Review of the treatment options for epilepsy in tuberous sclerosis complex: towards precision medicine

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***Abstract*:** Tuberous sclerosis complex (TSC) is a rare genetic disorder caused by mutations in the *TSC1* or *TSC2* genes, which encode proteins that antagonise the mammalian isoform of the target of rapamycin complex 1 (mTORC1) – a key mediator of cell growth and metabolism. TSC is characterised by the development of benign tumours in multiple organs, together with neurological manifestations including epilepsy and TSC-associated neuropsychiatric disorders (TAND). Epilepsy occurs frequently and is associated with significant morbidity and mortality; however, the management is challenging due to the intractable nature of the seizures. Preventative epilepsy treatment is a key aim, especially as patients with epilepsy may be at a higher risk of developing severe cognitive and behavioural impairment. Vigabatrin given preventatively reduces the risk and severity of epilepsy although the benefits for TAND are inconclusive. These promising results could pave the way for evaluating other treatments in a preventative capacity, especially those that may address the underlying pathophysiology of TSC, including everolimus, cannabidiol and the ketogenic diet (KD). Everolimus is an mTOR inhibitor approved for the adjunctive treatment of refractory TSC-associated seizures that has demonstrated significant reductions in seizure frequency compared with placebo, improvements that were sustained after 2 years of treatment. Highly purified cannabidiol, recently approved in the US as Epidiolex® for TSC-associated seizures in patients ⩾1 years of age, and the KD, may also participate in the regulation of the mTOR pathway. This review focusses on the pivotal clinical evidence surrounding these potential targeted therapies that may form the foundation of precision medicine for TSC-associated epilepsy, as well as other current treatments including anti-seizure drugs, vagus nerve stimulation and surgery. New future therapies are also discussed, together with the potential for preventative treatment with targeted therapies. Due to advances in understanding the molecular genetics and pathophysiology, TSC represents a prototypic clinical syndrome for studying epileptogenesis and the impact of precision medicine.

Keywords: Cannabidiol, epilepsy, epileptogenesis, everolimus, ketogenic diet, mTORC1, tuberous sclerosis complex; TSC-associated neuropsychiatric disorders

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Introduction

Tuberous sclerosis complex (TSC) is a rare genetic disorder caused by mutations in either the *TSC1* or *TSC2* tumour suppressor genes. *TSC1* and *TSC2* encode the proteins hamartin and tuberin, respectively, which form an intracellular complex that antagonises the mammalian isoform of the target of rapamycin complex 1 (mTORC1) through activation of the GTPase activity of RHEB (Ras homolog enriched in brain) (Figure 1a).[1] mTORC1 plays a central role in a whole range of fundamental cell processes, including lipid and nucleotide synthesis, protein synthesis and autophagy.[2] Mutations in *TSC1* or *TSC2* lead to

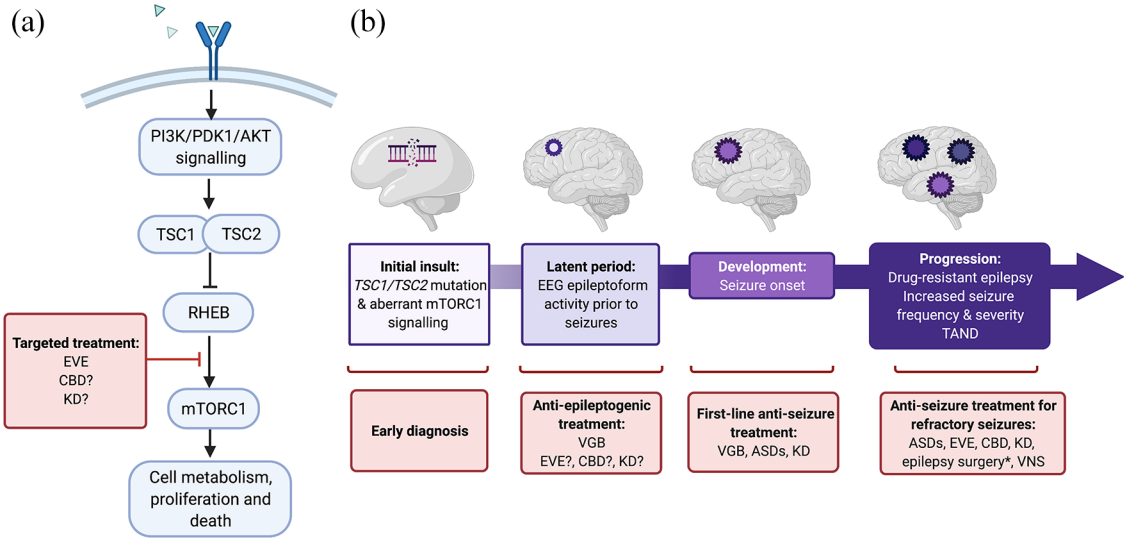

Figure 1. Simplified schematic of (a) targeted treatment in the mTOR pathway (b) the concept of epileptogenesis and the potential for early intervention in the treatment of TSC. Epileptogenesis describes the complex process by which the normal brain undergoes molecular, structural and functional changes that result in abnormal neuronal activity that promotes seizures (Jozwiak et al., 2020)[4]; it encompasses the evolution from the initial insult (in the case of TSC, mutation of the *TSC1* or *TSC2* gene), through what is known as a 'latent period' that occurs before the onset of seizures, to the development and progression of seizures. *Epilepsy surgery is indicated for carefully selected patients that underwent presurgical diagnostics; ? = possible, being/to be investigated. Created with BioRender.com.

