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The mTOR pathway in treatment of epilepsy: a clinical update

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Abstract

Nearly a third of patients with epilepsy have seizures refractory to current medical therapies. In the search for novel drug targets, the mTOR pathway has emerged as key in the regulation of neuronal function, growth and survival, and other cellular processes related to epileptogenesis.

Hyperactivation of the mTOR pathway has been implicated in tuberous sclerosis complex and other ‘mTORopathies’, clinical syndromes associated with cortical developmental malformations and drug-resistant epilepsy. Recently published clinical trials of mTOR inhibitors in tuberous sclerosis complex have shown that these drugs are effective at decreasing seizure frequency.

Future studies may establish whether mTOR inhibitors can provide effective treatment for patients with diverse genetic and acquired epilepsies, including preventative, disease-modifying therapies.

Keywords

epilepsy; everolimus; mTOR; rapamycin; seizure; tuberous sclerosis

Epilepsy is one of the most common neurologic diseases, affecting 1% of people across the lifespan. Unfortunately, nearly a third of patients with epilepsy will continue to have seizures despite appropriate medical therapy [1]. Although there are numerous US FDA-approved medications to treat seizures, their common mechanism of action is to decrease neuronal excitability through effects on neurotransmitter receptors or ion channels [2]. As pathophysiologic mechanisms of genetic and acquired epilepsies are elucidated, the need for precise molecular drug targets has become an area of intense interest. The mTOR pathway has been implicated as a mechanism by which diverse genetic mutations and acquired abnormalities lead to a final common pathway of seizures. As such, the use of drugs targeting mTOR holds promise as potent treatment for seizures and as a potential disease-modifying epilepsy therapy.

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The physiologic role of mTOR

mTOR is a serine-threonine protein kinase that forms part of two intracellular signaling complexes, mTORC1 and mTORC2 ^[3,4]. Under normal physiologic conditions, the proteins hamartin and tuberlin – encoded by the *TSC1* and *TSC2* genes, respectively – form a complex that inhibits the GTP-binding protein Rheb and thereby mTORC1 (Figure 1). mTORC1 has downstream effects on cell growth, proliferation and survival, primarily by controlling ribosomal biogenesis and protein translation. Upstream regulators of the hamartin/tuberlin complex or mTORC1 allow cells to respond to different metabolic conditions. In states of energy surplus, insulin and other growth factors stimulate the PI3K/Akt pathway, which in turn activates the mTOR pathway, leading to increased cell growth and metabolism. Similarly, high levels of amino acids, particularly leucine, trigger the DEPDC5 pathway, which leads to mTOR activation. In contrast, energy deficiency leads to activation of the LKB1/AMPK pathway, which in turn inhibits mTORC1 and limits cell growth. The other main mTOR signaling complex, mTORC2, regulates maintenance of actin cytoskeleton, somatic size and dendritic growth. Complex signaling mechanisms and feedback loops connecting mTORC1 and mTORC2 to each other and to upstream and downstream regulators, allow sensitive modulation of cell metabolism to different environments.

mTOR is ubiquitously expressed in mammalian tissues, and is particularly important in the nervous system. In the brain, mTORC1 and mTORC2 collectively regulate dendritic growth and morphology, synaptic transmission and plasticity, neurogenesis and neural network activity ^[5–8]. These functions are crucial for normal cortical brain development, as well as the maintenance of neuronal cells and neurotransmission across the lifespan.

The mTOR pathway in epilepsy

Given the critical role of mTOR in normal brain function, it is not surprising that dysregulation of the mTOR cascade has been implicated in numerous neurological diseases. The name ‘mTORopathies’ has been used to group a number of clinical syndromes in which mTOR regulation is altered. The classic mTOR-related disease is tuberous sclerosis complex (TSC), a genetic disorder characterized by benign tumors in multiple organs, including brain, kidneys, lungs, heart, eyes and skin. Mutations in the *TSC1* or *TSC2* genes cause loss of normal inhibition of mTORC1, leading to cell overgrowth and disruptions in synaptogenesis ^[9]. Many children and adults with TSC have neurologic abnormalities, including cortical malformations and subependymal giant cell astrocytomas (SEGAs) in the brain, as well as, frequently, drug-resistant epilepsy. There are multiple pathophysiological mechanisms by which mutations in *TSC1* or *TSC2* may cause epilepsy ^[10]. In animal models of TSC, deletions in *PTEN*, *TSC1* or *TSC2* genes lead to abnormal neuronal proliferation, differentiation and migration; altered expression of synaptic proteins; and abnormal axon and dendritic growth ^[8]. These changes most likely lead to decreased seizure threshold by promoting hyperexcitability of neuronal tissues at the cellular level, and by altering cortical morphology and neuronal connectivity at the systems level. Patients with *TSC1/TSC2* mutations develop cortical tubers, benign hamartomas found in cortex or subcortical white matter, which are thought to be the main structural abnormalities leading

