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REVIEW ARTICLE

mTOR inhibition in epilepsy: A literature review

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Abstract

Hyperactivation of the mammalian target of the rapamycin (mTOR) pathway causes epilepsies, neurodevelopmental disorders, and malformations of cortical development, collectively known as mTORopathies. These conditions arise from loss-of-function variants in negative regulators or gain-of-function variants in positive regulators of the mTOR pathway. Conventional antiseizure medications mainly target downstream effectors such as ion channels or neurotransmitter activity to suppress seizures. On the contrary, extensive pre-clinical and clinical evidence has demonstrated that mTOR inhibitors have anti-epileptogenic or disease-modifying effects, potentially preventing epilepsy or slowing disease progression rather than merely controlling seizures. In general, mTOR inhibitors bind to mTOR protein, preventing its interactions with substrates and disrupting mTOR complex assembly, thereby suppressing downstream activities. In this review, we provide a comprehensive overview of the mTOR signaling pathway, outline the spectrum of mTORopathies and its subset (GATORopathies), and highlight the clinical applications of mTOR inhibitors, particularly everolimus, along with other potential mTOR-modulating agents.

Keywords: mTOR; Epilepsy; Tuberous sclerosis; Rapamycin; Everolimus

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1. Introduction

Epilepsy contributes significantly to the global disease burden, particularly in children, accounting for 13.5 million disability-adjusted life years.[1] Since 1850, antiseizure medications have been introduced to suppress seizure activity, primarily by targeting the balance between inhibitory and excitatory neurotransmission.[2,3] D'Antuono *et al.*[4] observed that while antiseizure medications effectively controlled epileptiform synchronization and aborted seizures, interictal epileptiform activity often remained notoriously resistant, raising concerns about their potential negative impact on

cognition.[5] With the emergence and rapid advancement of genomic technologies, our understanding of epilepsy genetics has expanded, revealing critical pathways involved in epileptogenesis beyond the final common mechanisms of ion channels. This expanded understanding has opened new avenues for the development of precision medicine.[6,7] Both dysregulated mammalian or mechanistic targets of the rapamycin (mTOR) signaling pathway and heightened neuroinflammation have been implicated in epileptogenesis by causing hyperexcitability, altered synaptic transmission, and increased seizure susceptibility.[8] Inhibition of these processes could be a promising therapeutic option with potential antiepileptogenic or disease-modifying effects.[8]

Over the past two decades, there has been a drastic increase in research on the mTOR signaling pathway (Figure 1).[9] Rapamycin was first identified for its anticancer or antiproliferative effect in the 1980s, and in 1994, mTOR was discovered as its target.[10,11] For the past few decades, growing evidence from both animal and human studies has linked dysregulation of the mTOR pathway to a number of diseases, ranging from malignancies to neurological disorders, often due to loss-of-function or gain-of-function pathogenic variants in the mTOR pathway.

To further explore this area, we conducted a comprehensive search of PubMed and Google Scholar using the subject headings of "mTOR" and "Epilepsy," covering publications from inception through May 2024. The search was restricted to English-language full-text articles, including both human and animal studies. Our goal is to provide a comprehensive overview of the mTOR signaling pathway and its role in epileptogenesis, outline the spectrum of mTORopathies and its subset (GATORopathies), and highlight the clinical applications of mTOR inhibitors, particularly everolimus, along with other potential mTOR-modulating agents. In addition, we review recent advances in the modulation of mTOR inhibition.

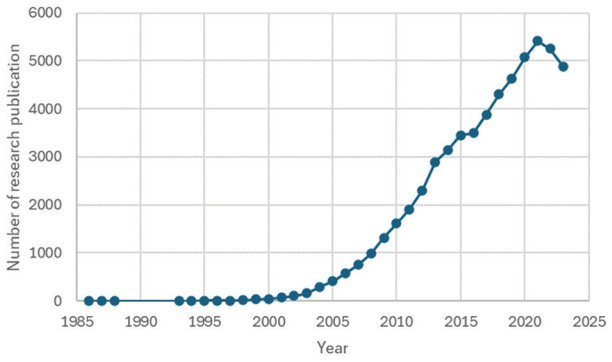

Figure 1. The number of research publications per year on the mTOR signaling pathway in PubMed from 1986 to 2023.[9]

2. mTOR signaling pathway

The mTOR signaling pathway is crucial for regulating cellular processes such as growth, proliferation, apoptosis, autophagy, metabolism, and cytoskeletal organization, as well as brain-specific functions such as synaptic plasticity, neurogenesis, and dendritic-axonal morphology.[12-14] The mTOR protein is a serine-threonine protein kinase belonging to the phosphatidylinositol-3-kinase (PI3K)-related protein kinase family. It consists of several domains, such as FAT (FK506-binding proteins [FKBP]-rapamycin-associated protein [FRAP]; ataxia-telangiectasia mutated [ATM]; transformation/transcription-domain-associated protein; and TRRAP), FKBP 12-rapamycin-binding (FRB), FATC (FRAP, ATM, and TRRAP C-terminal), kinase domains, and HEAT (Huntington, elongation factor 3, protein phosphatase 2A, and TOR) repeats.[13] The HEAT repeats are implicated in interactions with other proteins, cofactors, and kinase substrates. The kinase domain shares sequence similarity with the catalytic domain of PI3K, whereas the FAT and FATC domains interact to expose the catalytic domain. The FRB domain serves as the binding site for rapamycin (Figure 2).[15]

There are two mTOR complexes (mTORCs) with distinct upstream and downstream signaling pathways: mTORC1 and mTORC2 (Figures 2 and 3). mTORC1 is the central signaling node and consists of mTOR, the regulatory protein raptor, mammalian lethal with SEC13 protein 8 (mLST8), and G protein beta subunit-like (GβL).[16] Activation of tyrosine kinase receptors by trophic factors or insulin, mediated by *PI3K* and its negative regulator, phosphatase and tensin homolog (*PTEN*), leads to the activation of *Akt* proteins. These *Akt* proteins then phosphorylate and inactivate the *TSC1-TSC2* (tuberin-hamartin heterodimer) complex, which in turn inhibits *Rheb* (Ras homolog enriched in the brain) through the *PI3K/Akt* pathway.[16] In energy-depleted states, AMP-activated protein kinase (*AMPK*) phosphorylates and activates *TSC2* to reduce mTORC1 activity, leading to energy depletion-induced apoptosis through the *LKB1/AMPK* pathway. In addition, hypoxia activates the *TSC1-TSC2* complex through *REDD1* and *REDD2*.[16]

When amino acid levels, particularly leucine, are low, the GATOR1 complex (*DEPDC5, NPRL2,* and *NPRL3*) is activated, inhibiting mTORC1 activity.[16] The GATOR1 complex activity is downregulated by the GATOR2 complex in response to high amino acid levels.[16] Other negative regulators of mTORC1 activity include *PTEN, STRADA* (STE20-related kinase adaptor alpha), *NF1* (neurofibromin 1), and *p53* proteins.[16] mTORC1 plays an important role in lipid and nucleotide synthesis, lysosome biogenesis, ribosome biogenesis, mRNA translation, and

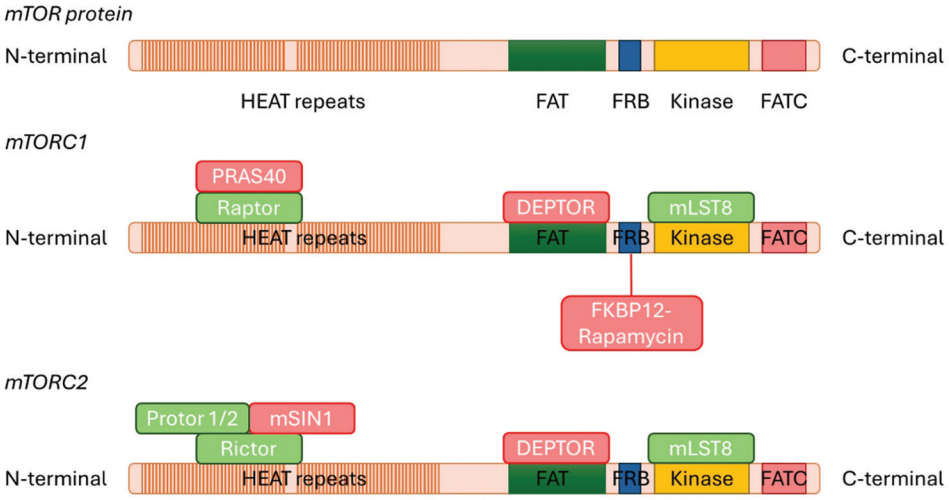

Figure 2. A schematic depiction of the main components of the mTOR protein, mTORC1, and mTORC2
Abbreviations: DEPTOR: DEP-domain-containing mTOR-interacting protein; FAT: FKBP-rapamycin-associated protein, ataxia-telangiectasia mutated, and transformation/transcription-domain-associated protein; FATC: FKBP-rapamycin-associated protein, ataxia-telangiectasia mutated, and transformation/transcription-domain-associated protein C-terminal; FRB: FKBP 12-rapamycin-binding; HEAT repeats: Huntington, elongation factor 3, protein phosphatase 2A, and TOR repeats; mLST8: Mammalian lethal with SEC13 protein 8; mSIN1: Mammalian stress-activated protein kinase interacting protein 1; mTOR: mammalian or mechanistic target of rapamycin; mTORC1: mTOR complex 1; mTORC2: mTOR complex 2; PRAS40: Proline-rich Akt substrate of 40 kDa; Protor ½: Proteins observed with rictor 1 and 2; Raptor: Regulatory-associated protein of mTOR; Rictor: Rapamycin-insensitive companion of mTOR.

