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Review

The role of mTOR inhibitors in preventing epileptogenesis in patients with TSC: Current evidence and future perspectives

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ABSTRACT

Tuberous sclerosis complex (TSC) is one of the most common genetic causes of epilepsy. Mutations in the TSC1 or TSC2 genes lead to the dysregulation of the mechanistic target of rapamycin (mTOR) pathway. This mTOR pathway hyperactivation is associated with several processes resulting in epileptic conditions. The occurrence of seizures and their treatment outcomes seem to play a crucial role in cognitive and behavioral developments in patients with TSC. Mechanistic target of rapamycin inhibitors have been proven to be effective in epilepsy treatment in individuals with TSC. Specifically, because of their disease-modifying mechanism of action, they have the capability to prevent epileptogenesis in patients with TSC. This article will provide an overview of the current evidence of and delineate future perspectives for mTOR inhibitors and their role in preventing epileptogenesis.

1. Tuberous sclerosis complex

Tuberous sclerosis complex (TSC) is an autosomal dominant genetic disease typically caused by mutations in the TSC1 or TSC2 genes. It is a rare disorder with an incidence of one in 6000 births affecting about 1.5 million patients worldwide [1]. Tuberous sclerosis complex mutations are characterized by the growth of hamartomas in multiple major organ systems due to an overactivation of the mechanistic target of rapamycin (mTOR) pathway.

Although TSC can affect organs such as the brain, skin, heart, eyes, kidneys, and lungs, it is primarily characterized by its neurological manifestations [1]. Epilepsy is one of the most frequent symptoms of TSC affecting about 80% of all individuals with TSC. More than 60% of all patients with TSC epilepsy turn out to be resistant to therapy despite the recent introductions of new antiepileptic drugs (AEDs) into clinical practice [2]. In contrast, classic AEDs only act by raising the seizure threshold and suppressing seizures. They have no disease-modifying mechanism for treating epilepsy in a curative way and are, therefore, anticonvulsants rather than true AEDs.

The idea of preventing epileptogenesis by inhibiting the underlying pathomechanism seems tempting. Several studies support this approach as mTOR inhibitors have been demonstrated to prevent epilepsy and reduce the underlying brain malformations in TSC animal models [3–5].

2. mTOR signaling pathway and epileptogenesis

Initially referred to as the mammalian target of rapamycin, mTOR was instead changed to the term “mechanistic target of rapamycin” to enhance the ubiquitous mechanisms of action [2]. This serine/threonine kinase plays a key role in regulating multiple physiological functions including cell proliferation, growth, survival, protein synthesis, neuronal morphology, and cortical development. Tuberous sclerosis complex 1 or TSC2 gene mutations lead to an overactivation of mTOR. The dysregulation of mTOR results in abnormal cell proliferation, growth, and the subsequent formation of hamartomas, giant cells, and disturbed connectivity of the brain. Furthermore, mTOR pathway hyperactivation leads to several epileptogenic brain malformation disorders or the so-called mTORopathies, which include TSC, focal cortical dysplasia, hemimegalencephaly, and ganglioglioma [6]. Hyperactivated mTOR seems to play a crucial role in epileptogenesis according to several animal models of acquired epilepsies. Mechanistic target of rapamycin regulates the inhibitory and excitatory neurotransmitter balance.

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Hyperactivation of mTOR is associated with impaired presynaptic transmission and increased synaptic transmission in glutamatergic and GABA (gamma-aminobutyric acid)-ergic neurons [2]. Ion channel expression and synaptic plasticity are also affected by dysregulated mTOR [7, 8].

3. mTOR inhibitors prevent epileptogenesis in animal models of genetic and acquired epilepsies

Mechanistic target of rapamycin is associated with various proteins to form the mechanistic target of rapamycin complex 1 (mTORC1) and TORC2 complexes. The mTORC1 pathway plays a major role in epileptogenesis, and seizures themselves seem to increase mTORC1 activity even in the absence of any other structural pathologies [6, 9]. Preclinical studies have demonstrated that treatment with mTOR inhibitors was beneficial for survival, seizure reduction, and developmental outcomes in animal models [4, 10].

A variety of different animal models of genetic or acquired epilepsies have demonstrated the antiepileptic effects of mTOR inhibitors [5].