AKT, protein kinase B; ASD, anti-seizure drugs; CBD, cannabidiol; EEG, electroencephalogram; EVE, everolimus; KD, ketogenic diet, including the medium-chain triglyceride (MCT) KD; mTOR, mammalian target of rapamycin; mTORC1, mammalian/mechanistic target of rapamycin complex 1; PDK1, phosphoinositide-dependent protein kinase-1; PI3K, phosphoinositide 3-kinase; RHEB, Ras homolog enriched in brain; TAND, TSC-associated neuropsychiatric disorders; TSC, tuberous sclerosis complex; TSC1/TSC2, hamartin-tuberin complex; VGB, vigabatrin; VNS, vagus nerve stimulation.

aberrant activation of this signalling pathway, resulting in the development of benign tumours (hamartomas or tubers) typically in the brain, kidneys, lungs, heart or skin.3 Given the numerous functions surrounding mTOR signalling, the full mechanisms underlying the pathogenesis of TSC are still being elucidated.

TSC is rare, affecting an estimated 1 in 5000 persons worldwide,[5] and an estimated annual incidence rate of 1:27,312 live births.[6] As the largest clinical case series of TSC patients, the International multicentre 'TuberOus SClerosis registry to increase disease Awareness (TOSCA)' has been pivotal in characterising the natural history of TSC: in 2093 patients, the most common manifestations of TSC included epilepsy, cortical tubers, subependymal nodules, intellectual disability, subependymal giant cell astrocytomas (SEGA), renal angiomyolipomas (AML), lymphangioleiomyomatosis (LAM), cardiac rhabdomyomas, facial angiofibromas, forehead plaque, ⩾3 hypomelanotic macules and shagreen patches.[7] Broadly speaking, *TSC2* mutations are associated with a more severe phenotype than *TSC1* mutations, including being more likely to have intractable focal epilepsy or infantile spasms (IS), SEGAs and intellectual disability,[8,9] although TSC is highly clinically heterogeneous, with both inter- and intra-familial phenotypic variability.

TSC has a substantial negative impact on the quality of life (QoL) of patients, that is worse than some other chronic conditions, affecting psychosocial factors with negative consequences for education and career.[10–13] TSC is also associated with increased mortality, predominantly due to complications from seizures and renal complications.[5] In addition, caregivers report negative impacts on family, social and work-related dynamics.[11] TSC is associated with a high burden of illness with consequent cost and resource implications for healthcare systems.[5,14,15]

TSC-associated epilepsy and neuropsychiatric disorders

Epilepsy is one of the most common manifestations of TSC, associated with significant morbidity and mortality and, as such, the management of seizures is an important treatment goal.[16–18] The TOSCA registry reported that 84% of TSC patients had epilepsy: 39% had IS and 68% had focal seizures.[19] TSC is associated with almost all seizure types and most patients develop multiple types including focal aware and focal impaired awareness seizures, tonic, atonic, tonic-clonic seizures, myoclonic and atypical absences.[20] Onset of seizures in TSC typically occurs in the first 2 years of life, although they can also develop in adulthood.[17,20] Treatment is particularly challenging because seizures are generally refractory to standard anti-seizure drugs (ASDs).

TSC is a common cause of West syndrome, an epileptic syndrome characterised by IS, hypsarrhythmia in an electroencephalogram (EEG) and developmental delay, that generally presents in the first year of life.[21] Of note, IS can also occur in TSC patients independently of West syndrome, without signs of hypsarrhythmia.[22] In either case, a TSC diagnosis should be investigated in infants with IS.[16,17] Indeed, TSC is often diagnosed due to the presence of IS and other seizures,[16,17] although there is now an increasing emphasis on diagnosing TSC before the onset of seizures by the early identification of other prevalent features including cardiac rhabdomyomas or hypomelanotic skin macules, with prenatal diagnosis even being possible in cases with cardiac rhabdomyomas that can be detected on ultrasound.[23,24] TSC is also associated with Lennox-Gastaut syndrome (LGS), another epileptic syndrome that generally begins in children aged 3–5 years, characterised by a clinical triad consisting of specific slow spike-and-wave EEG pattern, multiple seizure types and cognitive impairment/behavioural difficulties.[25]

TAND (TSC-Associated Neuropsychiatric Disorders) is a term that encompasses the neuropsychiatric comorbidities associated with TSC that includes a range of behavioural, psychiatric, intellectual, academic, neuropsychological and psychosocial manifestations including autism spectrum disorder and intellectual disability.[18,26,27] The pathogenetic mechanisms of TAND are poorly understood, and it is still not clear to what extent the underlying mechanisms of mTORC1 dysregulation contribute to their development and to what extent is the result of the epilepsy itself.[28–31] Seizures clearly have an important influence because a history of seizures, IS, early seizure onset, and refractory seizures are strongly associated with poor cognitive outcome.[19,20,32–34] Overall, the aim is to have targeted therapies that can reduce both seizure burden as well as the development of TAND.

There is now a growing body of evidence that early intervention before the onset of seizures has positive implications for the treatment of both epilepsy and TAND in TSC patients. To this end, studies have been focussing on elucidating the mechanisms of epileptogenesis, as well as improvements in early diagnosis, identifying biomarkers of later epilepsy, and developing targeted therapies for the prevention of seizure development (Figure 1).[17,4,35] In TSC, seizures are believed to arise due to the epileptogenic cortical tubers and the surrounding abnormally developed tissue that result as a consequence of increased mTORC1 signalling that occurs during embryonic brain development, although the full mechanisms are complex and are still being elucidated (Figure 1).[28,36] The 'latent period' may provide a window for initiating preventative treatment, while early diagnosis, biomarkers of epileptogenesis, and having appropriate treatments are all crucial to exploiting this opportunity (Figure 1).[4]

TSC is a prototypic clinical disorder for studying epileptogenesis and the impact of prophylactic interventions: not only are the underlying genetic mechanisms known, but suspected prenatal or early neonatal diagnosis of TSC is possible in some cases due to the visibility of cardiac tumours or cortical tubers on foetal ultrasounds, allowing for early diagnosis.[24] In addition, early abnormal EEG activity, which can be observed before the development of clinical seizures in TSC (i.e. in the latent period), has been determined to be a reliable prognostic biomarker for later epilepsy and indeed neurodevelopmental comorbidities (Figure 1).[37–39] Crucially, this discovery has been instrumental in the design of clinical trials focussed on epilepsy prevention, including the EPISTOP and PREVeNT trials that are described below. In addition, in line with current European guidelines for infants and children to receive regular EEG monitoring,[17] a recent study showed that conducting at least one EEG study in TSC

patients before epilepsy onset and continuing with regular EEG monitoring is now the standard of care in many centres, while it is becoming more common for clinicians to prescribe ASDs prophylactically when epileptiform discharges occur on EEG before the emergence of clinical seizures.[40]

