None of the serious adverse events reported by patients (n=237) in the two double-blind phase 3 studies or any other clinical study were assessed as study-drug related

Simufilam, a small molecule, reduced seizure activity in a mouse model of focal cortical malformations and demonstrated a favorable clinical safety profile

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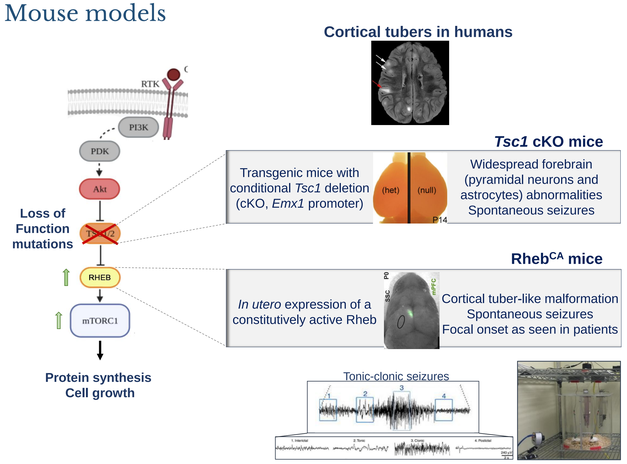
Abstract

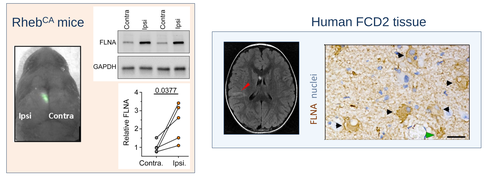
Epilepsy treatments for patients with mechanistic target of rapamycin (mTOR) disorders, such as tuberous sclerosis complex (TSC) or focal cortical dysplasia type II (FCDII), are urgently needed. In these patients, the presence of focal cortical malformations is associated with the occurrence of lifelong epilepsy, leading to severe neurological comorbidities. Recent evidence suggests that expression of the actin cross-linking protein filamin A (FLNA) is increased in resected cortical tissue from patients with TSC and FCDII, and in experimental cortical malformations leading to focal onset seizures in mice. Restoring proper FLNA expression in dysmorphic neurons in the focal cortical malformation reduced soma overgrowth and seizure activity. Treating mice with simufilam, a small molecule thought to modulate FLNA function, either before or after seizure onset, alleviated neuronal abnormalities and reduced seizure activity compared to vehicle-treated mice. Similarly preclinical studies are underway with the TSC Alliance preclinical consortium using conditional *Tsc1* knockout *(c*KO) mice. Simufilam shows high brain permeability in mice and rats and was measurable in CSF from human volunteers. Chronic oral toxicology studies found no adverse effects at doses up to 50 mg/kg/day in rats and 1250 mg/kg/day in mice. In two double-blind phase 3 studies in patients with mild-to-moderate Alzheimer's disease (n=1,929), simufilam demonstrated a favorable safety profile. Non-serious adverse events were typically mild and not considered study-drug related, and no specific adverse event was associated with simufilam administration. None of the reported serious adverse events in phase 3 (n=237) or any other clinical study were assessed as study-drug related. Based on these findings, Cassava Sciences, Inc. is preparing to submit an IND application to the FDA and enroll patients in a clinical trial of simufilam for the treatment of epilepsy in TSC

Mouse models



We used both transgenic conditional *Tsc1* KO mice (*Emx1*-Cre x *Tsc1*$^{fl/-}$) and constitutively active Rheb (Rheb^{CA}) mice. These display a focal cortical malformation generated using *in utero* electroporation (IUE) resulting in spontaneous seizures. Seizures were recorded using video-EEG (Pinnacle system) and tonic clonic seizures were quantified.

1. An unbiased screen identified increased Filamin A (FLNA) in TSC and FCDII mouse models and human brain tissue

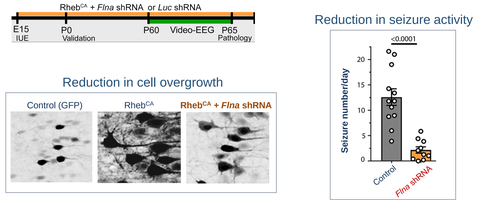


- An unbiased screen (PCR array) identified 5/84 dysregulated genes including *Flna* in the brain of *Tsc1* KO mice (not shown).
- We validated that FLNA is elevated at the protein level in the brain of *Tsc1* cKO mice (not shown) and Rheb^{CA} mice.
- We and others validated that FLNA is increased in FCD2 and TSC (not shown) cortical tissue

2. FLNA is a large multi-functional protein inside cells and its increased expression in TSC is mTOR-independent

- FLNA is an actin crosslinking molecule with dozens of binding partners, acting as a platform inside cells that regulates the function of its binding partners and the shape of the cytoskeleton
- FLNA controls many aspects of cell development
- The increase in FLNA is mTOR-independent
- Increased FLNA preceded seizure onset and may thus contribute to the development of a circuitry prone to seizures

