Apertura GENE THERAPY

#1060

Redefining TSC2 Gene Therapy: Development of a Novel Split Intein Payload and a Translational Potency Assay

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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.

Plain Language Poster Summary

Tuberous sclerosis complex (TSC) is a rare genetic condition that affects around 42,000 people in the United States. It is caused by mutations in the TSC1 or TSC2 genes which result in epilepsy, developmental challenges, and other symptoms. Developing a gene therapy for TSC2 is difficult because the gene is too large to fit into standard delivery systems, and treatment must reach many areas of the brain to be effective.

We created a specialized test, called a potency assay, to measure whether we can fix sick cells that have problems with TSC2. This type of test has been able to tell when a related gene, TSC1, would work and has also helped us when working on TSC2. We explored several strategies to fix sick cells that have problems with TSC2. We tested 21 different and smaller versions of the TSC2 gene, but none of these worked to fix the problems in cells or laboratory animals. Instead, we built a new version where TSC2 is broken into two pieces and delivered using two viruses; the two pieces come together inside of the cells. In this poster we show that this approach works to fix cells. Next steps will be to use the new approach for TSC2 in laboratory animals.

Introduction and Executive Summary

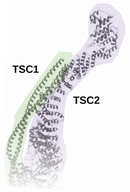
Tuberous sclerosis complex (TSC) is a haploinsufficiency disorder affecting approximately 42,000 individuals in the United States, with ~70% of cases caused by *TSC2* mutations. These mutations lead to hyperactive mTOR signaling, contributing to a range of disease manifestations, including epilepsy and neurodevelopmental symptoms. TSC2 mutations are highly heterogeneous, making a single gene-editing approach impractical; instead, gene supplementation is the most viable therapeutic strategy. Importantly, overexpression of *TSC2* has not been associated with toxicity, meaning that restoration of *TSC2* protein levels does not carry the risk of a "goldilocks effect" requiring strict dose control. However, gene therapy for *TSC2* presents two major challenges: (1) its coding sequence (5.4 kb) exceeds AAV packaging limits, and (2) its delivery requires widespread distribution across the brain, as no single brain region or cell type is uniquely responsible for seizure generation. While minigene-based approaches have been explored to overcome the size limitation, they have failed to restore mTOR regulation in our preclinical studies.

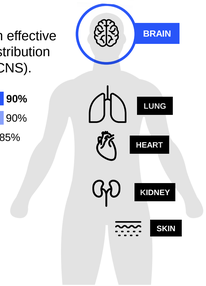
- Apertura developed a cell-based potency assay to assess *TSC2* function *in vitro* to support the development of a TSC2 therapeutic development program
- This potency assay revealed that a previously published TSC2 minigene was unable to restore mTOR function – likewise, that construct failed to demonstrate a therapeutic effect *in vivo* when assessed in an established animal model (data not shown)
- In parallel studies with TSC1, payloads that restored function in a similar potency assay also demonstrated therapeutic effects *in vivo*
- We present a novel split intein-based *TSC2* payload capable of full-length protein reconstitution and functional rescues of the mTOR pathway in a cell-based potency assay
- Apertura's BBB-penetrant capsid TfR1 CapX is ideal for this therapeutic program given its highly efficient CNS-wide delivery after IV administration

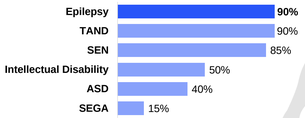
Background

- 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).

TSC 1/2 Complex



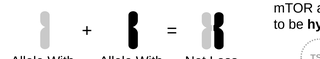




TAND = TSC-Associated Neuropsychiatric Disorders
SEN = Subependymal Nodules
ASD = Autism Spectrum Disorder
SEGA = Subependymal Giant Cell Astrocytoma

Genetics

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



Allele With Mutated *TSC2* Gene + Allele With Normal *TSC2* Gene = Net Loss of *TSC2* Function

Mode of Action

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

Normal Pathway

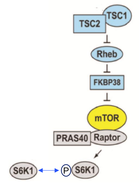
Loss of TSC function causes mTOR and downstream pathways to be **hyperactive**:

Dysfunctional Pathway

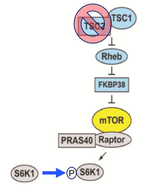
Motivation, Methods, and Approach

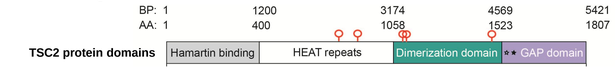
- A western blot-based expression assay and an ELISA-based phospho/total p70S6K potency assay were developed using commercially available reagents and a TSC2 knockout cell line (TSC2 KO) to assess therapeutic potential and guide payload development and optimization
- *In vitro* studies were conducted in the TSC2 KO cells via co-transfection and viral co-transduction to assess protein expression and mTOR regulation.
- Comparative studies included 19 novel minigene constructs as well as one previously published minigene.
- Five split intein TSC2 payloads were designed to splice within key domains, preserving critical folding and functional regions.