autophagy inhibition. In contrast, mTORC2 is involved in lipid and glucose metabolism, cytoskeletal integrity, and cell migration.[17] mTORC2 is less well understood but is thought to be regulated by growth factors or insulin.[17]

3. mTORopathy and GATORopathy

Proteins associated with the mTOR pathway are encoded by over 60 genes, of which 16 pathogenic variants have been identified in individuals with neurological disorders.[16,18] Loss-of-function variants in negative regulators such as *TSC1, TSC2, PTEN, and STRADA* or gain-of-function variants in positive regulators such as *PI3KCA, AKT3, RHEB, and MTOR*, result in mTORC1 hyperactivation. This hyperactivation causes epilepsies, neurodevelopmental disorders, and malformations of cortical development (MCD).[16] These diseases, collectively called mTORopathies, can be caused by germline variants, somatic variants, or a combination of both.[16]

The spectrum of mTORopathies includes disorders such as tuberous sclerosis, megalencephaly, hemimegalencephaly, focal cortical dysplasia (FCD), bottom-of-sulcus dysplasia (BOSD), or even magnetic resonance imaging-negative FCD. These disorders often share similar molecular and neurohistopathological phenotypes, including disorganized cortical lamination, dysmorphic cytomegalic neurons, balloon cells, strong pS6 immunohistochemical staining (a hallmark of mTOR kinase activity), and neuronal hyperexcitability.[19]

The most prototypical disorder associated with mTOR signaling dysregulation is the tuberous sclerosis complex, which is caused by somatic, germline, or both types of variants in either the *TSC1* or *TSC2* genes.[20] Disinhibited mTOR signaling stimulates excessive cellular proliferation, thus leading to cortical malformations (cortical and subcortical tubers) and tumorigenesis (subependymal giant cell astrocytomas or hamartomas in other organs) in tuberous sclerosis complex.[20]

Focal cortical dysplasia Type II is another well-known mTORopathy. Lim *et al.*[21] found that 15.6% of the studied patients with FCD type II (n=12/77) carried somatic mTOR variants. FCD Type II with somatic variants was also demonstrated to have a mutational gradient, with the highest mutational load in dysplastic tissues, lower levels in the surrounding epileptogenic zones, and absence in adjacent normal areas.[22] Somatic gain-of-function variants in the activating genes of mTOR pathway (*MTOR*, *AKT3*, *PIK3CA*, and *RHEB*), germline loss-of-function variants in inhibiting genes (*TSC1*, *TSC2*, and *DEPDC5*), somatic loss-of-function variants in *TSC1*/*TSC2* genes, and somatic second-hit loss-of-function variants in *DEPDC5* gene accounted for 63% of patients with FCD Type II and hemimegalencephaly.[23] The neurohistopathology of all other FCD Type II patients still demonstrated pronounced pS6 immunohistochemical staining, even in the absence of identifiable mTOR-related gene variants,[23] leading to the conclusion that all FCD Type II cases are mTORopathies, regardless of whether germline or somatic brain variants are presented.

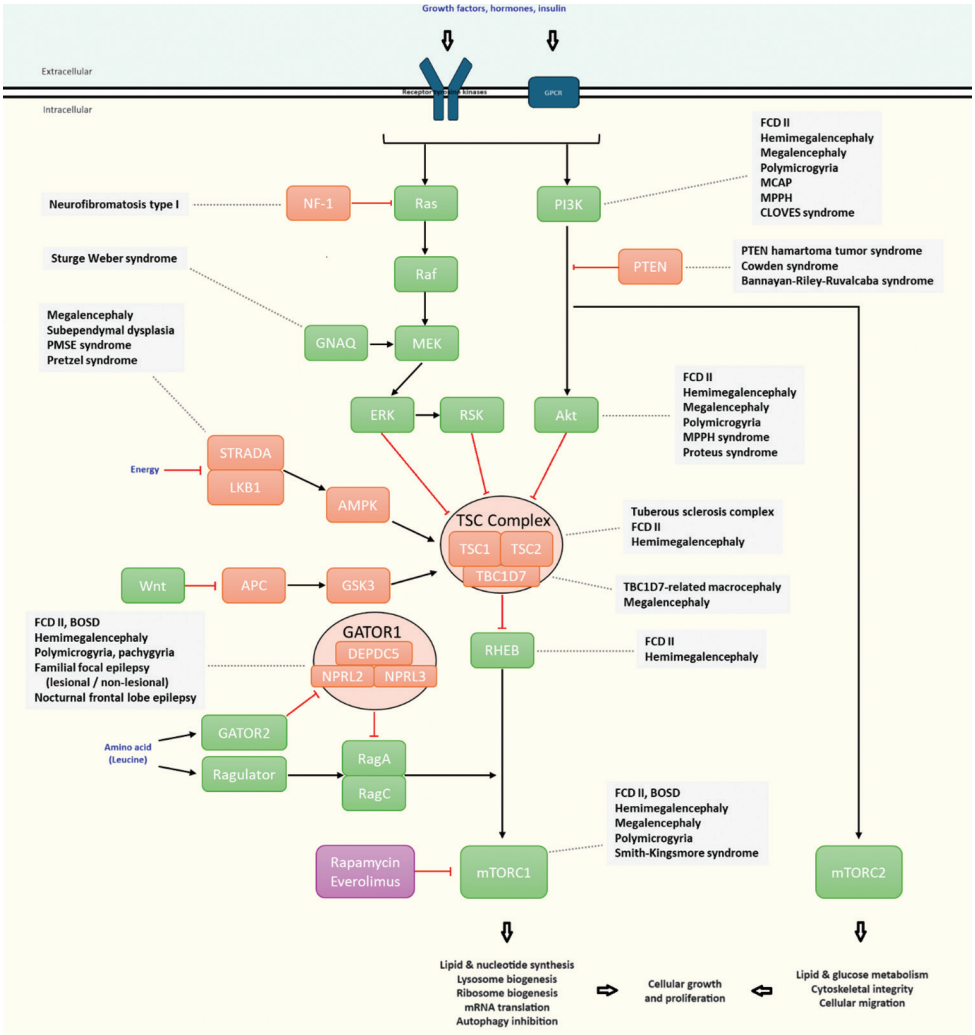

Figure 3. A summary of the mTOR upstream signaling pathway, integrating extracellular stimuli via receptor tyrosine kinases or G-protein-coupled proteins (GPCR) and intracellular amino acids or ATP. It involves complex interplay, either stimulation (black arrow) or inhibition (red, blunt arrow), between positive (pink) and negative regulators (green) of the mTOR signaling network, regulating mTORC activity. The grey boxes with dotted lines showed the mTORopathies and causative genes or proteins.