Zeng et al. provided the first evidence of the antiepileptogenic effect of mTOR inhibitors by preventing new onset seizures in TSC1 knockout mice [3]. The mice showed an improved level of survival and reduced seizure frequency when treated with the mTOR inhibitor. On the cellular level, rapamycin reversed the reduction of astrocyte-specific glutamate transporters, which also play a potential role in epileptogenesis in TSC. Meikle et al. demonstrated that early treatment with the mTOR inhibitors or everolimus led to sustained seizure freedom and normal survival rates in TSC1 knockout mice [4]. Further rodent models of mTORopathies like cortical dysplasia showed reduced seizure frequencies and a reduction of behavioral alterations following treatment with rapamycin. Mechanistic target of rapamycin inhibitors reversed the neuronal hypertrophy and megalencephaly in PTEN (phosphatase and tensin homolog; tumor suppressor) knockout mice. The authors hypothesized that changes in subcellular structures as well as synaptic excitability were possible mechanisms of action [11, 12]. Additionally, in a rodent absence epilepsy model, rapamycin blocked the development of absence seizures [13].

Furthermore, the mTOR pathway seems to play a crucial role in a variety of acquired epilepsies as well. The mTOR inhibitor was effective in prompting seizure reduction in animal models of temporal lobe epilepsies caused by kainic acid-induced status epilepticus. Treatment with rapamycin also successfully reduced mossy fiber sprouting and decreased neuronal degeneration [9]. Animal models of traumatic brain injury- or hypoxia-induced seizures showed decreased chronic seizures or the prevention of posttraumatic epilepsy following treatment with rapamycin [14–16].

4. Epilepsy and cognitive development in patients with TSC

Epilepsy is a common symptom in TSC with about 80% of all patients being affected. Epilepsy onset most frequently occurs within the first two years of life with a peak incidence during the first few months [1]. Initial seizures are focal or infantile spasms. There is usually a high seizure burden with multiple seizures per day. During brain maturation, all seizure semiologies can occur, and most patients with TSC epilepsy will develop more than one seizure type. Mental retardation is frequent in patients with TSC. Severe cognitive impairment in children with epilepsy onset during the first year of life ranges from 45% to 100% [17, 18]. Up to 45% of infants with TSC with infantile spasms develop autistic-like behavior [19]. Thus, epilepsy is an additional risk factor for mental and behavioral impairments in patients with TSC.

5. Prediction of epilepsy in patients with TSC

Although epilepsy is present in the majority of all patients with TSC, not every patient will be affected. To prevent epileptogenesis, it is essential to differentiate between affected and nonaffected patients prior to epilepsy onset. To avoid unnecessary treatment, it is also important to predict the earliest possible time of epilepsy onset.

Specific symptoms like cardiac rhabdomyoma, white spots, or genetic identification because of an affected family member allow for the early diagnosis of TSC before seizure onset in some patients [20, 21]. In an electroclinical study, later epilepsy was identified before seizure onset by preceding pathological electroencephalograms (EEGs). Out of 28 patients, 19 developed seizures (67.8%). Regarding onset, 14 of the 19 patients had their first abnormal EEG findings at a median of 1.9 months preceding epilepsy onset. The average time interval between the occurrence of abnormal EEG findings and the onset of seizures was 2.8 months $\pm$ 3.4 months. All patients with pathological EEG findings developed epilepsy subsequently [22]. Therefore, the positive predictive value of a pathological EEG for the diagnosis of later epilepsy was 100%. However, not all patients showed epileptic discharges prior to clinical onset. The negative predictive value was 64%, i.e., five out of 14 patients with normal EEG findings developed epilepsy. This could be due to prolonged EEG intervals, but a preventive treatment based on EEG findings will probably not successfully include all children with later epilepsy.

Jóźwiak et al. used EEGs to identify epilepsy in infants with TSC and treat their conditions before seizure onset. Out of 45 patients, 32 developed pathological EEG findings. In this study, all patients with pathological EEG developed epilepsy unless their conditions were treated preventively [23]. Although this study involved a small cohort only, it opens up the possibility for prophylactic treatment to be administered as soon as EEG pathologies are detected. Thus, EEG may represent a biomarker for predicting epilepsy in patients with TSC.