Vigabatrin and other conventional anti-seizure drugs used widely for TSC-associated epilepsy

Conventional treatment with vigabatrin

In the European Union (EU), vigabatrin (VGB) is indicated as monotherapy for IS, and in combination with other ASDs for patients with refractory focal epilepsy, with no age specifications (Table 1). In the US, VGB is indicated for IS as monotherapy in infants 1 month to 2 years of age, and as adjunctive therapy for refractory focal seizures from 2 years of age (Table 1); the latter is a recent welcome expansion whereby until January 2020 the indication for focal seizures was only for patients aged 10 years and older. VGB is the recommended first-line monotherapy for TSC-associated IS,[16–18] and for focal seizures in the EU.[17]

With regard to studies in TSC patients, VGB was reported to be the most efficacious for IS when prescribed as first treatment, with seizure freedom or 'treatment success' occurring in 78% of patients versus 50%–70% for the other ASDs.[46] VGB is also one of only a few treatments with evidence of efficacy in TSC patients from a randomised controlled trial (RCT), albeit in only 22 patients overall; over a 1-month period, all 11 patients (100%) were free of IS in the VGB group as compared with 5/11 (45%) patients treated with hydrocortisone.[41] Other studies, all of which are retrospective observational studies, have reported seizure-freedom rates of 27–89% for IS and 25–46% for other seizures, and ⩾50% response rates of 76–88% for IS and 31–88% for other seizures (Table 1).[53]

Despite VGB being the recommended first-line therapy, studies in Europe have suggested that it is not always the most commonly prescribed ASD in patients with TSC (Figure 2). Indeed there may be some reluctance to prescribe VGB due to the risk of visual field defects that have been reported to occur in up to a third of patients, which may not be reversible upon discontinuation[55]; regular vision testing is therefore required although this is not possible in infants and in patients with mental retardation. It should also be noted that VGB may have a relatively low retention rate compared with other ASDs,[56] which may be due to the development of tolerance as a result of its GABAergic mechanism.[57] However, it has been suggested recently that this risk of relapse could be reduced by using a high-dose of VGB, although further studies are warranted to confirm this.[58] Despite these limitations, VGB still remains a pivotal initial treatment option for IS and focal seizure in TSC patients.

Preventative treatment with VGB

Highly anticipated results from the EPISTOP trial [ClinicalTrials.gov identifier: NCT02098759] were published in November 2020: this trial, part RCT and part open-label trial depending on the trial site, followed 94 infants with TSC with epileptiform EEG abnormalities who received either conventional VGB treatment ($n=29$) (i.e. initiated after the first electrographic or clinical seizure) or preventative VGB (i.e. initiated after EEG epileptiform activity was seen but before seizures had occurred) ($n=25$).[61] The median time to onset of seizures was longer in infants who received preventive treatment than in children treated conventionally {Day 614 [95% confidence interval (CI): 474–infinity] versus Day 124 (95% CI): 114–200}. Overall, at 24 months, preventive VGB treatment was associated with reduced risks of clinical seizures [odds ratio (OR)=0.21 (95% CI: 0.04, 0.9); $p=0.032$], drug-resistant epilepsy [OR=0.23 (0.06, 0.83); $p=0.025$], and IS [OR=0 (0, 0.33); $p<0.001$]. In addition, there were no additional safety concerns with preventive treatment. EPISTOP is also designed to identify biomarkers of epileptogenesis and drug-resistant epilepsy, although these analyses are still ongoing.

This study comes off the back of an initial pilot open-label study in 45 infants that showed that the preventative group had a significantly higher proportion of seizure-free patients (93% vs. 35%; $p=0.004$), and a lower incidence of drug-resistant epilepsy (7% vs. 42%; $p=0.021$).[62] Furthermore, mental retardation was significantly less frequent and severe in the preventative group [14% vs 48%; $p=0.031$; mean intelligence quotient (IQ) score 92.3 vs. 68.7; $p<0.05$]. Based on this study, consensus guidelines recommend that infants with TSC should be monitored with serial

Table 1. Summary of conventional ASDs widely used for the treatment of seizures associated with TSC.

ASD	Generation (year of MA for epilepsy)	MOA via	Epilepsy indication(s) EU	Epilepsy indication(s) US	Studies reporting ⩾50% responder rate in TSC		Other considerations
					Study design	⩾50% responder rate	
Vigabatrine	Second generation (1992)	GABA-T	FS[b]; IS[a]	Refractory complex FS (⩾2 y)[b]; IS[a] (infants 1 mo to 2 y)	RCT (N = 22; 11 treated with VGB)[41]	100% for IS	Risk of visual field defects
					Retrospective (N = 42)[42]	73% for IS 34% for FS	
					Retrospective (N = 670)[43]	88% [c]	
					Retrospective (N = 49)[44]	31% for FS	
					Retrospective (N = 21)[45]	81% for ES and TC	
Valproate	First generation (1967)	• Voltage-gated sodium channels • T-type calcium channels • GABA	FS and GS or other epilepsy	FS*/[b]; Multiple seizure types[b]	Retrospective (N = 60)[46]	70%[d]	Risk of hepatotoxicity and pancreatitis Due to teratogenic effect a Pregnancy Prevention Program is in place
Lamotrigine	Second generation (1991)	• Voltage-sensitive sodium channels	FS and GS, including GTC and LGS (⩾2 y[b]; ⩾13 y[a])	FS, GTC and LGS (⩾2 y[b]; ⩾16 s[a])	Retrospective (N = 57)[47]	79% (42% seizure free + 37% >50% reduction)	Rare risk of life-threatening serious rash; mandatory slow titration reduces the risk
Levetiracetam	Second generation (2000)	• Synaptic vesicle protein SV2A	FS in newly diagnosed epilepsy[a] (⩾16 y) PS[b] with epilepsy (⩾1 mo); JME (myoclonic seizures)[b] (⩾12 y); IGE (GTC seizures)[b] (⩾12 y).	FS (⩾1 mo); JME (myoclonic seizures)[b] (⩾12 y); IGE (GTC seizures)[b] (⩾6 y)	Retrospective (N = 20)[48]	40%	Risk of psychobehavioural AEs, particularly in children or individuals with cognitive impairment
Oxcarbazepine	Second generation (1990)	• Voltage-sensitive sodium channels	FS with or without secondary GTC (⩾6 y[a/b])	FS (4–16 y[a]; 2–16 y[b])	Retrospective (N = 16)[46]	67%‡	–
Carbamazepine	First generation (1965)	• Voltage-sensitive sodium channels	GTC and FS	FS with complex symptomatology, GTC and mixed seizure patterns, other partial or generalised seizures.	Retrospective (N = 29)[46]	67%[d]	Risk of serious and sometimes fatal dermatologic reactions and aplastic anaemia and agranulocytosis.