to development of refractory seizures, and surgical removal of tubers frequently reduces or eliminates seizures [11]. Similar to the animal models, tubers in TSC patients have abnormal cortical lamination, enlarged and dysmorphic neurons, and alteration in receptor expression that likely promote hyperexcitability and seizures [12], although even perituberal and more remote cortex has similar histopathological abnormalities that may be epileptogenic [13,14]. Furthermore, beyond the classic cellular and pathological lesions of TSC, dysregulated mTOR can lead to numerous molecular changes in gene and protein expression that may affect neuronal excitability and promote seizures [15].

Other mTORopathies share with TSC common clinical features of cortical developmental malformations and medically refractory seizures. Hemimegalencephaly (HME) is a disorder of cortical development in which all or part of one cerebral hemisphere is abnormally enlarged, and can occur in isolation or as part of some neurocutaneous syndromes. Studies of brain tissue from HME patients have shown mTOR hyperactivation in some, but not all, neural tissue, suggesting that HME occurs following somatic mutation leading to hyperactivation of mTOR pathway during brain development [10]. More recent studies have shown specific causative somatic mutations in *AKT3*, *PI3K*, *PTEN* or *mTOR* itself in subsets of HME patients [16–20]. Megalencephaly is also seen in Pretzel syndrome, also known as polyhydramnios-megalencephaly-symptomatic epilepsy (PMSE), a rare autosomal recessive disorder described in Mennonite children from the area around Lancaster, PA, USA [21]. In addition to megalencephaly, these children have dysmorphic features, severe developmental delay and intractable epilepsy. PMSE results from a homozygous deletion in the *STRADA* gene [21,22]. Loss of STRADA function leads to decreased hamartin/tuberin inhibition of mTORC1, resulting in hyperactivation of the mTOR pathway.

Focal cortical dysplasias (FCDs) are the collective term for a diverse group of cortical developmental malformations. Although subtypes of FCDs have distinct histopathological features [23], they are all highly associated with medically intractable epilepsy. There is growing evidence for germline and somatic mutations in the mTOR pathway leading to focal dysplasias. In one series, somatic mutations in the *mTOR* gene accounted for 15% of patients with a subtype of FCD [24], a remarkably high yield, given that most if not all of these patients would be unlikely to have a causative genetic mutation found by conventional clinical testing. Many of the same somatic *AKT3/PI3K* mutations described in patients with HME have also been demonstrated in FCDs [18–20], suggesting that a single somatic mutation can lead to a spectrum of cortical abnormalities. Somatic mutations in *PTEN* gene have been shown cause FCDs without other tumor types typically seen in *PTEN*-related syndromes [25]. FCDs have also been described in patients with mutations of the *DEPDC5* gene. This gene encodes a protein that combines with NPRL2, and NPRL3 to form the GATOR1 complex, which acts as an inhibitor of mTORC1. *DEPDC5* mutations were first described in some Australian families with the syndrome Familial Focal Epilepsy with Variable Foci, in which individuals develop focal epilepsies localizing to different lobes [26]. Some, but not all family members have focal lesions associated with their epilepsies, and the same mutations were found in both lesional and nonlesional family members [27]. More recent studies have shown additional mutations in *TSC1/2*, *DEPDC5*, *NPRL2* and *NPRL3* that cause FCDs and nonlesional focal epilepsies, in both familial and sporadic forms [28–32], as well as epileptic spasms [33].

While mTOR-related syndromic epilepsies with associated non-neurological manifestations, such as TSC and PMSE, have been established as caused by germline mutations, recent advances in clinical genetics have allowed identification of a variety of somatic mutations in the mTOR pathway in cases of isolated, nonsyndromic cortical malformations, such as HME and FCDs as described above. While the diagnosis of these somatic mutations in brain tissue would otherwise be missed in patients by standard genetic testing in blood, these exciting discoveries have revolutionized concepts of the pathophysiology of a spectrum of cortical malformation as genetic mTORopathies and have suggested the possibility of treatment of many of these disorders with mTOR inhibitors.