Abbreviations: Akt: Ak strain transforming serine-threonine protein kinase; AMPK: Adenosine monophosphate-activated protein kinase; APC: Adenomatous polyposis coli protein; BOSD: Bottom-of-sulcus dysplasia; CLOVES: Congenital lipomatous overgrowth, vascular malformation, epidermal nevi, and skeletal/spinal abnormalities syndrome; DEPDC5: Disheveled, Egl-10, and Pleckstrin (DEP) homology domain containing 5; ERK: Extracellular signal-regulated kinase; FCD II: Focal cortical dysplasia type II; GATOR2: GTPase-activating protein activity towards Rags 2; GNAQ: Guanine nucleotide-binding protein G(q) subunit alpha; GPCR: G protein-coupled receptor; GSK3: Glycogen synthase kinase-3; LKB1: Liver kinase B1; MCAP: Megalencephaly-capillary malformation syndrome; MEK: Mitogen-activated protein kinase kinase; MPPH: Megalencephaly-postaxial polydactyly-polymicrogyria-hydrocephalus syndrome; mTORC1: mTOR complex 1; mTORC2: mTOR complex 2; NF-1: Neurofibromin 1; NPRL2: Nitrogen permease regulator 2-like protein; NPRL3: Nitrogen permease regulator-like 3 protein; PI3K: Phosphatidylinositol 3-kinase; PMSE: Polyhydramnios, megalencephaly, and symptomatic epilepsy syndrome; PTEN: Phosphatase and tensin homolog; Raf: Rapidly accelerated fibrosarcoma protein; RagA: Ras-related GTP-binding protein A; RagC: Ras-related GTP-binding protein C; Ragulator: Rregulator of lysosomal signaling with late endosomal/lysosomal adaptor, mapk, and mtor activator subunits, forming complexes with Rag GTPase; Ras: Rat sarcoma guanosine triphosphatase (GTPase); RHEB: Ras homolog enriched in brain protein; RSK: Ribosomal s6 kinase; STRADA: STE20-related kinase adaptor alpha; TBC1D7: TBC1 domain family member 7; TSC1: Tuberous sclerosis 1 or hamartin; TSC2: Tuberous sclerosis 2 or tuberin; Wnt: "wingless-related integration site" protein.

mTORopathies with multisystem involvement, other than tuberous sclerosis complex, are very rare. These conditions include Smith–Kingsmore syndrome caused by germline and somatic *MTOR* variants;[24] Pretzel syndrome, also known as polyhydramnios, megalencephaly, and symptomatic epilepsy (PMSE), caused by germline *STRADA* pathogenic variants;[25,26] megalencephaly capillary malformation–polymicrogyria syndrome caused by germline and somatic *PIK3CA* variants;[27] congenital lipomatous overgrowth, vascular malformation, epidermal nevi, scoliosis/skeletal

and spinal syndrome caused by somatic *PIK3CA* variants;[27] megalencephaly polymicrogyria–polydactyly hydrocephalus caused by germline *AKT3* and *PIK3R2* variants;[28] Proteus syndrome caused by somatic *AKT1* variants;[29] Cowden syndrome and Bannayan–Riley–Ruvalcaba syndrome both caused by germline *PTEN* variants;[30,31] and *TBC1D7*-related macrocephaly caused by germline *TBC1D7* variants.[32]

GATOR1 variants (*DEPD5*, *NPRL2*, and *NPRL3*) also cause a unique clinical subset collectively known as GATORopathy or GATOR1-related epilepsy, distinct from tuberous sclerosis complex, other mTORopathies, or overgrowth syndromes.[16,33] The paradigmatic epilepsy phenotypes include familial focal epilepsy with variable foci and sleep-related hypermotor epilepsy. Baldassari *et al.*[33] reported lesional and non-lesional focal epilepsy in 38% and 62% of the patients with GATORopathy, respectively. More than half of the patients with GATOR1-related epilepsy were drug-resistant, 26% had intellectual disability, and 43% had neuropsychiatric comorbidities such as oppositional disorder, attention deficit hyperactivity disorder, autism spectrum disorder, and mood disorder.[33] MCD was observed in 24% of the patients.[33] Among those who underwent epilepsy surgery, 80% had a favorable surgical outcome with Engel score I–II.[33] Emerging evidence also suggests a higher risk of sudden unexpected death in epilepsy associated with GATOR1 variants, with an incidence of approximately 12% in a cohort (*n*=9/73), highlighting the need for more efficacious treatments for GATOR1-related epilepsy.[16,33] Moloney *et al.*[16] recommended GATOR1 variant sequencing during epilepsy surgery evaluation for all patients with either lesional or non-lesional focal epilepsy, as the presence of a GATOR1 variant may suggest an underlying occult MCD and also potentially predict a favorable surgical outcome.

4. Epileptogenesis

In cases of mTOR hyperactivation, tau protein has been found to be upregulated and abnormally phosphorylated, which interferes with neuronal or glial growth and morphology, leading to altered cortical architecture.[34] Dysplastic cytomegalic neurons observed in mTORopathies have been demonstrated to possess abnormal intrinsic excitability, contributing to the generation and propagation of epileptic discharge.[16] On the other hand, Abs *et al.*[35] observed that acute biallelic deletion of the *TSC1* gene in healthy adult mice results in mTORC1 hyperactivation and neuronal hyperexcitability, without any evident histopathological changes or structural brain pathologies, which is sufficient to induce seizures.

Sosanya *et al.*[36] reported that elevated mTOR activity induced repression of the voltage-gated potassium channel Kv1.1 and hyperpolarization of the action potential threshold in an animal model, which led to hyperexcitability of CA1 pyramidal neurons. Hsieh *et al.*[37] concluded that mTOR-dependent ectopic expression of the hyperpolarization-activated cyclic nucleotide-gated potassium channel isoform 4 depolarizes dysmorphic neurons and enhances their cAMP-dependent excitability, contributing to seizure generation.

Other proposed epileptogenic mechanisms in mTORopathies include abnormal dendritic spine morphology, disrupted glutamatergic synaptic transmission or synaptic plasticity, dysregulated autophagy, astrogliosis, and possible ectopic neurogenesis.[8,38,39] Moreover, there is a complex interplay between the mTOR pathway and immune signaling. It has been shown that mTOR activation is crucial for the early development of the central nervous system's immune system, the maturation, and function of dendritic cells, T cell proliferation, as well as cytokine production and release.[8]

The precise mechanisms of epileptogenesis resulting from aberrant mTOR signaling networks are not yet fully established and are thought to be multifactorial. Overall, it is believed that mTOR hyperactivation, in conjunction with heightened neuroinflammation, triggers a cascade of downstream pathophysiological effects, including altered ion channel receptor expression, neurogenesis, apoptosis, exacerbated neuron damage, mossy fiber sprouting, and aberrant dendritic morphology. These changes lead to neuronal hyperexcitability, altered synaptic transmission, increased seizure susceptibility, and, ultimately, epileptogenesis.[8] In addition, different pathogenic variants in the mTOR pathway exhibit different impacts on mTORC1 activation, leading to a range of phenotypes.[40]

5. Anti-seizure effects of mTOR inhibition in animal models

Meikle *et al.*[41] demonstrated that effective pS6 reduction in the brain resulted in the restoration of Akt function, improvement in neurofilament abnormalities, myelination, and cell size, as well as enhanced behavior, phenotype, weight gain, and survival in *Tsc1*-ablated mice treated with rapamycin.

mTOR inhibition has also shown anti-seizure and anti-epileptogenic effects in animal models of tuberous sclerosis complex. Zeng *et al.*[42] investigated the outcomes of early and late rapamycin treatment in mice with *TSC1* gene inactivation, primarily in glia, which led to proliferation, progressive epilepsy, and premature death. Late treatment with rapamycin at 6 weeks of age suppressed seizures and improved survival in mice already manifesting seizures, whereas early treatment at postnatal day 14, before the

onset of neurological abnormalities, prevented epilepsy, and premature death.[42] However, the neurological phenotype and histopathological abnormalities reappeared after rapamycin cessation, resulting in death within approximately 2 months.[42] Similar findings were observed in *PTEN*-knockout mice with cortical dysplasia.[43]

Unlike its effects on genetic models of mTOR hyperactivity, mTOR inhibition exhibited significantly variable anti-seizure and anti-epileptogenic effects in acquired models of epilepsy.[14,20] Zeng *et al.*[44] reported that biphasic mTOR activities peaked 3 – 6 h after acute kainate-induced seizure and returned to baseline by 24 h in the neocortex and hippocampus, with a second peak 5 – 10 days later only in the hippocampus, in an animal model of status epilepticus-induced temporal lobe epilepsy (TLE). Early rapamycin use has been shown to prevent epilepsy, while late treatment reduces seizures.[44] In a mouse model of pilocarpine-induced status epilepticus and TLE, a 2-month treatment with rapamycin reduced mossy fiber sprouting – a common neuropathology of mesial TLE – but did not reduce seizure frequency.[45] Sliwa *et al.*[46] similarly found that rapamycin did not prevent epileptogenesis in a mouse model of amygdala stimulation-induced TLE. In rats with hypoxia-induced neonatal seizures, rapamycin reversed the early rise of glutamate neurotransmission and seizure susceptibility, reducing subsequent autistic-like behavior and epilepsy.[47] For traumatic brain injury, Guo *et al.*[48] reported that rapamycin did not prevent acute symptomatic seizures following controlled cortical impact injury in rats, but it significantly reduced the rate of developing post-traumatic epilepsy. On the other hand, rapamycin has been shown to have little or no effects on acutely induced seizures.[20,49,50]