(Continued)

Table 1. (Continued)

ASD	Generation (year of MA for epilepsy)	MOA via	Epilepsy indication(s) EU	Epilepsy indication(s) US	Studies reporting ⩾50% responder rate in TSC		Other considerations
					Study design	⩾50% responder rate	
Topiramate	Second generation (1995)	• Voltage-sensitive sodium channels • GABA-A receptor • AMPA/kainate glutamate receptors	FS and GTC (>6 y[a]; ⩾2 y[b]); LGS (⩾2y[b])	FS and GTC (⩾2 y[a/b]); LGS (⩾2 y[b])	-	-	Risk of cognitive side effect such as mental slowing and dysphasia
Lacosamide	Third generation (2010)	• Voltage-gated sodium channels	FS +/- GS (⩾4 y[a/b])	FS (⩾4 y)	Retrospective (N = 46)[49]	48%	–
Clobazam	Second generation (1995)	• GABA-A receptor	Epilepsy[b]	Seizures associated with LGS[b] (⩾2 y)	Retrospective (N = 29)[50]	69%	Risks from concomitant use with opioids that may result in profound sedation, respiratory depression, coma, and death Risk of physical and/or psychic dependence
Brivaracetam	Third generation (2016 adults; 2018 children)	• Synaptic vesicle protein SV2A	FS +/- secondary GS (⩾4 y[b])	FS (⩾4 y)	Retrospective (N = 44; 3 with TSC)[51]	43% for the overall population	–
Perampanel	Third generation (2012)	• AMPA glutamate receptor	FS ± secondary GS (⩾12 y[b]); IGE (GTC seizures) (⩾12 y[b])	FS ± secondary GS (⩾4 y); Primary GTC (⩽12 y[b])	Retrospective (N = 32; 6 with TSC)[52]	TSC 67% at 6 & 12 mo; overall population 44% & 31% at 6 & 12 mo	Risk of serious psychiatric and behavioural reactions including irritability and aggression especially in individuals with cognitive impairment

[a]Monotherapy.
[b]Adjunctive.
[c]Retention rate at 6 months;
[d]Seizure-freedom or treatment success; Adapted from van der Poest Clement et al 2020,[53] Málaga et al. 2019[54]; and the respective cited publications and Summary of Product Characteristics (SmPC) and US Highlights of Prescribing Information.
AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AS, absence seizures; ES, epileptic spasms; EU, European Union; FS, focal seizures; GABA, gamma aminobutyric acid; GABA-T, GABA-transaminase; GS, generalised seizures; GTC, generalised tonic clonic; IGE, idiopathic generalised epilepsy; IS, infantile spasms; JME, juvenile myoclonic epilepsy; LGS, Lennox-Gastaut syndrome; MA, marketing authorisation; Mo, month; MOA, mode of action; TS, tonic seizures; TSC, tuberous sclerosis; US, United States; y, year(s).

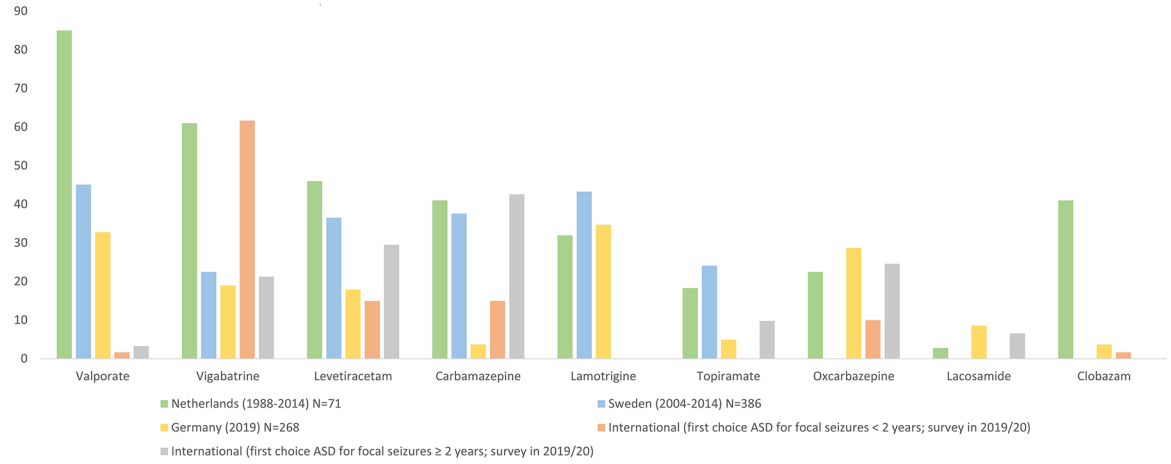

Figure 2. ASD treatment patterns in TSC patients. Data from Overwater et al. 2015 (Netherlands), Welin et al. 2017 (Sweden), Strzelczyk et al. 2021 (Germany) and Słowińska et al. 2020 (International).[40,46,59,60]
ASD, anti-seizure drugs; TSC, tuberous sclerosis complex.