Evidence for mTOR inhibitors in treatment of epilepsy

As a common pathway by which numerous sporadic and familial mutations cause epilepsy, mTOR represents a novel molecular target for antiseizure drugs. The compound rapamycin, an inhibitor of mTORC1, has been known for decades to have antimicrobial and antitumor effects. When it was approved for clinical use as sirolimus in 1999, its indication was as an immunosuppressant to prevent rejection of organ transplants. In 2010, the related mTOR inhibitor, everolimus, was approved for treatment of SEGAs in TSC patients [34]. More recently, there has been intense interest in mTOR inhibitors as potential antiseizure medications. Preclinical evidence for rapamycin's antiseizure effects in multiple animal models has been summarized previously [35]. Various animal models have shown a variety of mechanisms by which rapamycin may decrease seizure frequency or prevent development of epilepsy. These mechanisms include inhibiting abnormal cell growth and proliferation, inhibition of mossy fiber sprouting and restoration of normal glutamate signaling [35]. Some of these proposed mechanisms, particularly those on cell morphology and proliferation, may be less relevant to treatment of humans with epilepsy, in whom structural brain abnormalities nearly always precede development of seizures. mTOR inhibitors likely do not reverse preexisting structural lesions, such as tubers of TSC. Nonetheless, the potential for other cellular and molecular antiseizure effects of rapamycin has led to excitement for its use as a therapy in humans. In the current review, we focus on human studies and recent clinical trials of mTOR inhibitors (Table 1).

The most extensive use of mTOR inhibitors as antiseizure drugs has been in patients with TSC. A case series of the use of everolimus on a compassionate use basis for seven patients with severe, drug-resistant epilepsy, showed that four of six patients completing treatment had decrease in seizure frequency, although the response varied from a 25 to 100% reduction in seizures [36]. Another case series evaluating the use of sirolimus demonstrated a decrease in clinical seizure frequency in all patients, with five of seven patients having reduction greater than 50% [37]. A Phase I/II study of everolimus to treat SEGAs in TSC patients showed the drug-reduced seizure frequency in about half of patients, even though seizure effect was a secondary end point [34]. A follow-up analysis of these patients who received extended treatment for a median duration of 34.2 months (range 4.7–47.1 months) found that the percentage of patients who reported no seizures since their last visit (or more than 6 months since their last seizure from baseline) increased from 38.5% (10 of 26 patients) at baseline to 65.2% (15 of 23 patients) at 24 months [38]. In the subsequent Phase III trial of everolimus for SEGAs, the analysis of seizure control as a secondary end point was

inconclusive, as both the median number of seizures at baseline and median change in seizure frequency from baseline to follow-up was zero in both treatment and placebo groups [39]. These results prompted a Phase I/II study of everolimus as adjunctive therapy in TSC patients with the primary end point of reduction in seizure frequency [40]. This study showed that in 20 patients treated with 12 weeks of everolimus, seizure frequency was reduced in 17 patients, with median reduction 73% ($p < 0.001$), and 12 of 20 patients having seizure reduction greater than 50%. In a follow-up analysis of 18 patients in whom everolimus treatment was extended past 12 weeks, all but one of the 14 patients completing the study had greater than 50% reduction in seizure frequency at 48 months [41]. A separate open-label, single-center prospective trial of everolimus for epilepsy in children with TSC ages 1 through 18 years showed 12 of 15 children had treatment response, with seven patients seizure-free at median follow-up of 22 months [42]. These findings led to a Phase III, randomized placebo-controlled trial of adjunctive everolimus treatment for epilepsy, the EXIST-3 trial [43]. In this trial, 366 patients between age 2 and 65 years were randomized to placebo, low-exposure everolimus or high-exposure everolimus, in addition to concurrent standard medical therapy. 40% of patients in the high-exposure group showed a clinical response ($>50\%$ decrease in seizure frequency), compared with 15% in placebo group and 28% in low-exposure group. Median seizure reduction in the high-exposure group was 39.6%. In contrast to the EXIST-3 trial, a randomized controlled trial of sirolimus in children with TSC showed no effect of the drug on seizure frequency, although due to the slow recruitment rate, the study did not reach its target enrollment of 30 patients [44].