Wong[20] postulated that the anti-seizure and anti-epileptogenic effects of mTOR inhibition may depend on timing, duration, age, pathology, and the specific model used. Overall, early and long-term treatment with mTOR inhibitors may be crucial to achieving maximal anti-epileptogenic effects in both genetic and acquired models of mTOR hyperactive conditions.[20]

6. mTOR inhibitors and clinical studies

Targeting mTOR signaling is emerging as a promising approach for treating epilepsy, given that mTOR signaling networks play a crucial role in epilepsy development by influencing processes such as neuronal growth, synaptic changes, neurotransmitter release, energy metabolism, and autophagy. mTOR inhibitors are considered rational candidates as potential anti-epileptogenic or disease-modifying agents, with the ability to prevent epilepsy or slow disease progression rather than merely controlling seizures.[20] Targeting mTOR signaling represents a novel therapeutic strategy for epilepsy, with ongoing research focused on optimizing the use of mTOR inhibitors for treating this disorder.

To date, at least 60 mTOR inhibitors have been developed and are at various stages of clinical trials. Thus far, three mTOR inhibitors, namely rapamycin (also known as sirolimus), everolimus, and temsirolimus, have been approved by the United States Food and Drug Administration (U.S. FDA) for variable indications. However, only everolimus is specifically licensed for use in epilepsy, whereas rapamycin is used off-label for certain neurology-related conditions (Table 1).[16,51-55]

6.1. mTOR inhibitors used in tuberous sclerosis complex

6.1.1. Rapamycin (sirolimus)

Rapamycin was the first mTOR inhibitor identified. It was discovered by Vézina *et al.*[56] in 1975, isolated from *Streptomyces hygroscopius* in a soil sample from Rapa Nui, and initially demonstrated anti-fungal properties before its immunosuppressive effects were recognized.

Rapamycin is an allosteric inhibitor that binds to intracellular FKBP12 receptors, which in turn binds to the FRB domain of the mTOR protein. This binding allosterically alters the mTOR active site, inhibiting mTORC assembly, disrupting its interaction with substrates, and thereby suppressing kinase activity.[57-59] Rapamycin exhibits a differential sensitivity toward mTORC, being highly selective for mTORC1, whereas mTORC2 is relatively insensitive to rapamycin.[58] mTORC2's stronger interaction with phosphatidic acid compared to mTORC1 renders its stability against rapamycin,[60] resulting in the need for much higher concentration and longer exposure to rapamycin to suppress mTORC2 assembly and activity.[58,60,61]

Rapamycin was first approved by the U.S. FDA in 1999 as an immunosuppressant for rejection prophylaxis in renal transplant patients.[51] In the context of neurological disorders, the clinical response to rapamycin in patients with tuberous sclerosis complex was first reported in 2006. Significant volume reduction of subependymal giant cell astrocytomas (SEGAs) was observed in five tuberous sclerosis complex patients treated with rapamycin (Table 2).[62] When rapamycin was administered to six tuberous sclerosis complex patients with refractory epilepsy, all but one experienced a 50% or greater reduction in seizures with sirolimus.[63] In the study, the initial sirolimus dosage was 1.0 mg/m^2/d, with a target sirolimus level of 4 – 10 ng/mL. The initial sirolimus dosage of 1.0 mg/m^2/d is the recommended dosage for other established indications in patients weighing less than 40 kg. However, the safety of

Table 1. Overall characteristics of mTOR inhibitors

	Rapamycin (Sirolimus)[51]	Everolimus[52-54]	Temsirolimus[55]
FDA approval (Year)	• 1999: Renal transplant rejection prophylaxis (≥13 y, oral) • 2015: Pulmonary LAM (adult, oral) • 2021: PEComa (adult, iv) • 2022: TSC-associated facial angiofibroma (≥6 y, topical gel 0.2%)	• 2009: Advanced RCC (adult) • 2010: TSC-associated SEGA (adult) • 2011: Advanced pancreatic NET (adult) • 2012: TSC-associated renal AML (adult) • 2012: Advanced breast cancer (adult) • 2012: TSC-associated SEGA (age≥1 y) • 2016: GI and lung NET (adult) • 2018: TSC-associated partial-onset seizures (≥2 y)	2007
Indications (FDA)	• Renal transplant rejection prophylaxis • Pulmonary LAM • PEComa: locally advanced unresectable or metastatic • TSC-associated facial angiofibroma	• Breast cancer: Advanced, hormone receptor-positive, HER2 negative, in combination with exemestane after failure with letrozole or anastrozole • NET: GI or lung, unresectable, locally advanced, or metastatic disease • NET: Pancreatic, unresectable, locally advanced, or metastatic disease • RCC: advanced disease, after failure of treatment with sunitinib or sorafenib • TSC-associated renal AML • TSC-associated SEGA • TSC-associated partial-onset seizures (adjunct) • Liver transplant rejection: Combination therapy; Prophylaxis • Renal transplant rejection: Low-to-moderate risk, combination therapy; prophylaxis	Advanced RCC
Dosing information (adult)	• Renal transplant rejection, prophylaxis <40 kg: initial 3 mg/m^2/d POD1, maintenance 1mg/m^2/d POD 2 ≥40 kg: initial up to 15 mg POD 1, maintenance 5 mg/d POD 2 (high risk), 6 mg POD 1, maintenance 2 mg/d POD 2 (low to moderate risk) • Pulmonary LAM: initial 2 mg/d • PEComa: 100 mg/m^2 IV infusion on days 1 and 8 of each 21-day cycle until disease progression or unacceptable toxicity • TSC-associated facial angiofibroma: topical, max 800 mg/d (2.5cm)	• Breast cancer (advanced, hormone receptor-positive, HER2 negative, in combination with exemestane after failure with letrozole, or anastrozole): 10 mg/d (with exemestane 25 mg/d) • NET (GI or lung, unresectable, locally advanced, or metastatic disease): 10 mg/d qd • NET (Pancreatic, unresectable, locally advanced, or metastatic disease): 10 mg/d qd • RCC (Advanced disease, after the failure of treatment with sunitinib or sorafenib): 10 mg/d qd • TSC-associated renal AML: 10 mg/d qd • TSC-associated SEGA: Initial, 4.5 mg/m^2 qd, titrate to attain trough concentrations 5 – 15 ng/mL • TSC-associated partial-onset seizures (adjunct): Initial, 5 mg/m^2/d; titrate to attain trough concentration of 5 – 15 ng/mL at 1 – 2 weeks after initiation or modification of dose. • Liver transplant rejection (combination therapy; prophylaxis): initial 1 mg bid • Renal transplant rejection (low-to-moderate risk, combination therapy; prophylaxis): 0.75 mg bid with basiliximab induction with reduced-dose cyclosporine and tacrolimus	25 mg IV infusion over 30 – 60 min once weekly until disease progression or unacceptable toxicity
Dosing information (children)	• TSC-associated facial angiofibroma, maximum daily dose: • Age 6 – 11 y: 600 mg (2 cm) • Age ≥12 y: 800 mg (2.5 cm)	• TSC-associated SEGA: Initial, 4.5 mg/m^2 qd, titrate to attain trough concentrations 5 – 15 ng/mL • TSC-associated partial-onset seizures (adjunct): Initial, 5 mg/m^2/d; titrate to attain trough concentration of 5 – 15 ng/mL at 1 – 2 weeks after initiation or modification of dose.	-
Reference range (trough concentration)	• 10 – 15 ng/mL (renal transplant rejection, prophylaxis)	• 3 – 8 ng/mL (renal and liver transplant rejection, prophylaxis) • 5 – 15 ng/mL (SEGA, partial seizure)	-

(Contd...)