EEG in short intervals that cover wake and sleep phases and are accompanied by video recording. Preventive treatment with VGB in children within 24 months of age should be started if epileptiform activity should occur, with or without clinical manifestations,[17] which is becoming increasingly common in clinical practice.[40] More recently, the 5-year long-term follow up of this study reported that 50% (7/14) of patients in the preventive group remained completely seizure-free compared with only 5% (1/25) in the standard treatment group ($p = 0.001$). In addition, the median IQ remained higher in the preventive group (94 vs. 46; $p < 0.03$).[63]

Another study, the PREVeNT trial [ClinicalTrials.gov identifier: NCT02849457] has a primary completion date of May 2020.[64] This study is similar to the EPISTOP trial, evaluating preventative VGB treatment in infants <6 months with no history of seizures or IS, although it has the advantage of being a double-blind, placebo-controlled study.

Other conventional anti-seizure drugs

Besides VGB, a range of different ASDs are used to treat epilepsy associated with TSC (Figure 2), many of which have been evaluated in retrospective studies in TSC patients, demonstrating ≥50% responder rates of 37%–69% across studies (Table 1). Of note, brivaracetam (BRV) is a newer ASD approved for adjunctive therapy for focal onset seizures in patients with epilepsy aged ≥4 years. It is an analogue of levetiracetam (LEV), but is of interest because, in addition to being effective and generally well-tolerated in patients with epileptic encephalopathies, it appears to be associated with a lower incidence of psycho-behavioural adverse events (AEs) compared with LEV.[51,65,66]

Everolimus

Everolimus (EVE) is an oral protein kinase inhibitor of the mTOR signalling pathway developed over two decades ago. EVE is predominantly known as a cancer treatment; however, due to its mechanism of action as an mTOR inhibitor it also has application as a targeted treatment for TSC patients (Figure 1).[67] In 2017 in the EU and 2018 in the United States (US), EVE gained approval specifically for the treatment of refractory focal-onset seizures associated with TSC, representing an addition to earlier approvals to treat SEGA and renal AMLs.[68,69] Another mTOR inhibitor, sirolimus, is indicated for the treatment of patients with LAM, but has so far failed to show significant benefit for reducing seizures in TSC patients.[70]

Efficacy for TSC-associated seizures

Regarding TSC-associated seizures, the US Food and Drug Administration (FDA) and European Medicines Agency (EMA) approvals were based on data from a phase III, double-blind, placebo-controlled RCT (EXIST-3 [ClinicalTrials.gov identifier: NCT01713946]) in which 366 patients

Table 2. Efficacy of pharmacotherapies from the pivotal RCTs and OLE studies for TSC-associated epilepsy.

	Study design	Patient characteristics	Interventions + comparator	Median baseline monthly TSC-associated seizure frequency	Median percent reduction in TSC-associated seizure; *p*-value *versus* PBO	⩾50% responder rate; *p* value *versus* PBO	Improvement in overall condition/behaviour/QoL
EVE: EXIST-3[71,74]	Phase III RCT 18 wks	• Median (range) age: 10.1 (2.2–56.3) y • Failed >6 prior ASDs: 39%	EVE-LT (n = 117)	Per week: 8.6 (range: 1.4–192.9)	29.3% (95% CI 18.8–41.9); $p = 0.0028$	28.2% (95% CI 20.3–37.3); $p = 0.0077$	Mean (SD) change in QOLCE total score Responders: 5:8 (11.0); $p = 0.016$ *versus* non-responders Non-responders: 1.7 (10.7) Mean (SD) change in QOLIE-AD-48 total score: Responders: 8.2 (12.4); $p = 0.155$ Non-responders: 2.6 (10.7) Mean (SD) change in QOLIE-31-P total score Responders: 15.2 (15.7); $p = 0.021$ Non-responders: -0.6 (15.3)
			EVE-HT (n = 130)	9.5 (0.3–218.4)	39.6% (35.0–48.7); $p < 0.0001$	40.0% (31.5–49.0); $p < 0.0001$	
			PBO (n = 119)	10.5 (1.3–231.7)	14.9% (0.1–21.7)	15.1% (9.2–22.8)	
EVE: EXIST-3 OLE[75]	OLE: 48 mo (2 y)	• Median (range) age: 10.03 (2.2–56.3) y	EVE (target exposure, 3–15 ng/mL) (n = 361)	NR	31.7% (28.5–36.1), 46.7% (40.2–54), 56.9% (50–68.4) at 18 wk, 1 y and 2 y, respectively	31% (26.2–36.1), 46.6% (40.9–52.5), 57.7% (49.7–65.4) at 18 wk, 1 y and 2 y, respectively	NR
CBD: GWPCARE6[76]	Phase III RCT 16 wks: 4-wk titration and 12-wk maintenance phase.	• Median (range) age: 11.4 (1.1–56.8) y • Median current ASDs: 3 • Median prior ASDs: 4	CBD25 (n = 75)	Per 28 days: 56.0 (IQR: 21.2–101.0)	48.6% (40.4–55.8); $p < 0.00$	36%; $p = 0.07$	Percentage with improvement in overall condition S/CGIC 69%; OR = 2.25; $p = 0.0074$
			CBD50 (n = 73)	61.0 (36.0–117.0)	47.5% (39.0–54.8); $p = 0.002$	40%; $p = 0.02$	62%; OR = 1.77; $p = 0.0580$
			PBO (n = 76)	54.1 (26.4–102.0)	26.5% (14.9–36.5)	22%	40%
CBD: GWPCARE6 OLE[77]	OLE: 48 wks	Median (range) age: 10.8 (1.1–56.8) y	CBD25 with titration up to CBD50 (n = 199)	56.9 (28.0– 109.0)	12-wk windows over 48 wks: 54–68%	Wk 1-12: 53% Wk 13–24: 53% Wk 25–36: 58% Wk 37–48: 61%	S/CGIC: 87% of patients/caregivers at wk 26 P/GIC: 80% of physicians at wk 26