As most other mTORopathies have only been recognized in the past decade, clinical data regarding the effect of mTOR inhibitors for non-TSC epilepsies are limited. A prospective, open-label trial of sirolimus as adjunctive therapy in five infants with PMSE showed that four of five were seizure-free at follow-up (median 36 months, range 5–52 months), with the fifth child having had only one seizure in the prior 12-month period [45]. These patients also had improved receptive language compared with untreated historical controls, suggesting that mTOR inhibitors may have beneficial effects in mTORopathies beyond preventing seizures. Evidence for mTOR inhibitors in other syndromes of mTOR dysregulation is limited to animal models. As an example, rapamycin-suppressed epileptiform activity in EEG recordings of a *PTEN*-knockout mouse model [46]. With the recent experience using everolimus and sirolimus in TSC patients, prospective clinical studies of their use in other mTORopathies, particularly those causing severe refractory epilepsies, may be feasible.

The mTORopathies provide clinical cohorts for studies of mTOR inhibitors not only as antiseizure drugs in patients with drug-resistant epilepsy but also as possible preventative antiepileptogenic therapy. In mouse models of TSC, the use of postnatal rapamycin reversed histopathological sequelae of mTOR hyperactivation and prevented development of epilepsy [47, 48]. Infants diagnosed with TSC due to non-neurological findings represent an ideal clinical population for testing antiepileptogenic potential of drug therapies, including mTOR inhibitors, as the majority of patients with TSC will develop epilepsy. To date, however, there is limited published clinical evidence that mTOR inhibitors prevent epilepsy. In a follow-up study of eight children younger than age 3 years in EXIST-1 trial, three of five children who had refractory epilepsy at baseline saw significant improvement in seizures

with treatment [49], whereas, intriguingly, none of the other three children developed epilepsy.

Conclusion

The mTOR pathway plays a key role in normal neurologic cell morphology and physiology and has been implicated in TSC and other genetic epilepsies. Genetic mutations in mTOR and other key regulatory proteins have been shown to cause pathologic changes in cell size and function that lead to epileptogenesis. mTOR inhibitors may reverse some of these neuropathologic or functional abnormalities. Recent clinical trials of the mTOR inhibitors everolimus and sirolimus have shown potential for their use as antiseizure therapies in patients with TSC and other epilepsies caused by mTOR dysregulation. Future studies are needed to evaluate the long-term effects of mTOR inhibitors, as well as their role in other clinical syndromes.

Future perspective

The last few years have seen numerous studies showing that abnormal mTOR signaling is implicated in a growing number of epilepsies of a variety of etiologies. The most recent revision of the International League Against Epilepsy's classification of the epilepsies emphasizes that there is overlap – rather than division – between structural and genetic causes of epilepsies, citing TSC as a prime example of the dual etiology model [50]. Identification of genetic mutations in familial epilepsies and epilepsy syndromes, made possible by recent advances in technology and rapidly decreasing costs, has led to identification of germline and even somatic mutations in sporadic epilepsy, many of which are known or later shown to be involved in the upstream or downstream regulation of mTOR. There is even evidence that dysregulated mTOR signaling may play a role in nongenetic epilepsies, such as those that occur following traumatic brain injury or stroke. Histopathological analysis of tissue from patients with surgical resection of lesions for management of refractory epilepsy shows that mTOR hyperactivation occurs in acquired focal epilepsies such as Rasmussen's encephalitis and mesial temporal sclerosis [51]. Animal models of epilepsy following status epilepticus, traumatic brain injury and hypoxic ischemic injury in newborns suggest that mTOR inhibitors may prevent development in epilepsy following an epileptogenic insult to the brain [52–54].

There is a growing call for precision therapies for epilepsy, yet as a common signaling pathway, mTOR may be a precise molecular target with broad potential. Patient cohorts with similar genetic mutations will provide potential subjects for clinical trials. However, even with ideal target populations, such as children with TSC, there will remain many questions about the clinical use of mTOR inhibitors for epilepsy beyond how effectively they control seizures. For instance, will patients with a genetic mutation affecting the mTOR pathway whose seizures respond to everolimus require lifelong treatment, or a finite course of treatment within a certain therapeutic window? The latter is supported by a recent case report showing relapse of seizures following everolimus withdrawal, and subsequent seizure control with reintroduction of the drug [55]. The safety of chronic use of mTOR inhibitors is a concern, given the central role of mTOR in cell metabolism in all tissues. Although

adverse effects of everolimus treatment in patients with TSC, such as stomatitis and diarrhea, occur relatively frequently, most appear within the first year of treatment, are not serious and resolve without complication [56]. However, the immunosuppressive effects of mTOR inhibitors may leave treated patients vulnerable to opportunistic infections, which may cause significant morbidity, if not mortality [57].