6. Treatment of epilepsy in TSC

To date, classic AEDs are still the first-line treatment in TSC-related epilepsies. Antiepileptic drug treatment is age-related and dependent on seizure type and EEG syndrome. Treatment initiation is recommended in infants and children within the first two years of life as soon as ictal discharges in EEG occur with or without clinical manifestations. Epilepsy in children older than 24 months of age should be treated if clinical seizures become obvious [24]. According to international recommendations, vigabatrin is used as a first-line treatment in cases of infantile spasms or focal seizures caused by TSC before the age of one year [24]. Vigabatrin has an antiseizure effect on neuronal hyperexcitability by enhancing GABAergic inhibitory mechanisms. Furthermore, vigabatrin showed an impact on the mTOR pathway both in vivo and in vitro. It partially inhibited glial proliferation in TSC knockout mice and decreased mTOR activation in cultured astrocytes [25]. This additional effect may contribute to the unique effectiveness of vigabatrin in TSC epilepsies as compared with other antiseizure medications. Other AEDs used in TSC-related epilepsy include valproic acid, levetiracetam, carbamazepine, and clonazepam. Interestingly, there seems to be no difference in effectiveness among the classic AEDs apart from vigabatrin [26]. Rufinamide showed a positive effect on drop attacks and tonic seizures as well as in Lennox–Gastaut syndrome [27].

The ketogenic diet represents an important nonpharmacological treatment option. Smaller cohorts showed that the ketogenic diet was a generally effective therapeutic modality in children with TSC. Notably, it reduced seizure frequency as well as improved cognition and behavior [28, 29]. Other research indicates that the ketogenic diet inhibits the mTOR pathway in rodents [30]. This potential antiepileptic effect, in addition to anticonvulsant action and growth impairment, may be the cause of this effectiveness.

Epilepsy surgery can be curative if there is an unifocal epileptogenic zone in concordance with one hamartoma [31]. Epilepsy surgery can also be considered and performed in children with multiple epileptogenic tubers or dysplasia via one or several sequential procedures [32, 33]. However, despite current anticonvulsant and nonpharmacological

treatment options, TSC is still characterized by treatment-refractory epilepsy.

7. The current role of mTOR inhibitors in epilepsy treatment

The mTOR inhibitor everolimus has been established in the treatment of TSC-associated subependymal giant cell astrocytoma (SEGA) and renal angiomyolipoma [34, 35]. Occasional beneficial antiepileptic effects of mTOR inhibitors in patients with TSC and in a small case series have been reported since 2009 [36, 37]. A prospective study of patients with TSC strongly underlined improved seizure control during the treatment of SEGAs [34, 35]. Krueger et al. demonstrated the effective treatment of patients with TSC epilepsy in a prospective multicenter, open-label, phase I/II clinical trial [38]. In this study, seizure frequency was reduced by more than 50% in 12 out of 20 subjects. Overall, seizures were reduced in 17 out of the 20 subjects by a median reduction of 73% ($p < 0.001$).

In a single center prospective study, 80% (12/15) of the children involved were responders and 58% of them (7/12) were seizure-free. The overall reduction in seizure frequency was 60% in focal seizures, 80% in generalized tonic–clonic seizures, and 87% in drop attacks [39]. Most recently, a phase III study demonstrated the efficacy and safety of the mTOR inhibitor everolimus in patients with TSC epilepsy [40]. A total of 366 patients were enrolled and randomly assigned to placebo, low exposure everolimus (3–7 ng/ml), or high exposure everolimus (9–15 ng/ml) as an add-on treatment. The median seizure frequency reduction was 29.3% in the low exposure group and 39.6% in the high exposure group versus 14.9% in the placebo group after 18 weeks of treatment. The response rates (>50% seizure reduction) were 28.2% vs. 40% (low vs. high exposure). Grades 3 or 4 adverse events occurred in 18% in the low exposure group versus 24% in the high exposure group. Common adverse events included stomatitis, pyrexia, infections, vomiting, decreased appetite, hypercholesterolemia, and headache. Actual data recommend to target a concentration range of everolimus of 5–15 ng/ml [41]. Based on these results, the European Medicines Agency approved everolimus as adjunctive treatment of patients aged two years and older whose refractory partial onset seizures, with or without secondary generalization, are associated with TSC. Recently, in April 2018, the Food and Drug Administration approved everolimus for the adjunctive treatment of adult and pediatric patients aged two years and older with TSC-associated partial onset seizures.

8. Future perspectives for epilepsy treatment in patients with TSC

The importance of early and sustained seizure control for better developmental outcomes has been shown by several studies [42, 43]. Early epilepsy onset was a negative predictor for normal mental outcome in a cohort of 130 children [19]. Patients with TSC without epilepsy or those who have been successfully treated for epilepsy have a much better chance for normal development [42].