Table 1. ***(Continued)***

	Rapamycin (Sirolimus)[51]	Everolimus[52-54]	Temsirolimus[55]
Pharmacokinetics	• Tmax, oral solution: 2.1 h (adult); 5.88 h (age 6 to 11 y); 2.7 h (age 12 y or older) • Tmax, oral tablet: 3.5 h (adult) • Bioavailability, oral: 14% (solution), >27% than solution (tablet) • Protein binding: 92% • Vd: 12 L/kg • Metabolism: 7 major metabolites, substrate of CYP3A4 and P-glycoprotein • Excretion: renal 2.2%, feral 91%, elimination half-life 61.3 h (female), 72.3 h (male)	• Tmax, oral: 1 – 3 h (transplant), 1 – 2 h (advanced solid tumors) • Bioavailability: 20 – 36% • Protein binding: 74% • Vd: 107 – 342 L • Metabolism: liver, extensive, substrate of CYP3A4 and P-glycoprotein • Excretion: renal 5%, feral 80%, elimination half-life 30 h, 79 h (hepatic impairment)	• Tmax, Temsirolimus: 0.5 h (1 – 2.5 h sirolimus, primary metabolite) • Vd: 172 L • Metabolism: hepatic, substrate of CYP3A4 • Excretion: renal 4.6%, fecal 78%, total body clearance 16.2 L/h (adult), elimination half-life 17.3 h (adults), 31 h (children)
Adverse reactions[a]	• Renal transplant rejection prophylaxis: peripheral edema, dyslipidemia, hypertension, elevated creatinine, abdominal pain, diarrhea, headache, fever, urinary tract infection, anemia, nausea, arthralgia, pain, thrombocytopenia • Pulmonary LAM: stomatitis, diarrhea, abdominal pain, nausea, nasopharyngitis, acne, chest pain, peripheral edema, upper respiratory tract infection, headache, dizziness, myalgia, hypercholesterolemia • TSC-associated facial angiofibroma: dry skin, local irritation, pruritus, acne, acneiform dermatitis, ocular hyperemia, skin hemorrhage	• Breast cancer, NET, RCC: stomatitis, infections, rash, fatigue, diarrhea, edema, abdominal pain, nausea, fever, asthenia, cough, headache, anorexia • TSC-associated renal AML: stomatitis • TSC-associated SEGA: stomatitis, respiratory tract infection • TSC-associated partial-onset seizures: stomatitis	• Rash, asthenia, mucositis, nausea, edema, anorexia • Anemia, hyperglycemia, dyslipidemia, elevated alkaline phosphate, elevated serum creatinine, lymphopenia, hypophosphatemia, thrombocytopenia, elevated AST, leukopenia

Note: [a]The most common adverse reaction with incidence ≥30%, except for topical sirolimus with an adverse effect incidence of ≥1%.
Abbreviations: AML: Angiomyolipoma; CYP: Cytochrome; FDA: United States Food and Drug Administration; GI: Gastrointestinal; IV: Intravenous; LAM: Lymphangioleiomyomatosis; NET: Neuroendocrine tumor; PEComa: Perivascular epithelioid cell tumor; POD: Post-operation day; RCC: Renal cell carcinoma; SEGA: Subependymal giant cell astrocytoma; Tmax: Time to maximum concentration; TSC: Tuberous sclerosis complex; Vd: Volume of distribution; qd: Once a day; bid: Twice a day; y: Year.

rapamycin has not been established for children younger than 13 years old.[51]

Rapamycin has poor solubility and pharmacokinetic properties, limiting its use for other indications, such as epilepsy.[59] Rapamycin derivatives, or rapalogs, such as temsirolimus, everolimus, and ridaforolimus, act similarly as allosteric mTOR inhibitors but have been modified at the C-42 position of rapamycin to improve solubility, stability, and bioavailability.[59]

6.1.2. Everolimus

Everolimus, chemically known as 40-O-(2-hydroxyethyl)-rapamycin, is a derivative of rapamycin in which the hydroxyl group at the C-40 position is substituted by a hydroxyl ethyl group. This modification improves its pharmacokinetic properties, providing higher solubility, increased oral bioavailability, and a shorter half-life.[58] Everolimus is one of the most extensively studied mTOR inhibitors in clinical settings, particularly in the treatment of tuberous sclerosis complex.[64]

Everolimus was first approved by the U.S. FDA in 2010 for the treatment of surgically inaccessible SEGA in adults and children aged ≥3 years. This approval was expanded in 2012 to include children aged above 1 year, with a newly developed pediatric drug formulation (Table 1).[53] The effects of everolimus on tuberous sclerosis complex-related epilepsy have since been investigated in various clinical trials (Table 2).[65-68]

The first prospective, open-label clinical trial focused on everolimus use in tuberous sclerosis complex-related epilepsy was conducted by Krueger *et al.*[67] In this study, 20 patients were treated with everolimus for 12 weeks without any titration of their concurrent anti-seizure medication. The results showed a significant median

Table 2. Summary of the literature on everolimus use as adjunctive treatment in patients with tuberous sclerosis complex and refractory epilepsy

Authors	Study design	Disease of primary interest	*n*	Age (y)[a]	Durationa	Seizure outcomeb	Other findings
Krueger *et al.*[65]	Phase 1/2, prospective, open-labeled	TSC with SEGA	28	11 (3 – 34)	6 m	Sz reduced in 56.3% of pts No effects in 37.5% of pts Sz increased in 6.3% of pts	SEGA reduced by≥50% in 32% of pts. All patients had≥1 AE (Serious AE in 25% of pts)
Franz *et al.*[66]	Phase 1/2, prospective, double-blind, placebo-controlled (EXIST-1)	TSC with SEGA	117	9.5 (0.8 – 26.6)	6 m	No difference between Rx and placebo groups	SEGA reduced by≥50% in 35% of pts. AE mostly grades 1 – 2 (none discontinued due to AE)
Kotulska *et al.*[107]	Sub-study of EXIST-1	TSC with refractory epilepsy	8	2 (1 – 2.9)	median 2.9 y (2.8 – 3.2)	3 pts had no sz at baseline and throughout the study; Responder in 60% of pts (1 sz free)	89.4% AE were grade 1 – 2 (52% Rx-related)
Wiegand *et al*[108]	Prospective, cohort study	TSC with refractory epilepsy	7	5 (2 – 12)	48 w	Responders in 50% of pts	All pts had reduced AE over time. None withdrew.
Krueger *et al*. 67	Phase 1/2, prospective, open-labeled	TSC with refractory epilepsy	20	8 (2 – 21.3)	12 w (4 w titration, 8 w maintenance)	Responders in 60% of pts (20% sz free, 35% ≥90% sz reduction); Sz reduction by a median of 73%	All pts had AE but all were mild or moderate.
Franz *et al.*[68]	Open-labeled extension of EXIST-1	TSC with SEGA	111	9.5 (1.1 – 27.4)	median 29.3 m (19.4-33.8)	-	SEGA reduced by≥50% in 37% of pts at 24 w, 46% at 48 w, 47% at 96 w, 38% at 144 w. Rx-related AE grade 3-4 in 32% (Rx-related serious AE in 16%; 5% withdrew due to AE).
Cardamone *et al.*[63]	Prospective, open-labelled	TSC with refractory epilepsy and SEGA	7	6 (3 – 17)	median 18 m (6 – 38)	Responders in 71.4% of pts (14.3% had≥90% sz reduction)	SEGA was reduced by a mean of 33%. Well tolerated (dyslipidemia in 23%, gingivitis in 8%, anorexia in 8%, mild GI AE in 8%)

(Contd...)