Although there are many questions to be answered, there is reason to hope that we will be able to learn more about the clinical use of mTOR inhibitors in the near future. A major barrier to the use of mTOR inhibitors for epilepsy has been overcome by the approval of everolimus for treatment of refractory seizures in TSC patients in Europe in 2017, with the possibility of similar FDA approval now pending in the USA. Follow-up of patients in the EXIST-3 trial will provide evidence about the long-term safety and efficacy of everolimus. Finally, based on preclinical studies and mechanism of action, the potential of mTOR inhibitors for early preventative therapy for epilepsy may be even greater than standard treatment of drug-resistant epilepsy. Although no preventative studies of mTOR inhibitors have yet been initiated, there are two ongoing clinical trials investigating vigabatrin, a potent antiseizure medication that acts as a GABA transaminase inhibitor, as a therapy to prevent epilepsy (PREVeNT and EPISTOP) in infants with TSC. Regardless of their outcome, these trials will demonstrate feasibility of preventative trials of mTOR inhibitors in TSC and other mTORopathies.

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- of interest;
- of considerable interest

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Executive summary

Background

- Nearly a third of patients with epilepsy have seizures refractory to currently approved medications, which decrease neuronal excitability primarily through effects on neurotransmitter receptors or ion channels.
- The intracellular mTOR protein may be a precise molecular target for epilepsy treatment, as the pathway has been implicated in many seizure etiologies.

The physiologic role of mTOR

- The mTOR pathway plays a key role in normal cell physiology, affecting cell growth, proliferation and survival, particularly in the brain.
- Both upstream and downstream regulators of mTOR allow for tight regulation of neuronal cell size and function during normal cortical brain development and in response to changing environmental conditions across the lifespan.

The mTOR pathway in epilepsy

- The classic mTOR-related disease is tuberous sclerosis complex (TSC), in which genetic mutations in a key upstream mTOR regulator lead to development of characteristic clinical features, including cortical tumors and epilepsy.
- There is a growing list of genetic mutations elsewhere in the mTOR pathway that are now known to be associated with cortical developmental malformations and drug-refractory seizures in a number of other clinical diseases. Collectively referred to as mTORopathies, these syndromes include both sporadic and inherited forms of hemimegalencephaly, focal cortical dysplasias and even nonlesional epilepsies.

Evidence for mTOR inhibitors in treatment of epilepsy

- The mTOR inhibitors everolimus and sirolimus are approved agents for treatment of certain types of tumors.
- Early clinical data in TSC patients treated with mTOR inhibitors suggested these drugs could effectively decrease seizure frequency.
- A recent Phase III, randomized placebo-controlled trial of everolimus as adjunctive therapy for epilepsy in patients with TSC, the EXIST-3 trial, showed that 40% of treated patients had a greater than 50% reduction in seizure frequency.
- Limited clinical data suggest that mTOR inhibitors also may be effective to treat seizures in other mTORopathies.
- Future studies will define the role of mTOR inhibitors in the treatment of seizures and prevention of epileptogenesis in genetic and acquired epilepsies.

Figure 1. The mTOR protein kinase forms part of two cell signaling complexes – mTORC1 and mTORC2

Downstream effects of mTORC1, which include modulation of cell growth, proliferation, metabolism and ion-channel expression, are primarily mediated by ribosomal biogenesis (proteins S6K and S6) and protein translation (4E-BP1 and eIF4E). mTORC2 also has effects on cell growth and function, but unlike mTORC1, is not inhibited by the drug rapamycin, at least with short-term use. Regulation of cell growth and maintenance in different energy states is mediated by upstream regulators of mTORC1, including the hamartin/tuberlin complex encoded by *TSC1* and *TSC2* genes, the PI3K/AKT pathway, the GATOR1 complex pathway and the STRAD α /AMPK pathway. Genetic mutations affecting function of these regulatory pathways have been shown to cause mTOR hyperactivation in animal models and human tissues. Dysregulation of mTOR cell signaling is, therefore, thought to be a common mechanism for cortical developmental malformations and symptomatic epilepsy seen in a diverse group of neuroclinical syndromes.

Table 1

Summary of clinical evidence for mTOR inhibitors as adjunctive therapy in epilepsy.

