ho CN, et al. The Voice of the Patient: A Report from the Tuberous Sclerosis Alliance's Externally-Led Patient-Focused Drug Development Meeting. 2017.
2. Please see presentation 305 by Jorge Santiago-Ortiz on Friday May 16: "FDA-Aligned Strategy for AAV-Based TSC1 Gene Therapy: Advancing Preclinical Development Without Non-Human Primates".
3. Uhlmann EJ, et al. Astrocyte-specific TSC1 conditional knockout mice exhibit abnormal neuronal organization and seizures. Ann Neurol. 2002 Sep;52(3):285-96. PMID: 12205640.
4. Prabhakar S, et al. Survival benefit and phenotypic improvement by hamartin gene therapy in a tuberous sclerosis mouse brain model. Neurobiol Dis. 2015 Oct;82:22-31. PMID: 26019056.

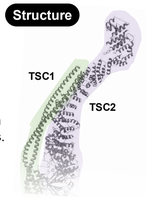
Background

- Tuberous sclerosis complex (TSC) is a neurodevelopmental disorder caused by loss of function mutations in the genes *TSC1* or *TSC2*, which encode regulators of mTOR activity.
- Mutations in *TSC1* or *TSC2* lead to hyperactivation of the mTOR pathway, resulting in abnormal cell growth, gliosis, and epileptogenesis.
- While options exist for the treatment of TSC-associated epilepsy, ~60% of TSC patients develop recalcitrant epilepsy resistant to multiple anti-seizure medications, underscoring the need for improved treatments for TSC.[1]
- Given the neurological impact of TSC, an effective therapeutic would require widespread distribution throughout the central nervous system (CNS).

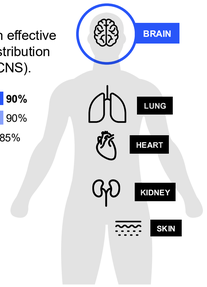


TAND = TSC-Associated Neuropsychiatric Disorders
SEN = Subependymal Nodules
ASD = Autism Spectrum Disorder
SEGA = Subependymal Giant Cell Astrocytoma

Structure

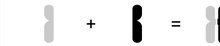


TSC1/2 Complex



Genetics

- **Most cases** (~70%) are **spontaneous**, with only a minority (~30%) inherited
- **Haploinsufficiency, loss-of-function mutation**: 50% level of TSC function is insufficient
- **Autosomal dominant**: when inherited, single copy of the mutated TSC gene from one parent is enough to cause the disorder:



Allele With Mutated *TSC1* Gene + Allele With Normal *TSC1* Gene = Net Loss of *TSC1* Function

Mode of Action

Normally, TSC plays a **negative regulatory role** in the mTOR pathway:

Normal Pathway

Loss of TSC function causes mTOR and downstream pathways to be **hyperactive**:

Dysfunctional Pathway

Approach

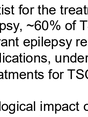
Apertura has developed AGTX-1111, an AAV designed to deliver the *TSC1* gene to the CNS after intravenous delivery using TfR1 CapX, a capsid engineered to cross the BBB via the human transferrin receptor (hTfR1).

Preclinical studies for AGTX-1111 make use of a surrogate approach[2] wherein a mouse-specific BBB-crossing capsid, PHP.B, is used in place of TfR1 CapX for mouse disease models lacking expression of hTfR1.

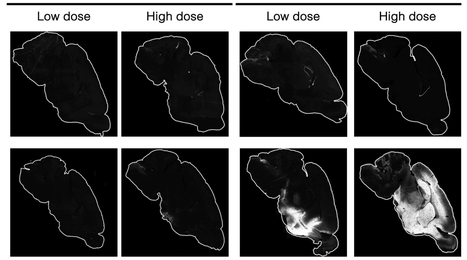
Confirmation of Delivery in Disease Model

- We utilize a previously described loss of function mouse model in which *Tsc1* is conditionally disrupted in GFAP-expressing cells (*Tsc1 fl/fl GFAP-Cre*; "CKO"), leading to loss of function in astrocytes and neurons, resulting in reduced lifespan and seizures.[3]
- Using a GFP reporter transgene, we confirmed the ability of PHP.B to transduce the mouse CNS after intravenous dosing in this strain.

Study details



- **Mouse strain:** *Tsc1 fl/fl GFAP-Cre*
- **Dose levels:** Low, High
- **Age at dosing and ROA:**
  - P3 (facial vein)
  - P10 (retro-orbital)
- **Readout:** Imaging detecting GFP fluorescence



Evaluation of TSC1 Transgene Efficacy

TSC1 Pre-Proof of Concept Study Design

Goals

Confirm reproducibility of published data
✓ Can prophylactic (prior to seizure onset) delivery of AAV carrying published TSC1 transgene cassette[4] demonstrate benefit?

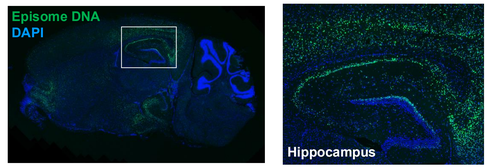
Novel insights
✓ Can treatment at seizure onset recover the model?