Jóźwiak et al. compared early treatment with vigabatrin after seizure onset with treatment when epileptiform discharges were seen on EEG but before epilepsy onset [23]. The preventively treated group had a significantly higher number of seizure-free patients (93% vs. 35%) while mental retardation was more likely in the standard treatment group (48% vs. 14%). The results regarding mental retardation might show some bias because there were no pretreatment data available and the preventive treatment group was small. Four out of the 14 infants in the preventive group presented with neither epileptiform discharges nor seizures. Because of this systematic failure and the small cohort, this study can only speculate on the hypothesis that preventing epilepsy in TSC is associated with a better developmental outcome.

9. mTOR inhibitors in very young infants

Human data on early postnatal administration of mTOR inhibitors are only anecdotal [44–47]. In these studies, neonates were treated with everolimus because of cardiac rhabdomyoma or giant cell subependymal astrocytoma. One infant developed drug-resistant infantile spasms under everolimus treatment at the age of seven months. One neonate with cardiac rhabdomyoma and SEGA was treated with everolimus and did not develop epilepsy during a one year observation period [47]. All available studies, to our knowledge, on patients with TSC treated with mTOR inhibitors before seizure onset are listed in Table 1.

10. The prevention of epileptogenesis in children with TSC

From the pathomechanistic side, the hypothesis of preventing epileptogenesis by inhibiting the mTOR pathway is very tempting. There are preclinical data showing clear evidence of the prevention of epileptogenesis and cognitive impairment by the use of mTOR inhibitors.

Mechanistic target of rapamycin dysregulation may represent a final common pathway in epilepsies of various causes. Therefore, mTOR inhibition is an exciting potential antiepileptogenic strategy.

However, many questions are raised regarding the possibility of the preventive treatment of epilepsy in patients with TSC. For example, the optimal timing of treatment initiation seems to be crucial. Early seizure onset is often combined with encephalopathy resulting in neurodevelopmental deficits. Evidence has arisen that encephalopathy in TSC might be genetically determined and that epilepsy is merely one of the symptoms rather than the cause of the encephalopathy. Thus, the development of encephalopathy starts very early during prenatal brain development and so the window for inhibiting the mTOR hyperactivation effects preceding epileptogenesis might be very limited [48].

The early identification of patients who will develop epilepsy seems to be possible by pre- or early postnatal diagnosis of TSC and closely spaced EEG monitoring intervals during the first year of life. However, in a high proportion of patients, TSC is diagnosed with epilepsy onset

Table 1
Clinical studies with treatment in patients with TSC before seizure onset.

Study	Study type	Cause for treatment	Drug and dose	Number + age of patients	Duration of treatment	Clinical result
Jóźwiak et al. [23]	Prospective, open-label, parallel group	Epileptic discharges in EEG	Vigabatrin 100–150 mg/kg/d	N = 10 2.5–15 months	9–21.5 months	9/10 seizure-free, 8/10 normal EEG outcome, significant better cognitive outcome compared with standard care group
Goyer et al. [44]	Case report	Cardiac rhabdomyoma Subependymal giant cell astrocytoma	Everolimus 5–15 ng/ml	N = 3 neonates	7 months	1/3 infantile spasms under everolimus treatment
Chang et al. [45]	Case report	Cardiac rhabdomyoma	Everolimus 3–7 ng/ml	N = 3 neonates	6 months	1/3 had TSC1 mutation and developed seizures one day after everolimus treatment initiation
Aw et al. [46]	Case report	Cardiac rhabdomyoma	Everolimus 5–15 ng/ml	N = 4 neonates	73 days	2/4 neurological manifestations (not further specified) no report of seizures
Hoshal et al. [47]	Case report	Cardiac rhabdomyoma	Everolimus 0.5 mg	N = 1 neonate	12 months	Seizure-free at 12 months

as infantile spasms are frequently the first sign in patients with TSC. It is questionable whether mTOR inhibitors will be successful concerning seizure freedom and normal cognitive development when introduced after seizure onset.

The duration of treatment in very young and possibly asymptomatic patients remains unclear. Several animal models showed dysfunction relapse as soon as the mTOR inhibitor treatment was discontinued. Seizures reoccurred and both neurological and histological abnormalities reappeared within a few weeks after treatment was stopped [3, 4]. Seizure relapse was also shown in a clinical study after withdrawal [49]. This indicates that long-term and possibly lifelong treatment is required to achieve sustainable effects on epilepsy and developmental outcomes. The first publications on long-term treatment with mTOR inhibitors show a tolerable safety profile during an observational period of up to four years [35, 50]. Also, the first long-term safety data especially those for young children are available and in a small observation group of eight children under the age of three years, the incidence of adverse events was similar to that observed in older children and adults [51]. There were no negative effects on neuropsychological evaluation, growth velocity, or weight development over a mean follow-up of 35 months. However, studies involving large populations to prove the safety of long-term treatment with mTOR inhibitors especially in young children are lacking at this point in time.