Study (year)	Study type	Study drug	Patients	Duration of treatment	Summary of results	Ref.
<i>TSC</i>						
Wiegand <i>et al.</i> (2013)	Open-label case study/series	Everolimus	Seven patients, mean age 5 years (range 2–12 years)	36 weeks, with extended treatment in six patients up to 36 months	One patient stopped treatment due to rash. One patient achieved seizure freedom, three had reduction in seizure frequency between 25 and 59%, and two had no change from baseline	[36]
Cardamone <i>et al.</i> (2014)	Open-label case series	Sitrolimus	13 patients, seven of which had seizures at baseline patients, median age 6 years (range 3–17 years)	Median 18 months (range 6–36 months)	One patient had more than 90% reduction in seizure frequency, four had 50–90% reduction, two had less than 50% reduction	[37]
Krueger <i>et al.</i> (2010)	Prospective, open-label Phase I/II study (primary end point SEGA reduction; secondary end point seizure frequency)	Everolimus	28 patients, median age 11 years (range 2–34 years)	6 months, with extended treatment in 27 patients, median 21.5 months (range 4.7–34.4 months)	At 6-month follow-up, nine patients had a decrease in seizure frequency, six patients had no change in frequency (five of whom had no seizures at baseline or follow-up) and one patient had an increase in frequency. At 24-month follow-up, 15 of 23 patients receiving extended treatments in whom seizure data were available reported no seizures since last visit [38]	[34]
Franz <i>et al.</i> (2013), EXIST-1	Multicenter, Phase III, randomized, placebo-controlled trial (primary end point SEGA reduction; secondary end point seizure frequency)	Everolimus	78 patients received everolimus, median age 9.5 years (range 1.0–23.9 years)	Median 41.9 weeks	Both median number of seizures at baseline and median change in seizure frequency from baseline to follow-up was 0 in both treatment and placebo groups	[39]
Kotulska <i>et al.</i> (2013)	Subgroup analysis of EXIST-1 trial	Everolimus	Eight patients under the age of 3 years, five of whom had active epilepsy at baseline	Median treatment duration and follow-up 35 months (range 33–38 months)	One child achieved seizure freedom and two had at least 50% reduction in seizure frequency. One child had decrease, then increase in seizure frequency and another had no change from baseline. None of the three children who were seizure free at baseline developed epilepsy	[49]
Krueger <i>et al.</i> (2015)	Prospective, multicenter, open-label, Phase I/II study (primary end point seizure frequency)	Everolimus	20 patients, median age 8 years (range 2–21 years)	12 weeks	Seizure frequency was reduced in 17 patients, with median reduction 73% ($p < 0.001$), and 12 of 20 patients having seizure reduction more than 50%. In a follow-up analysis of 18 patients receiving extended treatment (median 50.9 months, range 2.7–53.9 months), all but one of the 14 patients completing the study had more than 50% reduction in seizure frequency at 48 months [41]	[40]
Samueli <i>et al.</i> (2016)	Open-label, single-center prospective trial	Everolimus	15 children, median age 6 years (range 1–18 years)	Median 22 months (range 6–50 months)	Twelve of 15 children had treatment response, with seven patients achieving seizure freedom at last follow-up	[42]
French <i>et al.</i> (2016), EXIST-3	Phase III, randomized, placebo-controlled trial (primary end point seizure frequency)	Everolimus, low-exposure (target serum trough 3–7 ng/ml) versus	366 patients, median age 10.1 years (range 2.2–56.3 years)	18 weeks	Forty percent of patients in the high-exposure group showed a clinical response (defined as greater than 50% reduction in seizure frequency from baseline), compared with 28% in low-exposure group and 15% in placebo group. Median percentage reduction in seizure	[43]

Study (year)	Study type	Study drug	Patients	Duration of treatment	Summary of results	Ref.
		high-exposure (target trough 9–15 ng/ml)			frequency was significantly higher in the high-exposure group (mean reduction 39.6%, $p < 0.0001$) and low-exposure group (29.3%, $p = 0.0028$) compared with placebo (mean reduction 14.9%)	
Overwater <i>et al.</i> (2016)	Prospective, randomized, open-label cross-over	Sirolimus (early vs late treatment)	23 children, median age 5 years (range 1.8–10.9 years)	6 months	Sirolimus treatment showed a 41% decrease in seizure frequency, although the results were not statistically significant ($p = 0.11$). The study did not reach target enrollment of 30 patients	[44]
PMSE						
Parker <i>et al.</i> (2013)	Open-label, prospective trial	Sirolimus	Five children, median 4 months (range 3–8 months) at onset of treatment	Median 36 months (range 5–52 months)	Four of five patients were seizure free in the past 12 months, with the fifth having had one seizure in 12 months	[45]

PMSE: Polyhydramnios-megalocephaly-symptomatic epilepsy; SEGA: Subependymal giant cell astrocytoma; TSC: Tuberous sclerosis complex.