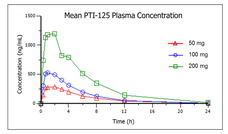
3. Reducing FLNA levels limited cell overgrowth and seizure activity



A short-hairpin (sh) RNA was expressed to reduce FLNA expression in Rheb^{CA}-expressing neurons. *Flna* shRNA reduced cell overgrowth compared to control shRNA (against luciferase) and significantly reduced tonic-clonic seizure activity. P = postnatal day

4. Simufilam is a small molecule with high brain permeability and an excellent safety profile

Single dose in healthy volunteers 18-46 yo



1,929 enrolled AD subjects for 76 weeks

	Simufilam 50 mg bid N = 376	Simufilam 100 mg bid N = 773	Placebo N = 771
Any Adverse Event (AE)	288 (76.6%)	570 (73.7%)	551 (71.5%)
AE Assessed by Investigator as Related to Study Drug	50 (13.3%)	138 (17.9%)	102 (13.2%)
Serious Adverse Event	61 (16.2%)	95 (12.3%)	81 (10.5%)
Death	6 (1.6%)	3 (0.4%)	6 (0.8%)
AE Leading to Study Discontinuation	34 (9.0%)	58 (7.5%)	34 (4.4%)

- Half-life at 100 mg single dose in healthy volunteers was 4.45 hrs
- CSF/plasma ratio was 0.61 (0.41 SD) in Alzheimer's disease (AD) patients following 100 mg BID for 28 days (not shown)
- Simufilam was safe and well-tolerated. There were no serious adverse events, and no adverse events were related to 10 mg BID simufilam in >1,929 AD patients

Conclusion

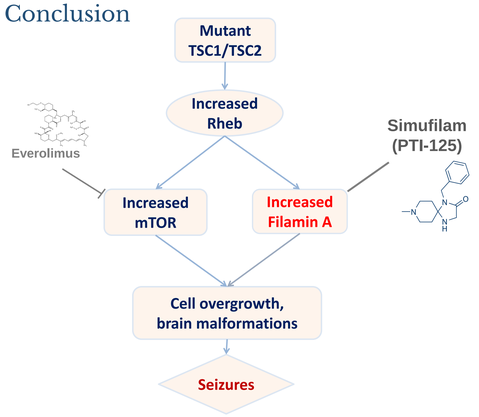


Diagram illustrating our findings and hypothesis

References

- Convulsive seizures from experimental focal cortical dysplasia occur independently of cell misplacement. Hsieh LS et al. 2016 Nat. Comms 7:11753.
- MEK-ERK1/2-dependent FLNA overexpression promotes abnormal dendritic patterning in tuberous sclerosis independent of mTOR. Zhang L, et al. 2014. Neuron 84:78.
- Filamin A inhibition reduces seizure activity in a mouse model of focal cortical malformations. Zhang L, et al. 2020 Sci. Trans. Med. 12:eaay0289.

Acknowledgements and COI

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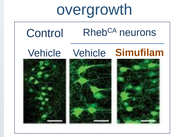
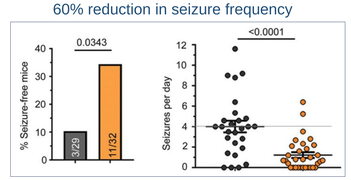
Dr. Bordey is a professor at Yale and an employee of Cassava Sciences since 2025.

5. Simufilam reduced cell overgrowth and seizure activity in Rheb^{CA} mice

Reduction in cell overgrowth

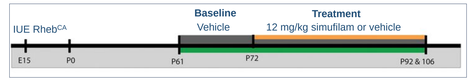


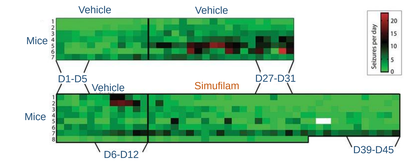
60% reduction in seizure frequency

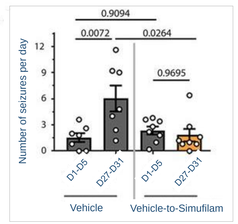


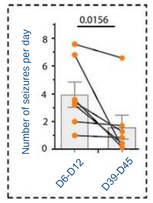
1 dose:	12 mg/kg (salt), intraperitoneal, BID
3 treatment ages:	Neonate (P8-P66), Young adult (P29-P54), Adult (P61)
Treatment length:	>3 weeks (analysis of seizures during 5 last treatment days
Video-EEG:	5 days at the end of treatment or continuous (adult group)
Control groups:	Vehicle (saline) for each

6. Simufilam's effect takes time to reduce seizure activity in Rheb^{CA} mice









- Seizures were recorded from P61 to P92 (vehicle) or to P106 (drug)
- Seizure activity increased over time (5 last days vs first 5 days) in vehicle-treated mice consistent with an increase in cell overgrowth and network abnormalities
- Simufilam alleviated the increase in seizure activity observed in vehicle treated mice
- Simufilam significantly reduced seizure activity when comparing the last 5 days of treatment to the 5 days preceding treatment (baseline phase)

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