Table 2. ***(Continued)***

Authors	Study design	Disease of primary interest	*n*	Age (y)[a]	Durationa	Seizure outcomeb	Other findings
Krueger *et al.*[109]	Open-labeled extension of Krueger *et al.* 67	TSC with refractory epilepsy	14	8 (2 – 21.3)	48 m	Responders in 93% of pts	AE grade 1 – 2 in 94% of pts (72.5% of reported AE were Rx-related and 2% serious AE)
Samueli *et al.*[110]	Prospective, open-labeled	TSC with refractory epilepsy	15	6 (1 – 18)	median 22 m (6 – 50)	Responders in 80% of pts (58% sz free)	Transient AE in 93% of pts. None withdrew due to AE.
French *et al.*[69]	Phase 3, prospective, double-blind, placebo-controlled (EXIST-3)	TSC with refractory epilepsy	366	10.1 (2.2 – 56.3)	18 w (6 w titration, 12 w maintenance)	Responders: 15.1% in the placebo group 28.2% in LE 40% in HE	Serious AE (Rx withdrawal): 3% of pts (2%) in placebo 14% (5%) in LE 14% (3%) in LE
Franz *et al.*[64]	Open-labeled extension of EXIST-3	TSC with refractory epilepsy	361	10	21 m (2 – 165)	Responders in 31% of pts at 18 w, 46.6% at 1 y, and 57.7% at 2 y	AE grade 3 – 4 in 40.2% of pts; 13% withdrew due to AE; 2 deaths were Rx-related (pneumonia, septic shock)
Curatolo *et al.*[71]	*Post hoc* study of EXIST-3	TSC with refractory epilepsy	299	4.1 (2.2 – 5.9)	18 w (6 w titration, 12 w maintenance)	Responders aged <6 y (≥6 y): 17.6% (12.9%) in placebo group 30.3% (27%) in LE 59.5% (30%) in HE	AE grade 3 – 4 reported in: 45% of pts aged <6 y 38% of pts aged ≥6 y
Mizuguchi *et al.*[111]	Sub-study of EXIST-3	TSC with refractory epilepsy	35	8.8 (2.9 – 16.6)	18 w (6 w titration, 12 w maintenance)	Responders: 0% in the placebo group 30% in LE 28.6% in HE	36.4% of pts with ASD in the Rx group and 12.5% in the placebo group showed PARS drop by ≥5 points.
Franz *et al.*[70]	Open-labeled, post-extension of EXIST-3	TSC with refractory epilepsy	244	9.7 (2.2 – 52.3)	48 w	At 12 w: 18.9% sz free, 64.8% improved sz At 24 w: 18.2% Sz free, 64.5% improved sz At 36 w: 17.1% sz free, 70.1% improved sz, At 48 w: 20% sz free, 61.8% improved sz.	98.6% of pts had AE; AE grade 3 – 4 in 45.2% of pts; 13.9% withdrew Rx due to AE.
Stockinger *et al.*[112]	Retrospective chart review	TSC with refractory epilepsy	45	31.6±11	42±28 m	Responders in 33% of pts (4 had sz free)	42.2% of pts had AE; serious AE in 13.3%.

Notes: [a]Age and duration were expressed in median years (range) or mean±*SD*, unless indicated otherwise; [b]Responders were referred to those with seizure reduction of at least 50%.
Abbreviations: AE: Adverse events; ASD: Autism spectrum disorder; EXIST: Examining everolimus in a study of tuberous sclerosis complex trial; GI: Gastrointestinal; HE: High everolimus exposure group with a blood trough target of 9 – 15 ng/mL; LE: Low everolimus exposure group with a blood trough target of 3 – 7 ng/mL; PARS: Pervasive developmental disorders autism society Japan rating scale; pts: Patients; Rx: Treatment; SEGA: Subependymal giant cell astrocytoma; sz: Seizure; TSC: Tuberous sclerosis complex; m: Month (s); w: Week (s); y: Year (s).

seizure reduction of 73% at 12 weeks. In addition, 60% of the patients experienced a ≥50% seizure reduction in seizures, 35% had at least a 90% reduction, and 20% achieved seizure freedom.[67]

The landmark EXIST-3 trial, a phase-3, double-blind study, involved 366 patients with tuberous sclerosis complex-related refractory epilepsy. Patients were randomly assigned to receive either a placebo, low-exposure everolimus (trough concentration of 3 – 7 ng/mL), or high-exposure everolimus (9 – 15 ng/mL).[69] The trial demonstrated that significantly more patients in the low-exposure (28.2%; $P = 0.0077$) and high-exposure everolimus (40%; $P < 0.0001$) groups achieved a ≥50% seizure reduction after 18 weeks of therapy compared to the placebo group (15.1%).[69] The open-label extension of the EXIST-3 trial ($n = 361$), conducted over 2 years, showed sustained benefits of long-term everolimus use, with an increasing response rate over time: 31% at week 18, 46.6% at 1-year, and 57.7% at 2 years.[64]

In the post-extension phase study of the EXIST-3 trial, Franz *et al.*[70] observed that the efficacy of everolimus is usually maintained once a response is achieved. A *post hoc* analysis by Curatolo *et al.*[71] on the pediatric patients in this cohort showed findings similar to those in the adult population. In fact, younger children aged <6 years appeared to derive greater benefits than older children aged ≥6 years, likely due to lower everolimus clearance in younger patients.[71]

In 2018, the U.S. FDA approved everolimus for the treatment of tuberous sclerosis complex-related focal-onset seizures in adults and children aged ≥2 years.[54] The recommended starting dose of everolimus is 4.5 mg/m^2/d for SEGA and 5 mg/m^2/d for tuberous sclerosis complex-related focal-onset seizures. Although everolimus has a better pharmacokinetic profile than rapamycin, both drugs share a narrow therapeutic index. Dose titration based on whole blood trough concentration from serial therapeutic drug monitoring is recommended, with a target range of 5 – 15 ng/mL.[16,54,72] Franz *et al.*[73] concluded that seizure reduction with everolimus improves progressively over time. Due to its disease-modifying effects, patients receiving higher doses of everolimus may achieve a faster clinical response, whereas those on lower doses may reach a similar response more slowly over a longer period.[73] Franz *et al.*[73] recommended starting everolimus at a low dose and gradually increasing it, as tolerated, to minimize adverse events and enhance adherence. Temporary discontinuation of everolimus is also advisable during febrile illnesses, before receiving live vaccines, and before surgical procedures.[73]

In the post-extension phase of EXIST-3, everolimus demonstrated a tolerable long-term safety profile, with most side effects being transient and mild-to-moderate, and manageable with dose adjustments or temporary discontinuation.[70] Adverse events occurred more frequently within the first 6 months of everolimus treatment. Common adverse events included stomatitis pyrexia (38.2%), stomatitis (36.0%), diarrhea (33.2%), oral ulceration (28.8%), upper respiratory tract infection (26.6%), nasopharyngitis (26.0%), vomiting (22.2%), and cough (21.6%).[70] The severity of adverse events was comparable across all age groups, except for pneumonia, which was more common and severe in children aged <3 years. In this cohort, 38% of patients reported serious adverse events, with 21.3% considered to be everolimus-related.[70] Common adverse events included pneumonia (10.5%), seizures (5.0%), and status epilepticus (4.2%). Four deaths were reported, two of which were due to treatment-related pneumonia and septic shock.[70] Two other deaths were attributed to non-treatment-related sudden unexpected death in epilepsy.[70] Non-infectious adverse events during everolimus treatment were less common but included non-infectious pneumonitis,[72] a pro-coagulant state,[74] angioedema with concurrent use of angiotensin-converting enzyme inhibitors,[75] renal failure,[76] myelosuppression,[77] impaired wound healing,[78] hyperglycemia,[77] dyslipidemia,[77] and acne.[73]

A significant challenge during everolimus treatment is managing drug-drug interaction. Everolimus has significant interactions with CYP3A4/p-glycoprotein (p-GP) inducers and inhibitors.[79] CYP3A4/p-GP inducers, such as phenobarbital, phenytoin, rifampicin, and glucocorticoids, reduce everolimus bioavailability, while CYP3A4/p-GP inhibitors, such as erythromycin, ketoconazole, ritonavir, and verapamil, tend to increase everolimus serum levels.[79] Consequently, dose modifications are often necessary, with frequent therapeutic drug monitoring to guide management.[79]

6.1.3. Other mTOR inhibitors

Several other mTOR inhibitors are currently under development, progressing through various stages of clinical trials. Non-rapalog allosteric inhibitors suppress the phosphorylation of both S6K1 and 4E-BP1, substrates of mTORC1.[59] ATP-competitive mTOR inhibitors are gaining popularity in research as they target the ATP-binding site of the catalytic domain of the mTOR protein, thereby inhibiting both mTORC1 and mTORC2.[59] To overcome resistance that may develop with long-term mTOR inhibitor use, RapaLink-1, a dual-binding site inhibitor, has been designed to simultaneously bind to the FRB domain and the ATP binding site of the mTOR protein.[59] Dual-target mTOR inhibitors, such as dual mTOR/PI3K inhibitors, dual mTOR/HDAC inhibitors, and ATR/

mTOR dual kinase inhibitors, not only suppress mTOC1 and mTORC2 but also target additional pathways—either vertically along the same pathway by inhibiting *PI3K* activity or horizontally by targeting HDAC or ATR proteins in different pathways.[59] Despite these advancements, the clinical use of these drugs is still far from being established.