Furthermore, the long-term effects of mTOR inhibition on early human brain development are hardly predictable. A total blockade of the fundamental physiological actions of mTOR seems to hold a risk of widespread complications such as insulin tolerance, the stimulation of autophagy, and many other problems [6]. The ubiquitous mTOR pathways involve substantial and necessary mechanisms of normal brain development like normal cell growth and proliferation. No first-line antiepileptic treatment with mTOR inhibitors in infants or children have been investigated or reported yet.

The ongoing European Union-funded long-term, prospective study evaluating clinical and molecular biomarkers of epileptogenesis in a genetic model of epilepsy – tuberous sclerosis complex (EPISTOP) initiative will address some of these questions by comparing standard vs. preventive treatment with vigabatrin in children with TSC in a prospective multicenter study (study no. NCT02098759). The EPISTOP study aims to target the risk factors for drug-resistant epilepsy and finds new therapeutic targets to block epileptogenesis. Further goals will be to identify biomarkers and secure a better understanding of underlying basic mechanisms in epileptogenesis. Molecular analysis of blood biomarkers like microribonucleic acid (microRNA) profiling will be correlated to EEG and seizures in order to obtain new insights into ictogenesis and epileptogenesis in TSC [52]. It is hoped that these projects will provide some answers to the questions of presymptomatic treatment of TSC-related epilepsies by mTOR inhibitors and open up new opportunities for preventive and causative treatments.

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Conflicts of interest

Susanne Schubert-Bast reports personal fees from UCB, Eisai, Desitin Pharma, LivaNova, and Zogenix outside the scope of the submitted work.

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We confirm that we have read the journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