ASD, anti-seizure drug; CBD, cannabidiol; CBD25, CBD 25 mg/kg/day; CBD50, CBD 50 mg/kg/day; CI, confidence interval; EVE, everolimus; IQR, interquartile range; HT, high trough (range of 9–15 ng/ml); LT, low trough (range of 3–7 ng/ml); mo, months; NR, not reported; OLE, open-label extension trial; OR, adds ratio; PBO, placebo; P/GIC, Physician Global Impression of Change; QoL, quality of life; QOLCE, Quality of Life in Childhood Epilepsy Questionnaire; QOLIE-AD-48, Quality of Life in Epilepsy Inventory-Adolescents-48; QOLIE-31-P, Patient Weighted Quality of Life in Epilepsy Inventory-Form 31; RCT, randomised controlled trial; S/CGIC, Subject/Caregiver Global Impression of Change; SD, standard deviation; wk, week; y, year(s).

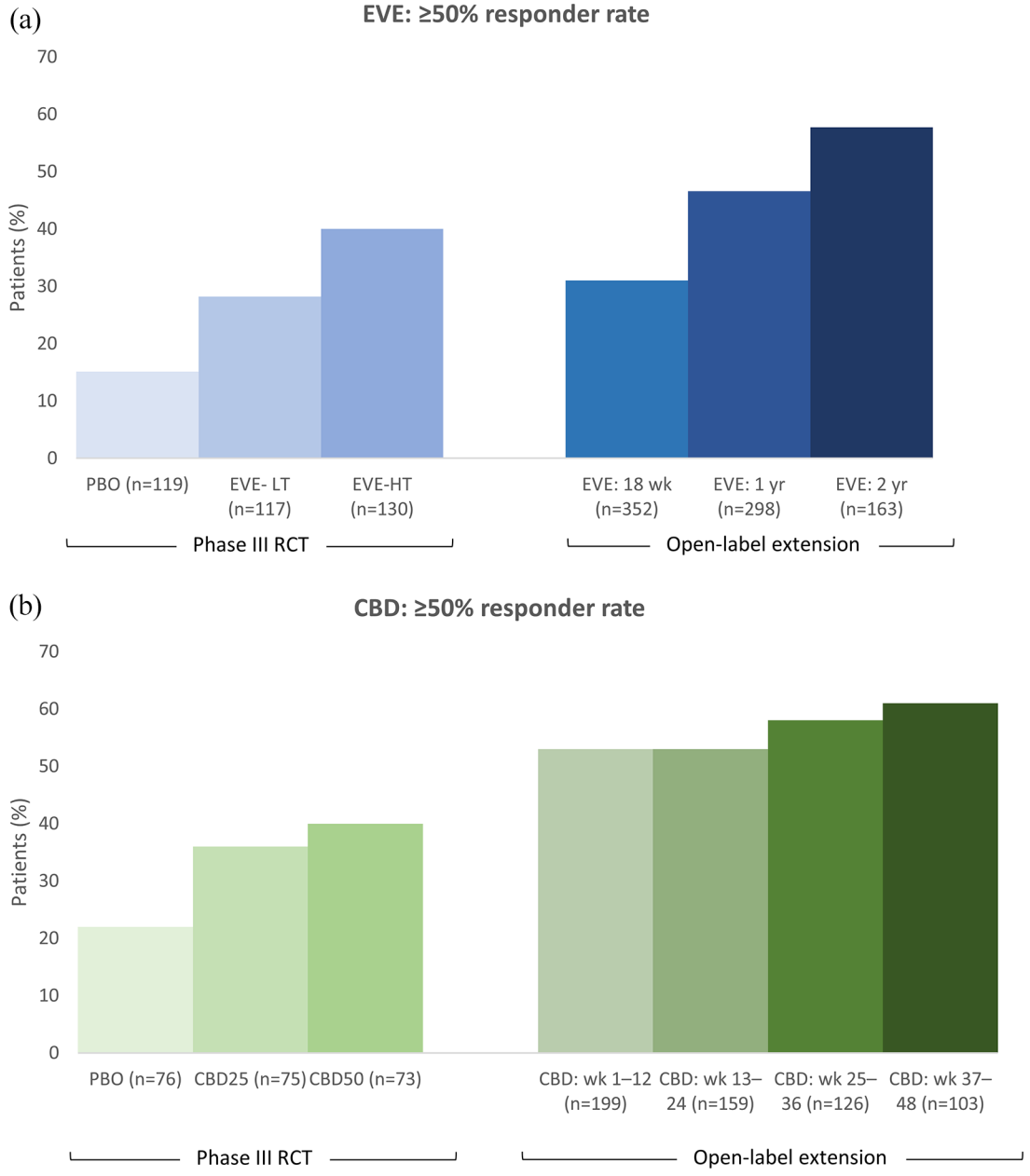

Figure 3. Responder rates for (a) EVE and (b) CBD from the pivotal RCTs and OLE studies for TSC-associated epilepsy. There are no head-to-head trials of EVE versus CBD, nor any published indirect comparisons. Therefore, any comparisons of the efficacy of the two treatments should be made with caution due to differences in baseline patient demographics and clinical characteristics in the trial populations, and a difference in the placebo effect.