Temsirolimus, an intravenous rapalog, was approved by the U.S. FDA in 2007 for the treatment of advanced renal cell carcinoma (Table 1).[55] Deforolimus has shown promising clinical responses in metastatic soft tissue or bone sarcoma, but further studies on its safety and efficacy are still needed.[58]

6.2. mTOR inhibitor used in non-tuberous sclerosis complex-related epilepsy

6.2.1. PMSE and hemimegalencephaly

One of the earliest descriptions of mTOR inhibitor use in non-tuberous sclerosis complex-related epilepsy was reported in 2013.[80] Parker *et al.*[80] demonstrated that fibroblasts from skin biopsies of patients with PMSE had higher mTORC1 activity compared to those from the control group and their asymptomatic heterozygous parents. The mTORC1 activity level and migration defects in these fibroblasts were normalized with sirolimus. The authors further described the clinical experience of sirolimus use in five patients with PMSE. Three patients achieved seizure freedom, whereas one experienced a 75% reduction in seizures (Table 3).[80] All treated patients were more interactive or socially engaged than untreated historical controls.[80] In this cohort, a 3-month-old patient who received prophylactic sirolimus treatment at 3 months of age remained seizure-free throughout the follow-up period, highlighting its potential role as a disease-modifying agent.[80]

Xu *et al.*[81] reported a 6-day-old girl with hemimegalencephaly due to a mosaic MTOR variant with 16% mosaicism. Her seizures were initially resistant to nine antiseizure medications. While awaiting for hemispherectomy, sirolimus treatment led to a >50% reduction in seizures within 1 week and improved neurodevelopment in 2 weeks.[81]

On the other hand, Hadouiri *et al.*[82] reported a conflicting case involving a 12-year-old girl with hypomelanosis of Ito, focal cerebral hypertrophy involving the left hemisphere, and an *MTOR* missense variant with 41% mosaicism in her skin-derived DNA. No seizure reduction was observed after 5 months of everolimus treatment, and her clinical condition continued to deteriorate.[82] The authors hypothesized that the degree of mutation mosaicism, as well as the severity of epilepsy, might impact the efficacy of mTOR inhibitors, underscoring the challenges in treating this heterogeneous clinical entity of mTORopathies.[82]

6.2.2. Focal cortical dysplasia

In recent years, there has been growing interest in the use of mTOR inhibitors for treating FCD Type II. Leitner *et al.*[83] observed lower ribosomal protein S6 phosphorylation in the brain tissues of patients with FCD type II (n=9) and tuberous sclerosis complex (n=5) who had undergone surgical resection and were treated with everolimus, compared to the control group. These patients also exhibited higher synaptic transmission and cellular respiration, as well as lower levels of neuron ensheathment, nuclear mRNA catabolism, and organophosphate metabolism (Table 3). Shiraishi *et al.*[84] reported a case of a 2-year-old girl who had surgical resection of FCD type II at the age of 4 months. Her seizures recurred at the age of 1 year and 10 months and were refractory to various antiseizure medications. Treatment with sirolimus resulted in a 95% seizure reduction over 92 weeks.[84]

An open-label, multicenter clinical trial involving 16 patients with FCD type II initiated oral sirolimus at an initial dose of 1 mg/d for patients with a weight of <40 kg and 2 mg/d for those weighing ≥40 kg, with subsequent titration to achieve a blood trough level of 5 – 15 ng/mL.[85] The trial reported a response rate of 33%, defined as the percentage of patients showing a seizure reduction of ≥50% during the 12-week maintenance period.[85]

A recent double-blinded, crossover, randomized clinical trial involved 22 patients with FCD type II-related seizures, in which a population pharmacokinetic model of everolimus was developed to explore its optimal dosage regime.[86] The authors suggested that an initial everolimus dose of 7 – 9 mg/m^2 should be used for patients with a body surface area of <1 m^2, and 6 – 7 mg/m^2 for those with a body surface area between 1 and 2 m^2, to meet the target trough concentration of 5 – 15 ng/mL.[86] In a prospective, randomized, double-blinded, placebo-controlled, crossover, phase 2 trial (ClinicalTrials.gov: NCT03198949), the anti-epileptic efficacy of everolimus was evaluated in patients aged 4 – 40 years who had pathologically confirmed FCD Type II and drug-resistant epilepsy with at least three seizures per month for 2 months.[87] In the 4-week baseline phase, antiseizure medications were kept constant, and seizure burden was monitored. Patients were then randomly assigned in a 1:1 ratio to either the everolimus-first or placebo-first group for the 12-week core phase 1,[87] followed by a crossover to the other treatment arm for another 12 weeks of core phase 2. A 29-week unblinded extension phase was offered to all patients, during which they received everolimus if they consented.[87] In the 1st 4 weeks (titration period) of both core phases, everolimus was started at a daily dose of 4.5 mg/m^2 and titrated to attain a trough concentration

Table 3. Summary of the literature on mTOR inhibitor use in non-TSC related epilepsy.

Authors	Study design	Disease of primary interest	*n*	Age (y)[a]	mTOR inhibitor	Durationa	Seizure outcome	Other findings
Parker *et al.* [80]	Case series	PMSE syndrome	5	0.3 (0.3 – 0.7)	Sirolimus	36 m (5 – 52)	Three achieved sz freedom One had sz reduction by 75% One remained sz freedom from birth	• No mortality within this cohort. • More interactive than the untreated historical counterparts.
Xu *et al.*[81]	Case report	Hemimegalencephaly (MTOR mosaic variant)	1	0.3	Sirolimus	3 m	Sz reduction by>50%	• Improved development
Triana Junco *et al.*[92]	Case report	Sturge Weber syndrome	1	0.8	Sirolimus	23 m	Sz free	• Normal psychomotor development • No significant ophthalmologic changes from baseline
Hadouiri *et al.*[82]	Case report	Hypomelanosis of Ito with lesional refractory epilepsy (MTOR mosaic variant)	1	12	Everolimus	5 m	No beneficial effect	-
Kearney *et al.*[89]	Case report	*DEPDC5*-related refractory epilepsy (non-lesional)	2	33; 48	Everolimus; everolimus	6 m; 6 m	Sz reduction by 33%; sz reduction by 85%	• Improved cognition
Sun *et al.*[93]	Retrospective study	Sturge-Weber syndrome	6	1.5 (0.5 – 7.5)	Sirolimus	16 m (4 – 26)	All had seizure freedom throughout the follow-up period.	• Lightened facial capillary malformation • Improved hypertrophy of pathological tissue in all patients.
Sebold *et al.*[94]	Prospective study	Sturge-Weber syndrome	10	12 (5 – 20)	Sirolimus	6 m	No significant change	• Significant increase in individual processing speed score from neuropsychological test. • Significant improvement in quality-of-life subscale on anger, cognitive function, and depression. • Shortened recovery time from stroke-like episodes.
Leitner *et al.*[83]	Clinical trial	FCD II; TSC	9; 5	12 (3 – 45)	Everolimus	7 d	-	• Lower phospho-S6 in the brain tissues obtained from surgical resection in the everolimus-treated group than the control group with higher synaptic transmission, cellular respiration, and lower neuron ensheathment, nuclear mRNA catabolism as well as organophosphate metabolism.
Kato *et al.*[85]	Open-labeled multicenter clinical trial	FCD II	16	13 (0 – 53)	Sirolimus	12 w	33% achieved sz reduction ≥50%	-

(Contd...)

Table 3. ***(Continued)***

Authors	Study design	Disease of primary interest	*n*	Age (y)[a]	mTOR inhibitor	Durationa	Seizure outcome	Other findings
Moloney *et al.*[90]	Open-labeled observational study	GATOR1-related epilepsy (non-lesional) • *DEPDC5*, *n*=4 • *NPRL3*, *n*=1	5	35 (15 – 49)	Everolimus	12 m (7 – 31)	Three achieved sz reduction by 74.3-86.1% (DEPDC5 LoF variant). One achieved sz reduction by 43.9% (DEPDC5 missense variant). One had no improvement (NPRL3).	-
Shiraishi *et al.*[84]	Case report	FCD II	1	2	Sirolimus	92 w	Sz reduction by 95%	-
Park *et al.*[86]	Double-blinded, crossover, randomized clinical trial	FCD II	22	13.5 (4 – 32)	Everolimus	57 w	-	The optimal initial dose of everolimus was recommended for FCD-related seizures • 7 – 9 mg/m^2 if BSA 0.5 – 1 m^2 • 6 – 7 mg/m^2 if BSA was ≤1.5 m^2

Note: [a]Age and duration were expressed in median (range) or mean±*SD*, unless indicated otherwise.
Abbreviations: BSA: Body surface area; FCD II: Focal cortical dysplasia; Phospho-S6: Ribosomal protein S6 phosphorylation; sz: Seizure; LoF: Loss-of-function variants; TSC: Tuberous sclerosis complex; NS: Not clearly specified; d: Day (s); m: Month (s); w: Week (s); y: Year (s).

of 5 – 15 ng/mL. This dose was subsequently maintained throughout the remaining 8 weeks (maintenance period) of core phases.[87] Thus far, 21 patients have completed the core phases. Although the final results of the trial have yet to be published, the findings will undoubtedly be valuable for the future establishment of guidelines on the use of everolimus for patients with FCD.