References

[1] Curatolo P, Bombardieri R, Jozwiak S. Tuberous sclerosis. Lancet 2008;372:657–68. https://doi.org/10.1016/S0140-6736(08)61279-9.
[2] Curatolo P. Mechanistic target of rapamycin (mTOR) in tuberous sclerosis complex-associated epilepsy. Pediatr Neurol 2015;52:281–9. https://doi.org/10.1016/j.pediatrneurol.2014.10.028.
[3] Zeng L-H, Xu L, Gutmann DH, Wong M. Rapamycin prevents epilepsy in a mouse model of tuberous sclerosis complex. Ann Neurol 2008;63:444–53. https://doi.org/10.1002/ana.21331.
[4] Meikle L, Pollizzi K, Egnor A, Kramvis I, Lane H, Sahin M, et al. Response of a neuronal model of tuberous sclerosis to mammalian target of rapamycin (mTOR) inhibitors: effects on mTORC1 and Akt signaling lead to improved survival and function. J Neurosci 2008;28:5422–32. https://doi.org/10.1523/JNEUROSCI.0955-08.2008.
[5] Citraro R, Leo A, Constanti A, Russo E, De Sarro G. mTOR pathway inhibition as a new therapeutic strategy in epilepsy and epileptogenesis. Pharmacol Res 2016;107:333–43. https://doi.org/10.1016/j.phrs.2016.03.039.
[6] Bockaert J, Marin P. mTOR in brain physiology and pathologies. Physiol Rev 2015;95:1157–87. https://doi.org/10.1152/physrev.00038.2014.
[7] Jaworski J, Spangler S, Seeburg DP, Hoogenraad CC, Sheng M. Control of dendritic arborization by the phosphoinositide-3′-kinase–Akt–mammalian target of rapamycin pathway. J Neurosci 2005;25:11300–12. https://doi.org/10.1523/JNEUROSCI.2270-05.2005.
[8] Von Der Brelie C, Waltereit R, Zhang L, Beck H, Kirschstein T. Impaired synaptic plasticity in a rat model of tuberous sclerosis. Eur J Neurosci 2006;23:686–92. https://doi.org/10.1111/j.1460-9568.2006.04594.x.
[9] Zeng L-H, Rensing NR, Wong M. The mammalian target of rapamycin signaling pathway mediates epileptogenesis in a model of temporal lobe epilepsy. J Neurosci 2009;29:6964–72. https://doi.org/10.1523/JNEUROSCI.0066-09.2009.
[10] Ehninger D, Han S, Shilyansky C, Zhou Y, Li W, Kwiatkowski DJ, et al. Reversal of learning deficits in a TSC2+/− mouse model of tuberous sclerosis. Nat Med 2008;14:843–8. https://doi.org/10.1038/nm1788.
[11] Zhou J, Blundell J, Ogawa S, Kwon C-H, Zhang W, Sinton C, et al. Pharmacological inhibition of mTORC1 suppresses anatomical, cellular, and behavioral abnormalities in neural-specific Pten knock-out mice. J Neurosci 2009;29:1773–83. https://doi.org/10.1523/JNEUROSCI.5685-08.2009.
[12] Ljungberg MC, Bhattacharjee MB, Lu Y, Armstrong DL, Yoshor D, Swann JW, et al. Activation of mammalian target of rapamycin in cytomegalic neurons of human cortical dysplasia. Ann Neurol 2006;60:420–9. https://doi.org/10.1002/ana.20949.
[13] Russo E, Citraro R, Donato G, Camastra C, Iuliano R, Cuzzocrea S, et al. mTOR inhibition modulates epileptogenesis, seizures and depressive behavior in a genetic rat model of absence epilepsy. Neuropharmacology 2013;69:25–36. https://doi.org/10.1016/j.neuropharm.2012.09.019.
[14] van Vliet EA, Forte G, Holtman L, den Burger JCG, Sinjewel A, de Vries HE, et al. Inhibition of mammalian target of rapamycin reduces epileptogenesis and blood–brain barrier leakage but not microglia activation. Epilepsia 2012;53:1254–63. https://doi.org/10.1111/j.1528-1167.2012.03513.x.
[15] Guo D, Zeng L, Brody DL, Wong M. Rapamycin attenuates the development of posttraumatic epilepsy in a mouse model of traumatic brain injury. PLoS ONE 2013;8. https://doi.org/10.1371/journal.pone.0064078.
[16] Talos DM, Sun H, Zhou X, Fitzgerald EC, Jackson MC, Klein PM, et al. The interaction between early life epilepsy and autistic-like behavioral consequences: a role for the mammalian target of rapamycin (mTOR) pathway. PLoS ONE 2012;7. https://doi.org/10.1371/journal.pone.0035885.
[17] Winterkorn EB, Pulsifer MB, Thiele EA. Cognitive prognosis of patients with tuberous sclerosis complex. Neurology 2007;68:62–4. https://doi.org/10.1212/01.wnl.0000250330.44291.54.
[18] Jansen FE, Vincken KL, Algra A, Anbeek P, Braams O, Nellist M, et al. Cognitive impairment in tuberous sclerosis complex is a multifactorial condition. Neurology 2008;70:916–23. https://doi.org/10.1212/01.wnl.0000280579.04974.c0.
[19] Capal JK, Bernardino-Cuesta B, Horn PS, Murray D, Byars AW, Bing NM, et al. Influence of seizures on early development in tuberous sclerosis complex. Epilepsy Behav 2017;70:245–52. https://doi.org/10.1016/j.yebeh.2017.02.007.
[20] Datta AN, Hahn CD, Sahin M. Clinical presentation and diagnosis of tuberous sclerosis complex in infancy. J Child Neurol 2008;23:268–73. https://doi.org/10.1177/0883073807309250.
[21] Słowińska M, Jóźwiak S, Peron A, Borkowska J, Chmielewski D, Sadowski K, et al. Early diagnosis of tuberous sclerosis complex: a race against time. How to make the diagnosis before seizures? Orphanet J Rare Dis 2018;13:25. https://doi.org/10.1186/s13023-018-0764-z.