CBD, cannabidiol; CBD25, CBD 25 mg/kg/day; CBD50, CBD 50 mg/kg/day; EVE, everolimus; HT, high trough (range 9–15 ng/ml); LT, low trough (range 3–7 ng/ml); OLE, open-label extension trial; RCT, randomised controlled trial; wk, week; yr, year. The recommended dosage in the OLE trial for EVE was a target trough concentration of 3–15 ng/ml (median dose intensity: 6.76 mg/m^2/day [range, 1.1–27.8]) and for CBD it was 25 mg/kg/day with titration up to 50 mg/kg/day (mean modal dose: 27 mg/kg/day).

were assigned randomly to low-exposure EVE [low trough (LT) range of 3–7 ng/ml; $n=117$ (EVE-LT group)], high-exposure EVE [high trough (HT) range of 9–15 ng/ml $n=130$ (EVE-HT group)] or placebo ($n=119$).[71] The median percentage reduction in seizure frequency was significantly higher in the EVE groups compared with placebo, and significantly higher

percentages of patients in the EVE groups achieved 50% or more reductions in seizure frequency (Table 2; Figure 3). In addition, EVE was associated with improvements in the seizure-free rate and the median number of seizure-free days versus placebo. Importantly, EVE has shown efficacy in both younger and older subgroups of children (Supplemental Table S1).[72,73]

Of the 366 patients in the core EXIST-3 study, 361 continued in an open-label extension (OLE) to determine the long-term outcomes.[75] Reductions in TSC-associated seizures were sustained with adjunctive EVE, with ⩾50% responder rates increasing over the 2-year period from 31% to 57%, and median percent reductions in seizure frequency increasing from 32% to 57%, although it should be noted that the number of evaluable patients decreased (Figure 3). New responders emerged with a longer EVE treatment duration and 50% of patients experienced persistent responses. The median number of additional seizure-free days (per 28-day period) increased from 2.5 days at week 18, to 4.32 days at 1 year, and 6.15 days at 2 years of EVE treatment, while 15/275 (5%) of patients were seizure-free over the previous 6 months at year 1 and 13/117 (11%) were seizure-free over the previous 6 months at year 2.

The efficacy of EVE for the treatment of seizures in TSC patients has also been reported in a few single-arm trials and real-world retrospective studies, with ⩾50% responder rates ranging from 27% to 100% across studies of different follow-up periods and different age ranges (including children <2 years and adults ⩾18 years),[78–86] together with increases in seizure-free days and seizure freedom rates of 7%-58% (Supplemental Table S2).[78,82–85] As with the EXIST-3 study, longitudinal studies, up to 48 months, reported increasing responder rates over time, providing further support that EVE is associated with long-term efficacy for TSC-associated seizures, with the caveat of these studies being smaller, single arm trials (Supplemental Table S2).[81,83]

TAND outcomes

Health-related QoL (HRQoL) scales were analysed as secondary endpoints in EXIST-3, with patients classified as 'responders' having the greatest mean change scores for the majority of domains across a range of HRQoL measures. However, there were low completion rates for the two self-report patient-reported outcome (PRO) measures in the older age groups, which may also have been related to the individuals' intellectual disability impeding their self-reporting.[74] In a subgroup analysis of Japanese patients in the EXIST-3 study, a positive trend towards an improvement of autism spectrum disorder symptoms, evaluated using the Pervasive Developmental Disorders Autism Society Japan Rating Scale, was observed.[73]

In contrast, two RCTs have failed to demonstrate significant differences between EVE and placebo with regard to a wide range of assessments for autism, social and communication skills, IQ, behavioural and emotional problems, sleep quality, QoL, learning and memory, visual motor and fine motor skills, executive functioning, sensory processing and other neuropsychological deficits.[87,88] However, the jury is still out on mTOR inhibitors and their potential for improving TAND outcomes: in particular, these two RCTs were conducted in older children (6 years and older) (Supplemental Table S2) whereas, to have an impact, treatment may be needed at earlier stages of brain development. Even in a study of younger children (aged 1.7–13 years), the vast majority already had severe developmental disorder at baseline, and therefore developmental impairment continued to decline, albeit possibly at a slower pace.[78] In this respect, it may not be possible to reverse or significantly delay developmental impairment that is already present, but rather a strategy of prevention before onset may be required (Figure 1).[78]

Safety

The most common AEs in the EVE groups included stomatitis, diarrhoea, nasopharyngitis, pyrexia and upper respiratory tract infection, although nasopharyngitis and upper respiratory tract infection were equally common in the placebo group.[71] AEs leading to discontinuations were rare (⩽5%), with stomatitis being the most common reason. In the OLE, the safety profile was generally consistent with that reported in the core study.[75] However, there were two deaths due to pneumonia and septic shock that were suspected to be treatment related. Indeed, EVE has immunosuppressive properties and may therefore predispose patients to infections. Despite this concern, EVE has generally been found to be well tolerated across studies, with the majority of AEs

being mild to moderate, including in younger patients.[72,82,89]

Since the treatment of TSC usually requires polypharmacy, possible interactions with other ASDs and other medications are also important aspects to be taken into consideration. EVE was found to have no effect on pre-dose concentrations of CYP3A4 substrate ASDs such as clonazepam, diazepam, felbamate and zonisamide (ZNS), but has been associated with small (approximately 10%) increases in pre-dose concentrations of the ASDs CBZ, clobazam (CLB) and the CLB metabolite N-desmethylclobazam; these increases may not be clinically significant although dose adjustments for ASDs with a narrow therapeutic index, e.g. CBZ, may be considered.[68,69] A clinically significant drug–drug interaction has been reported between EVE and cannabidiol (CBD),[90,91] as described in more detail in the CBD section below.

Cannabidiol

Evidence suggests that CBD exerts its anti-convulsive actions through multiple mechanisms including modulation of intracellular calcium and adenosine-mediated signalling,[92] although the understanding of the molecular mechanisms underlying these processes is still in its infancy. Regarding TSC, data from a zebrafish model of the disease showed that CBD was associated with modulation of rpS6, a downstream target of the mTOR pathway, in the brain. In this respect, *in vitro* and *in vivo* studies of other disease models including multiple sclerosis, Parkinson's disease, schizophrenia and cancer have also suggested a role of CBD in modulating the mTOR pathway.[93–97] However, CBD appears to have contrasting effects in different environments (i.e. upregulation of the mTOR pathway in some studies and downregulation in others), and more research is needed to further our understanding.[93–97] This research is particularly pertinent given that, despite this potential role of CBD in mediating the mTOR pathway, preliminary evidence suggests that CBD treatment at a therapeutic dose for refractory epilepsy does not decrease the volume of SEGAs or AMLs in TSC patients, in contrast with mTOR inhibitors.[98]