6.2.3. GATOR1-related epilepsy or GATORopathy

GATOR1 is an important modulator in the mTOR signaling network, responsible for inhibiting mTORC1 activity. Pathogenic variants in all three genes encoding GATOR1 complex proteins, namely *DEPDC5*, *NPRL2*, and *NPRL3*, have been shown to cause both lesional and non-lesional focal epilepsy.[88]

Kearney *et al.*[89] reported two cases of non-lesional *DEPDC5*-related refractory epilepsy that showed seizure reduction of 33% and 85% after 6 months of everolimus treatment (Table 3). An open-label observational study by Moloney *et al.*[90] (*n*=5) further suggested that everolimus could be a potential precision therapy for GATOR1-related epilepsy. In this study, three patients with *DEPDC5* loss-of-function variants achieved the best seizure reductions, ranging from 74.3 – 86.1%, with everolimus.[90] Another patient with a *DEPDC5* missense variant experienced a 43.9% reduction in seizures, whereas no improvement was observed in a patient with an *NPRL3* variant.[90] Myers and Scheffer[88] postulated that *DEPDC5* could be an exciting potential therapeutic target, with *DEPDC5* agonists likely having anti-epileptogenic properties and potentially working synergistically with the ketogenic diet.

6.2.4. Sturge-Weber syndrome (SWS)

SWS is a congenital neurocutaneous disorder caused by somatic activating mutations in the *GNAQ* gene.[91] Somatic *GNA11* variants can also result in distinctive features beyond the classical presentation of SWS, thereby expanding the phenotypic spectrum of the syndrome.[91] Both *GNAQ* and *GNA11* encode alpha subunits of the G_q protein, which are linked to G-protein-couped receptors.[91] Pathogenic variants in these genes can lead to the dysregulation of several signaling pathways, including the phospholipase C pathway, the Hippo-YAP pathway, and the *MEK/ERK/mTOR* pathway.[91]

Triana Junco *et al.*[92] reported the use of sirolimus in combination with aspirin in a 3-week-old infant with bilateral SWS (Table 3). The patient remained seizure-free with normal neurodevelopment over a 23-month follow-up period.[92] A 2021 retrospective study involving six patients with SWS and uncontrolled epilepsy observed

that sirolimus treatment not only lightened facial capillary malformations but also resulted in seizure freedom for all patients throughout the follow-up period, with a median follow-up duration.[93] On the other hand, a prospective study by Sebold *et al.*[94] reported no significant changes in seizure after 6 months of sirolimus. However, the study did note significant improvements in individual processing scores from neuropsychological tests, quality-of-life subscales related to anger, depression, and cognitive function, as well as a shortened recovery time from stroke-like episodes.

7. Other seizure treatments with mTOR signaling modulation

7.1. Ketogenic diet

The ketogenic diet is a well-established treatment modality for refractory epilepsy. There are many hypotheses about its mechanism of action, with modulation of the insulin/Akt/mTORC1 pathway thought to be one of the complex synergic mechanistic interplays that occur following the initiation of the ketogenic diet.[95] Kossoff *et al.*[96] demonstrated that 92% of children with tuberous sclerosis complex-related refractory epilepsy experienced a >50% reduction in seizures, and 67% had a >90% reduction in seizures after 6 months on the ketogenic diet. In 2018, the International Ketogenic Diet Study Group included tuberous sclerosis complex as one of the epilepsy syndromes or conditions that consistently benefit from the ketogenic diet.[97]

7.2. Vigabatrin

Vigabatrin is primarily known as an irreversible GABA transaminase inhibitor. However, Zhang *et al.*[98] demonstrated that vigabatrin not only increases brain GABA levels but also reduces mTOR downstream S6 phosphorylation activity in a *TSC1*-knockout mouse model. It is particularly effective against tuberous sclerosis complex-related epileptic spasms in early childhood.[99]

In 2011, findings by Jóźwiak *et al.*[100] sparked significant interest in the concept of preventive treatment for tuberous sclerosis complex. Their prospective, open-label trial involved 45 infants with an early diagnosis of tuberous sclerosis complex before seizure onset. In the conventional treatment group, vigabatrin was started within a week after seizure onset, whereas in the preventive group, vigabatrin was initiated before seizure onset, within a week after the appearance of epileptiform activity.[100] The authors observed that the preventive group had significantly lower rates of mental retardation, more seizure-free patients, and a lower incidence of refractory epilepsy at 24 months of age compared to the conventional treatment group.[100]

Another prospective study, EPISTOP, conducted across six sites as a randomized controlled trial and four sites as an open-label trial, investigated 94 infants with tuberous sclerosis complex.[101] This study similarly found that the preventive vigabatrin group had significantly fewer patients with clinical seizures, refractory epilepsy, and infantile spasms.[101] However, the authors observed no significant difference in neurodevelopmental delay between the preventive and conventional groups.[101]

Most recently, Bebin *et al.*[102] published their observations from the PREVENT trial, a phase 2b, multicenter, randomized, double-blinded, placebo-controlled trial (n=84) comparing the use of vigabatrin at the first epileptiform electroencephalogram and seizure onset in tuberous sclerosis complex infants. Contradictorily, the study found that preventive vigabatrin only reduced the incidence of infantile spasms at 24 months, without significantly impacting other seizure types or drug-resistant epilepsy, unlike the EPISTOPS trial.[102] It also did not improve neurocognitive outcomes at 24 months. The authors concluded that while prophylactic vigabatrin use could prevent infantile spasms, it was insufficient to prevent long-term negative neurocognitive outcomes.[102] We postulate that this observation could be due to insufficient mTOR modulation by vigabatrin to address the underlying ongoing epileptogenesis in the tuberous sclerosis complex. At present, a phase 2 randomized, double-blind, placebo-controlled multicenter study (TSC-STEPS) is underway to evaluate the efficacy of preventive sirolimus use in infants with tuberous sclerosis complex (ClinicalTrials.gov: NCT05104983).[103] In addition, another two-arm, randomized, double-blind, double-dummy, placebo-controlled phase 2/3 study (ViRap trial) is comparing the efficacy, tolerability, and safety of prophylactic sirolimus versus vigabatrin in infants with tuberous sclerosis complex (ClinicalTrials.gov: NCT04987463).[104]

7.3. Metformin

There is growing evidence supporting the use of metformin in oncological conditions.[105] Metformin is also thought to influence the mTOR signaling pathway.[105] It has been postulated to inhibit mTORC1 activities through several mechanisms, including the activation of the AMPK pathway, reduction in IGF1 and insulin signaling, upregulation of *p53* and *DICER1* gene expression, and downregulation of *c-MYC* and *HIF-1α* expression.[105] Amin *et al.*[106] conducted the first randomized, double-blind, placebo-controlled trial (MiTS trial) to investigate the safety and efficacy of metformin in 55 patients with tuberous sclerosis complex aged 10 – 65 years. After 12 months of therapy, metformin was found to significantly reduce SEGA volume by 20.8% (vs. 3.0% in the placebo group; P = 0.03) and reduce seizure frequency by 43.7% (vs. 3.1% in the placebo group; P = 0.03), with 25% of the patients becoming seizure-free

compared to none in the placebo group.[106] Metformin was well-tolerated, with no cases of hypoglycemia, lactic acidosis, or treatment-related serious adverse events.[106] The authors concluded that although metformin may be less potent than rapamycin or everolimus, it offers several advantages, such as a more favorable safety profile and fewer drug-drug interactions, without interfering with the metabolism of other mTOR inhibitors or antiseizure medications.[106] The authors also raised the possibility of combined therapy using metformin and a more potent mTOR inhibitor, which could allow for lower doses of the mTOR inhibitor, thereby minimizing side effects.[106]

8. Conclusion

In this review, we provide a comprehensive overview of the mTOR signaling pathway, outline the spectrum of mTORopathies and GATORopathies, and highlight the clinical use of mTOR inhibitors and other potential mTOR-modulating agents. Modulation of the mTOR pathway holds promise for providing anti-epileptogenic and disease-modifying effects in mTORopathies, with the potential to reverse underlying neuropathology.

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Writing – original draft: Lip-Yuen Teng
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Consent for publication

Not applicable.

Availability of data

Not applicable.

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