**Systematic review of mTOR inhibitor treatment, biomarkers and prophylaxis for tuberous sclerosis complex-associated seizures**

**Introduction**
- Mutations in Tsc1 and Tsc2 genes result in hyperactivation of mammalian target of rapamycin (mTORC1) pathway, linked to epileptogenesis in tuberous sclerosis complex (TSC).
- Everolimus: mTOR inhibitor, FDA-approved for TSC-associated seizures in patients older than 2 years in 2018

**Outline**
1. Effectiveness of mTOR inhibitors in TSC-associated seizures [PubMed]
2. Predictive, diagnostic and prognostic biomarkers of epilepsy in TSC [PubMed]
3. Seizure prophylaxis with mTOR inhibitors

### Effectiveness of mTOR inhibitors in TSC-associated seizures

**Everolimus:**
- **Long-term safety:** 94% of 48 TSC patients with refractory epilepsy maintained improved seizure control over 4 years; adverse effects decreased over time

**White matter modification:** pharmacologically modify the genetic defect of TSC (incl. normal-appearing white matter), 28 pts., 12-18 months; longer exposure & younger age (< 10) -> greater effect

**Dosing and response:** 5-7 mg/NI initially and 5-15 mg/NI if inadequate clinical response; more difficult for pts with higher baseline seizure frequency to respond

**Patient stratification for treatment with mTOR inhibitors:**
- **Age:** more effective in <18, greatest in <6; longer exposure & early initiation -> long-term efficacy; critical time windows; more calcification in cortical tubers with age -> resistance
- **Baseline seizure frequency:** higher baseline seizure frequency -> more difficulty becoming a responder to adjunctive everolimus

**Calcification in cerebral parenchyma:** cerebral parenchymal calcification in epileptic discharge sites -> more likely resistant to appropriate AEDs and adjunctive rapamycin

**Refractory seizures:** refractory TSC-associated epilepsy -> higher diffusivity increase, i.e., greater response to everolimus

### Predictive, diagnostic & prognostic biomarkers of epilepsy in TSC

**Electroencephalogram (EEG):** epileptiform discharges in video EEG monitoring; serial routine EEGs; presence of interictal epileptiform discharges; increased neural connectivity in TSC infants

**Genetics:** no mutation or mosaic mutation -> reduced risks of seizures; Tsc1 mutation was associated with a milder phenotype than Tsc2; missense mutations in the central region of Tsc2 (exons 23 and 23) -> reduced incidence of infantile spasms; increased expression of genes for cell adhesion (VCAM1, integrins and CD44) and inflammatory responses (complement factors, serpinA3, CCL2 and several cytokines); decreased expression of genes for synaptic (glial glutamate transporter GLT-1 and voltage-gated channel); different gene expression in peri-tuberal cortex from control cortex

**miRNAs:** 4 candidate biomarker miRNAs for seizure development in TSC: miR 199a-5p, miR 21-5p, miR 29b-3q and miR 324-5p

**Inflammation:** increased CSF nerve growth factor; blood brain barrier dysfunction and perivascular inflammation; prenatal key inflammatory pathway activation in developing brain lesions in TSC

### Other biomarkers identified by EPISTO:
- Brain lesions in prenatal MRI; 3 SNPs were associated with epilepsy onset among 58 SNPs implicated in epilepsy GWAS: rs1046276 (T/C), rs3743123 (G/A) and rs1801133 (G/A); serum proteins showed small differences between TSC patients w/ & w/o seizures; oxidative stress

### Prognostic biomarkers
- Cyst-like tubers – aggressive seizures; predominance of poorly organized tubers; increased tuber count – strongly associated with infantile spasms; white matter mean diffusivity; cerebellar lesions – more severe clinical and neuroradiological phenotype

### Seizure prophylaxis with mTOR inhibitors

**Critical time windows for mTOR inhibition**
- mTOR inhibitors to modify neural migration and synaptogenesis caused by mTOR hyperactivation in humans, synaptogenesis lasts until around 3.5 years old
- Dysfunction relapse after mTOR inhibitor withdrawal was shown by animal studies – seizures occurred a few weeks after stopping treatment

### Clinical evidence of seizure prophylaxis in TSC
- Effectiveness of prophylaxis for epilepsy in TSC established for vigabatrin: the risk of developing clinical seizures by school age was 50% w/ vigabatrin prophylaxis (vs 96% w/o prophylaxis)
- Everolimus on seizure control in TSC patients before seizure onset was mentioned by a few anecdotal reports (Guyer et al. 2015; Chang et al. 2017; Hoshal et al. 2015)

### Conclusion
- Clinical trials have proven the efficacy and safety of mTOR inhibitors, principally everolimus, for seizure control in TSC.
- Effects of everolimus were shown to be mediated by duration- and dose-dependent mechanisms and more pronounced in patients with young age, low baseline seizure frequency, low level of cerebral parenchymal calcification and refractory seizures.
- Predictive biomarkers, incl. EEGs, genetics, miRNAs and immuno-inflammation changes, could identify high-risk patients and prompt initiation of prophylaxis.
- Diagnostic and prognostic biomarkers could confirm diagnosis and monitor response to treatment and disease progression.
- Animal studies have shown that mTOR inhibitors could modify neural migration and synaptogenesis caused by mTOR hyperactivation, if given within critical time windows.
- Widespread effects of mTOR blockade are unknown and case reports of everolimus prophylaxis in TSC patients were inconclusive.
- Future clinical trials needed to study everolimus prophylaxis in young, asymptomatic patients and in combination with other AEDs.

**References**
Available at https://tinyurl.com/mTORinhibitor

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**Small-scale clinical trials**

<table>
<thead>
<tr>
<th>Author</th>
<th>Medication</th>
<th>Condition</th>
<th>No. of Pts</th>
<th>Results</th>
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<tr>
<td>Krueger et al. 2010</td>
<td>Everolimus</td>
<td>TSC-associated SEGA</td>
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<td>Improved seizure control over 34.2 months (median)</td>
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<td>Krueger et al. 2013</td>
<td>Everolimus</td>
<td>TSC-associated refractory epilepsy</td>
<td>20</td>
<td>No. of pts with no seizures reported since last visit (or more than 6 months since last seizure) increased from 38.5% at baseline to 65.2% at 24 months</td>
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<td>Cardamone et al. 2014</td>
<td>Sirolimus</td>
<td>TSC-associated refractory epilepsy</td>
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<td>Patient receiving everolimus had a 25-50% seizure frequency reduction</td>
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<tr>
<td>Overwart et al. 2016</td>
<td>Sirolimus (adjunctive)</td>
<td>TSC-associated refractory epilepsy</td>
<td>23</td>
<td>Despite seizure frequency reduction, significant benefits could not be proven</td>
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**EXIST-3 clinical trial**
- **Fereres et al. 2016** | Everolimus (adjunctive, low/high exposure) | TSC-associated refractory epilepsy | 366 | Greater response rate, median reduction in seizure frequency and number of seizure-free days |
- **Duration-response and dose-response**
- **2019 Mizuguchi et al.: Japanese study confirmed results**
- **2018 Curatolo et al.: patients’ greatest benefit among paediatric population (<18)**

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