[22] Wu JY, Peters JM, Goyal M, Krueger D, Sahin M, Northrup H, et al. Clinical electroencephalographic biomarker for impending epilepsy in asymptomatic tuberous sclerosis complex infants. Pediatr Neurol 2016;54:29–34. https://doi.org/10.1016/j.pediatrneurol.2015.09.013.
[23] Jóźwiak S, Kotulska K, Domańska-Pakieła D, Łojszczyk B, Syczewska M, Chmielewski D, et al. Antiepileptic treatment before the onset of seizures reduces epilepsy severity and risk of mental retardation in infants with tuberous sclerosis complex. Eur J Paediatr Neurol 2011;15:424–31. https://doi.org/10.1016/j.ejpn.2011.03.010.
[24] Curatolo P, Jóźwiak S, Nabbout R. Management of epilepsy associated with tuberous sclerosis complex (TSC): clinical recommendations. Eur J Paediatr Neurol 2012;16:582–6. https://doi.org/10.1016/j.ejpn.2012.05.004.
[25] Zhang B, McDaniel SS, Rensing NR, Wong M. Vigabatrin inhibits seizures and mTOR pathway activation in a mouse model of tuberous sclerosis complex. PLoS ONE 2013;8. https://doi.org/10.1371/journal.pone.0057445.
[26] Overwater IE, Bindels-de Heus K, Rietman AB, ten Hoopen LW, Vergouwe Y, Moll HA, et al. Epilepsy in children with tuberous sclerosis complex: chance of remission and response to antiepileptic drugs. Epilepsia 2015;56:1239–45. https://doi.org/10.1111/epi.13050.
[27] Crumrine PK. Management of seizures in Lennox–Gastaut syndrome. Paediatr Drugs 2011;13:107–18. https://doi.org/10.2165/11536940-000000000-00000.
[28] Kossoff EH, Thiele EA, Pfeifer HH, McGrogan JR, Freeman JM. Tuberous sclerosis complex and the ketogenic diet. Epilepsia 2005;46:1684–6. https://doi.org/10.1111/j.1528-1167.2005.00266.x.
[29] Park S, Lee EJ, Eom S, Kang H-C, Lee JS, Kim HD. Ketogenic diet for the management of epilepsy associated with tuberous sclerosis complex in children. J Epilepsy Res 2017;7:45–9. https://doi.org/10.14581/jer.17008.
[30] McDaniel SS, Rensing NR, Thio LL, Yamada KA, Wong M. The ketogenic diet inhibits the mammalian target of rapamycin (mTOR) pathway. Epilepsia 2011;52:e7–11. https://doi.org/10.1111/j.1528-1167.2011.02981.x.
[31] Fallah A, Guyatt GH, Snead OC, Ebrahim S, Ibrahim GM, Mansouri A, et al. Predictors of seizure outcomes in children with tuberous sclerosis complex and intractable epilepsy undergoing resective epilepsy surgery: an individual participant data meta-analysis. PLoS ONE 2013;8. https://doi.org/10.1371/journal.pone.0053565.
[32] Weiner HL, Carlson C, Ridgway EB, Zaroff CM, Miles D, LaJoie J, et al. Epilepsy surgery in young children with tuberous sclerosis: results of a novel approach. Pediatrics 2006;117:1494–502. https://doi.org/10.1542/peds.2005-1206.
[33] Ibrahim GM, Fallah A, Carter Snead O, Rutka JT. Changing global trends in seizure outcomes following resective surgery for tuberous sclerosis in children with medically intractable epilepsy. Epilepsy Res Treat 2012;2012. https://doi.org/10.1155/2012/135364.
[34] Franz DN, Belousova E, Sparagana S, Bebin EM, Frost M, Kuperman R, et al. Efficacy and safety of everolimus for subependymal giant cell astrocytomas associated with tuberous sclerosis complex (EXIST-1): a multicentre, randomised, placebo-controlled phase 3 trial. The Lancet 2013;381:125–32. https://doi.org/10.1016/S0140-6736(12)61134-9.
[35] Bissler JJ, Kingswood JC, Radzikowska E, Zonnenberg BA, Belousova E, Frost MD, et al. Everolimus long-term use in patients with tuberous sclerosis complex: four-year update of the EXIST-2 study. PLoS ONE 2017;12. https://doi.org/10.1371/journal.pone.0180939.
[36] Muncy J, Butler IJ, Koenig MK. Rapamycin reduces seizure frequency in tuberous sclerosis complex. J Child Neurol 2009;24:477. https://doi.org/10.1177/0883073808324535.
[37] Wiegand G, May TW, Ostertag P, Boor R, Stephani U, Franz DN. Everolimus in tuberous sclerosis patients with intractable epilepsy: a treatment option? Eur J Paediatr Neurol 2013;17:631–8. https://doi.org/10.1016/j.ejpn.2013.06.002.
