Treating Epilepsy in an Orphan Genetically-defined Seizure Disorder, Tuberous Sclerosis Complex (TSC)
Tuberous Sclerosis Complex, a genetically-defined (TSC1/TSC2) life-long epilepsy disorder

TSC diagnosis: First by pediatricians, referred to specialists
- 1-100 Daily Seizures: 85% of all patients (neurologists)
- Median age of seizure onset: 3 months
- Skin patches (dermatologists)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Current SOC</th>
<th>Efficacy</th>
<th>Comorbidities</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Brain Malformations</td>
<td>• Brain surgery</td>
<td>• Limited efficacy</td>
<td>• Insomnia</td>
</tr>
<tr>
<td>• Childhood onset seizures</td>
<td>• Everolimus</td>
<td>• Side-effects</td>
<td>• Learning disabilities</td>
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<td>• Life-long epilepsy</td>
<td></td>
<td></td>
<td>• Behavior issues (e.g., anxiety)</td>
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<td>• AED resistant</td>
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- High burden on care givers and patients: We need new drugs to treat seizures and comorbidities
TSC is an orphan disorder with a high societal cost

**Incidence:** 1/6,000 new births
50,000 TSC pts with epilepsy in the US
30,000-40,000 TSC pts with drug-resistant epilepsy (60-80% all pts)

**Cost of Everolimus (SOC):** $16K/mo/pt, $192K/year/pt
For 30,000 patients this represents **a US market opportunity of $5-6B/year**
We will use everolimus trial’s design and clinical endpoints

**Primary endpoint Phase III:** Percent change in seizure frequency
- [core phase (18 wks) vs baseline (8 wks)]

**Secondary endpoints:** Impact on behavior and quality of life (and more)

**Brain surgery:** In only 10-15% of pts
- Seizures remain in ~40% of operated pts
- Seizures return in 50% of seizure-free pts post-op

**Everolimus:** Limited efficacy (40% of pts respond at high dose)
(Afinitor) Major side-effects
## Competition

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Efficacy</th>
<th>Formulation</th>
<th>Side-effects</th>
<th>Mode of action</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Conventional AED</strong></td>
<td>Seizure reduction in 30-40% pts</td>
<td>Liquid, pill,</td>
<td>e.g., Sleepiness, nausea depending on the drug</td>
<td></td>
<td>several</td>
</tr>
<tr>
<td></td>
<td></td>
<td>suppository</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Everolimus (SOC)</strong> (Afinitor)</td>
<td>40% pts with &gt;50% seizures reductions</td>
<td>Liquid suspension</td>
<td>Many and serious: e.g., stomatitis, diarrhea, infections (bone loss)</td>
<td>mTOR inhibitor</td>
<td>Novartis</td>
</tr>
<tr>
<td><strong>Under development</strong></td>
<td>Unknown (failed phase II for Fragile X syndrome)</td>
<td>unknown</td>
<td>Unknown but widespread expression</td>
<td>mGluR5 antagonist</td>
<td>Noema Pharma</td>
</tr>
<tr>
<td><strong>Epidiolex (cannabidiol)</strong></td>
<td>Age 1-57 years, 201 pts 20% reduction (vs placebo)</td>
<td>Liquid solution, twice daily</td>
<td>serious: e.g. diarrhea, suicidal thoughts, elevated liver enzymes, sleepiness, fever, vomiting, rash</td>
<td>Cannabinoid receptor mTOR inhibition</td>
<td>Greenwich Biosciences Inc.</td>
</tr>
</tbody>
</table>
Our Mouse Model: Competitive Advantage for Drug Discovery

Using in utero electroporation to model human brain malformation in TSC

- Plasmid DNA expression via in utero electroporation
- Seizure monitoring via video-EEG
- Histology

Embryonic day 15, 6-8 weeks, 9 weeks

Brain malformation

Ipsi, Contra

Our mouse model: Definitive and only model for TSC seizures
- Hsieh, Bordey 2016
- Validated through collaborations with Biotechs
Three New Validated Targets & Four Yale Solutions – Three patents

- Mutant TSC1/TSC2
  - Increased Rheb
  - Increased mTOR
  - Increased MEK

- Everolimus

- Increased Filamin A

- Abnormal HCN4
  - Enhanced excitability
  - Cell overgrowth, brain malformations
  - Seizures

- Yale HCN4 Gene Therapy
  - Patent Filed

- Increased 4EBP1 activity
  - Increased EIF4E activity

- Yale 4EBP Gene Therapy and/or EIF4E RNAi
  - Patent Filed

- Mirdametinib
  - MEK1/2 Inhibitor (Ph 2 Solid Tumors)

- Increased EIF4E activity

- Yale FLNA NCE and RNAi
  - Patent Filed

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Solution 1: Targeting Filamin A (FLNA) for seizure reduction is validated in adult mice

- FLNA is an actin-binding molecule that is increased in TSC patients and mouse models.
- Normalizing (shRNA) or blocking (drug) FLNA shrinks cell size and brain malformation and reduces seizure activity in the most relevant and accepted mouse model (Yale generated).

Goal: CNS Injection of Flna siRNA
Solution 2: Overexpressing 4EBP1 for seizure reduction is validated in adult mice

- 4EBP1 activity is decreased in TSC patients and mouse models.
- Decreased 4EBP1 activity results in increased protein synthesis, cell overgrowth, and brain malformation.
- Overexpression a constitutively active 4EBP1 shrunk brain malformation and reduced seizures.

Goal: Focal delivery of 4EBP1-AAV Gene Therapy
Mouse *in vivo* efficacy studies of RNAi and AAV will enable our IND application

Mouse *in vivo* studies

- Cell size analysis
- Monitor daily seizures
- Change in seizure frequency

Translate well into clinical studies

Genetically-Defined Patient Population

Clinical endpoints

- MRI every 2 months
- Monitor daily seizures
- Change in seizure frequency
RNAi and AAV efficacy on seizures is gating to pre-IND meeting

**Completed**
- Target validation FLNA and 4EBP1
- Clinical collaboration
- Animal model
- Clinical endpoints established

**Flna RNAi solution - $70K**

Deliverables Q4 2022
- RNAi being generated (commercial source)
- Efficacy on seizures via CNS injections in Yale Model
- Validation of knockdown in human neurons

**4EBP1 AAV solution - $70K**

Deliverables Q3 2022
- 4EBP1-AAV being produced (commercial source)
- Efficacy on seizures via CNS injection in Yale Model

**Partnership - $5M Seed**

Q2 2023
- Efficacy on seizures via second model
- Final Tox study
- Pre-IND package

- **4EBP1 AAV solution - $70K**
  - Q2 2023
  - Efficacy on seizures via second model
  - Final Tox study
  - Pre-IND package