TSC2 Wildtype Cells: Basal phospho-S6K



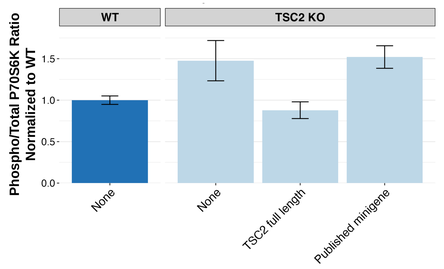
TSC2 KO Cells: High phospho-S6K



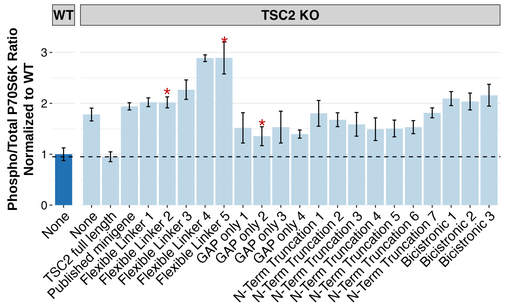


Minigene group	Hamartin binding domain (HBD)	HEAT domain repeats	Dimerization domain/motif	GAP domain	Constructs evaluated	Protein expression	Phospho-S6K rescue
Flexible linker	Present in 4/5 constructs	Present in 2/5 constructs	Yes	Yes	5	3/5 constructs	No
GAP only	No	No	Yes	Yes	4	3/4 constructs	No
Bicistronic	Yes	No	Yes	Yes	3	Yes	No
N-terminal truncation	No	Yes	Yes	Yes	7	Yes	No

Previously published minigene fails to restore mTOR function *in vitro*



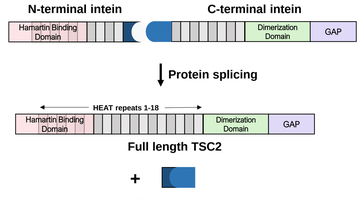
Novel minigenes fail to restore mTOR function *in vitro*



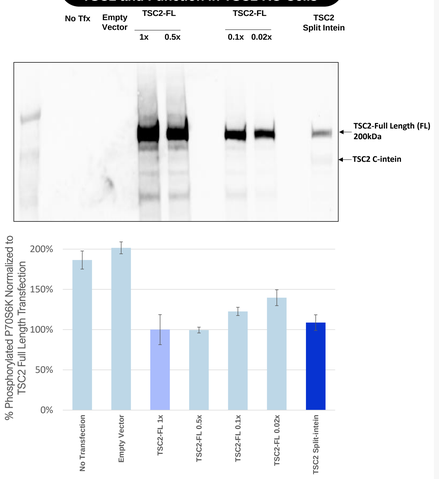
Asterisks indicate constructs for which expression was not detected by western

Results and Transgene Confirmation

Despite efficient protein expression in TSC2 KO cells, none of the 19 novel minigenes nor the previously published minigene restored mTOR regulation. Likewise, the previously published minigene failed to demonstrate a therapeutic effect in an established animal model (data not shown). The split intein payloads tested demonstrated expression and function *in vitro*, paving the way for future *in vivo* studies.



TSC2 Split Intein Results in Full Length TSC2 and Function in TSC2 KO Cells



Conclusions

We report the successful development of a functional TSC2 payload using split inteins, supported by a cell-based potency assay with clear preclinical and regulatory applications. The potential predictive value of this potency assay has been reinforced by successful *in vivo* studies in TSC1, supporting its utility for gene therapy development (see Poster #1531). These findings establish a viable path forward for TSC2 gene therapy, with next steps including *in vivo* validation.

Apertura's TfR1 CapX's inherent efficiency in crossing the blood-brain barrier and achieving robust CNS-wide delivery after intravenous administration provides a viable translational path to clinical applications of this TSC2 split intein payload.