[38] Krueger DA, Wilfong AA, Holland-Bouley K, Anderson AE, Agricola K, Tudor C, et al. Everolimus treatment of refractory epilepsy in tuberous sclerosis complex. Ann Neurol 2013;74:679–87. https://doi.org/10.1002/ana.23960.
[39] Samueli S, Abraham K, Dressler A, Gröppel G, Mühlebner-Fahrngruber A, Scholl T, et al. Efficacy and safety of everolimus in children with TSC - associated epilepsy – pilot data from an open single-center prospective study. Orphanet J Rare Dis 2016;11. https://doi.org/10.1186/s13023-016-0530-z.
[40] French JA, Lawson JA, Yapici Z, Ikeda H, Polster T, Nabbout R, et al. Adjunctive everolimus therapy for treatment-resistant focal-onset seizures associated with tuberous sclerosis (EXIST-3): a phase 3, randomised, double-blind, placebo-controlled study. Lancet 2016;388:2153–63. https://doi.org/10.1016/S0140-6736(16)31419-2.
[41] Franz DN, Lawson JA, Yapici Z, et al. Everolimus dosing recommendations for tuberous sclerosis complex–associated refractory seizure. Epilepsia 2018;00:1–10. https://doi.org/10.1111/epi.14085.
[42] Bombardieri R, Pinci M, Moavero R, Cerminara C, Curatolo P. Early control of seizures improves long-term outcome in children with tuberous sclerosis complex. Eur J Paediatr Neurol 2010;14:146–9. https://doi.org/10.1016/j.ejpn.2009.03.003.
[43] Chung CWT, Lawson JA, Sarkozy V, Riney K, Wargon O, Shand AW, et al. Early detection of tuberous sclerosis complex: an opportunity for improved neurodevelopmental outcome. Pediatr Neurol 2017;76:20–6. https://doi.org/10.1016/j.pediatrneurol.2017.05.014.
[44] Goyer I, Dahdah N, Major P. Use of mTOR inhibitor everolimus in three neonates for treatment of tumors associated with tuberous sclerosis complex. Pediatr Neurol 2015;52:450–3. https://doi.org/10.1016/j.pediatrneurol.2015.01.004.
[45] Chang J-S, Chiou P-Y, Yao S-H, Chou I-C, Lin C-Y. Regression of neonatal cardiac rhabdomyoma in two months through low-dose everolimus therapy: a report of three cases. Pediatr Cardiol 2017;38:1478–84. https://doi.org/10.1007/s00246-017-1688-4.
[46] Aw F, Goyer I, Raboisson M-J, Boutin C, Major P, Dahdah N. Accelerated cardiac rhabdomyoma regression with everolimus in infants with tuberous sclerosis complex. Pediatr Cardiol 2017;38:394–400. https://doi.org/10.1007/s00246-016-1528-y.
[47] Hoshal SG, Samuel BP, Schneider JR, Mammen L, Vettukattil JJ. Regression of massive cardiac rhabdomyoma on everolimus therapy. Pediatr Int Off J Jpn Pediatr Soc 2016;58:397–9. https://doi.org/10.1111/ped.12816.
[48] Curatolo P, Aronica E, Jansen A, Jansen F, Kotulska K, Lagae L, et al. Early onset epileptic encephalopathy or genetically determined encephalopathy with early onset epilepsy? Lessons learned from TSC. Eur J Paediatr Neurol 2016;20:203–11. https://doi.org/10.1016/j.ejpn.2015.12.005.
[49] Mingarelli A, Vignoli A, Briola FL, Peron A, Giordano L, Banderali G, et al. Dramatic relapse of seizures after everolimus withdrawal. Eur J Paediatr Neurol 2018;22:203–6. https://doi.org/10.1016/j.ejpn.2017.07.018.
[50] Krueger DA, Wilfong AA, Mays M, Talley CM, Agricola K, Tudor C, et al. Long-term treatment of epilepsy with everolimus in tuberous sclerosis. Neurology 2016;87:2408–15. https://doi.org/10.1212/WNL.0000000000003400.
[51] Kotulska K, Chmielewski D, Borkowska J, Jurkiewicz E, Kuczyński D, Kmieć T, et al. Long-term effect of everolimus on epilepsy and growth in children under 3 years of age treated for subependymal giant cell astrocytoma associated with tuberous sclerosis complex. Eur J Paediatr Neurol 2013;17:479–85. https://doi.org/10.1016/j.ejpn.2013.03.002.
[52] Auvin S, Walker L, Gallentine W, Jozwiak S, Tombini M, Sills GJ. Prospective clinical trials to investigate clinical and molecular biomarkers. Epilepsia 2017;58:20–6. https://doi.org/10.1111/epi.13782.