Study details

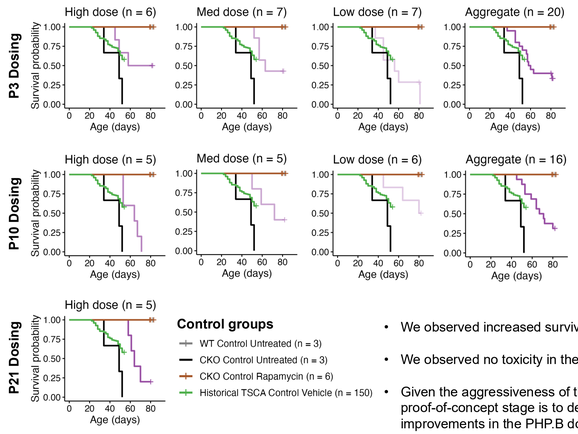
- **Mouse strains:**
  - CKO: *Tsc1 fl/fl GFAP-Cre* (n = 6/dose level)
  - WT: *Tsc1 fl/fl* (n = 3/dose level)
- **Dose levels:** Low, Med, High
- **Age at dosing and ROA:** P3 (facial vein), P10 (retro-orbital), P21 (retro-orbital)
- **Capsid:** PHP.B
- **Controls:**
  - CKO + rapamycin (n = 6)
  - CKO + no treatment (n = 6)
  - Control + no treatment (n = 3)
- **Readouts:** Distribution evaluation by *in situ* hybridization (ISH), survival

Distribution evaluation by ISH

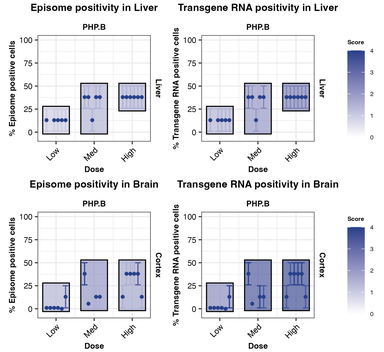
- *In situ* hybridization was used to confirm distribution of test article and transgene expression in the brain (see example image below).
- We observed a dose response in transduction, where increasing test article dosage yielded an increasing percentage of cells positive for episome and transgene RNA (see plots below for quantification).



Survival



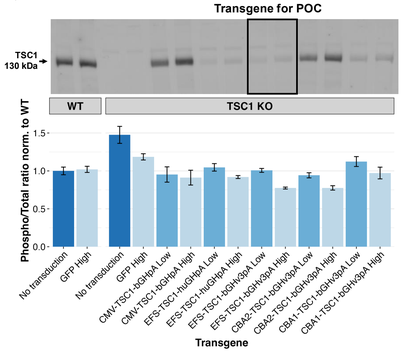
Control groups
- WT Control Untreated (n = 3)
- CKO Control Untreated (n = 3)
- CKO Control Rapamycin (n = 6)
- Historical TSCA Control Vehicle (n = 150)



- We observed increased survival in our PHP.B dosed groups relative to the untreated CKO group.
- We observed no toxicity in the WT mice (*Tsc1 fl/fl*) for all PHP.B doses evaluated (data not shown).
- Given the aggressiveness of this disease model, our initial criteria for advancing this program to the proof-of-concept stage is to demonstrate any benefit in any group. We believe that the survival improvements in the PHP.B dosed group relative to the control untreated group satisfies this criteria.

TSC1 Transgene Development

- We systematically evaluated AAV-driven TSC1 expression by screening promoter/polyA pairs in TSC1 KO 293Ts, assessing expression via Western blot and mTOR activity via p-P70S6K ELISA.
- mTOR pathway restoration observed with lower expression level from EFS promoter, suggesting high expression of TSC1 is not necessary for therapeutic benefit.
- The EFS-TSC1-bGHv3pA construct (boxed) was advanced for evaluation in the Tsc1 CKO mouse model in a Proof-of-Concept study (see design below).



TSC1 Proof-of-Concept Study Design

Goals

Novel insights
✓ Can prophylactic (prior to seizure onset) delivery of AAV carrying Apertura's TSC1 transgene cassette demonstrate benefit?
✓ Does this treatment improve epilepsy phenotype in the disease model?

Study details

- **Mouse strains:**
  - CKO: *Tsc1 fl/fl GFAP-Cre* (n = 14 / dose level)
  - WT: *Tsc1 fl/fl* (n = 6 / dose level)
- **Dose levels:** Low, Med, High
- **Age at dosing and ROA:** P10 (retro-orbital)
- **Capsid:** PHP.B
- **Controls:**
  - CKO + rapamycin (n = 6)
  - CKO + no treatment (n = 16)
  - Control + no treatment (n = 6)
- **Readouts:** Seizure frequency (EEG), Distribution evaluation by *in situ* hybridization (ISH), survival

Conclusions

- These preliminary efficacy data of the nominated development candidate payload motivate its advancement to proof-of-concept *in vivo* efficacy evaluations for the developed TSC1 gene therapy.
- These data will fit within a broader regulatory strategy to identify TfR1 CapX dose levels that achieve similar levels of CNS distribution as an efficacious dose of the surrogate capsid PHP.B.
- We anticipate that this strategy will help enable identification of AGTX-1111 dose levels appropriate for treatment of TSC patients, as the dose scaling from mouse to human is 1:1 for TfR1 CapX..