In addition to its prior indications for Dravet syndrome (DS) and LGS, CBD has recently gained approval by the FDA and the EMA for the treatment of seizures associated with TSC that includes patients 1 year of age and older in the US and 2 years of age and older in the EU.[99,100] Of note, CBD is indicated in the EU in conjunction with CLB for DS and LGS, but is licensed without CLB for TSC.[100]

Efficacy for TSC-associated seizures

The efficacy and safety of add-on CBD has been evaluated in a phase III RCT (GWPCARE6 [ClinicalTrials.gov identifier: NCT0254476]) conducted in 224 patients with drug-resistant epilepsy associated with TSC, whereby patients were randomised to CBD 25 mg/kg/day (CBD25; $n=75$) and 50 mg/kg/day (CBD50; $n=73$) or placebo ($n=76$) (Table 2).[76] Compared with placebo, CBD was associated with a significantly greater reduction in the percentage change from baseline in the frequency of TSC-associated seizures (Table 2). Higher percentages of patients in the CBD groups achieved a ⩾50% reduction in seizures versus placebo (Table 2; Figure 3). CBD was also associated with a significantly greater reduction in total seizure frequency compared with placebo. In addition, during the 12-week maintenance period, the CBD groups had mean gains of additional seizure-free days over placebo. Furthermore, improvement in overall condition, evaluated using the subject/caregiver global impression of change (S/CGIC), was reported by more patients/caregiver in the CBD groups than with placebo (Table 2). Findings from a *post hoc* analysis suggested that the onset of the treatment effect occurred early, within the first 2 weeks.[101]

Longer-term adjunctive CBD treatment has been evaluated in an OLE to the GWPCARE6 study, involving 199 of the 201 patients who completed the RCT.[77] Reductions in seizures were maintained through 48 weeks, at least 6% of patients remained seizure free during the 12-week windows, and improvement in S/CGIC continued to be reported by a high proportion of proportion of patients/caregivers (Table 2; Figure 3).

Safety

The safety profile of the 25 mg/kg/day dose was found to be superior to 50 mg/kg/day; as such the recommended starting dose for TSC patients is 5 mg/kg/day, which can be increased as tolerated to a maintenance dose of 25 mg/kg/day.[99] The most common AEs included diarrhoea, decreased

appetite and somnolence.[76] Treatment discontinuations due to an AE were reported in 11% patients in the CBD25 group. Elevated liver transaminases occurred in 9 (12%) patients in the CBD25 group, with the vast majority (81%) of patients with elevations being on concomitant valproate (VPA). A *post hoc* analysis reported that AEs lasted longer for CBD versus placebo but resolved within the 16-week study in most patients.[101] Furthermore, results from a real-world study have suggested that slow titration of CBD can deliver improved tolerance with comparable efficacy.[102] The AE profile over the long-term in the OLE was similar to that observed previously.[77]

With regard to drug–drug interactions, it has been observed that CBD results in increased serum levels of the mTOR inhibitors EVE and sirolimus, requiring reduced mTOR inhibitor dosing to avoid potentially serious AEs.[90,91] Also of note is that the risk of transaminase elevations may be increased with concomitant use of VPA and higher doses of CBD, whereby CBD and/or VPA should be reduced or discontinued if clinically significant increases of transaminases occur.[99] In addition, the interaction with CLB, which leads to increased levels of its active metabolite N-desmethylclobazam, may result in increased somnolence and sedation, requiring a dose reduction of CLB.[99] While increases in serum levels of some other ASDs, including TPM, rufinamide (in adults and children), ZNS and eslicarbazepine (in adults) have been reported with increasing doses of CBD, they were within the accepted therapeutic range.[103] Increases in BRV levels in five patients that led to mild AEs in two patients and a reduction of BRV in one patient have also been reported in a small case series.[104]

Non-pharmacologic agents

Ketogenic diet and medium-chain triglycerides

The ketogenic diet (KD) is a low carbohydrate, high-fat diet that is designed to mimic the physiological process of fasting whereby the liver produces ketones as an alternative energy source for the brain. Although not fully understood, various mechanisms have been implicated in the role of the KD in treating refractory epilepsy.[105,106] In the 'classic' KD (a strict diet that can be difficult to adhere to, with very low carbohydrate content and 60–80% of dietary energy provided by long-chain fats) ketone bodies are believed to have a central role.[105,106] However, for the more popular, less strict medium-chain triglyceride (MCT) KD, that comprises of the triglycerides heptanoic acid, octanoic acid and decanoic acid, additional key mechanisms of action that are independent of ketones have been reported.[105,106] *In vivo* studies have provided evidence that decanoic acid can directly and selectively inhibits AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors to reduce neuronal excitability, binds PPARγ (peroxisome proliferator-activated receptor gamma) involved in mitochondrial biogenesis and, of particular relevance for TSC, inhibits mTORC1 activity.[107–109] Other studies have also demonstrated that the KD can attenuate mTOR signalling pathways,[110,111] providing a biological basis for its demonstrated efficacy in controlling seizures in TSC patients. In studies in patients with TSC, the KD is associated with ⩾50% response rates of 68%–83.3%, and seizure-free rates of 33–42% across studies over the short term (3–5 months).[112–114] In addition, Youn et al. further demonstrated the long-term efficacy of the KD but only in a proportion of patients, with the number of patients with a >50% response decreasing over time from 58.1% at 6 months to 32.3% at 24 months.[114] Importantly, the KD has also been associated with improvements in cognition and behaviour,[113] although further studies are needed to confirm this observation. Overall, the consensus TSC guidelines from Europe recommend that the KD be considered in early infancy and early childhood when surgery is not an option.[17] The guidelines from the International Ketogenic Diet Study Group from 2018 also propose that the KD be initiated early in the TSC treatment pathway.[115] With this in mind, the KD has been reported to be effective and well tolerated in very young infants with refractory epilepsy, including in infants maintaining a breast milk diet.[116]

Vagus nerve stimulation

The consensus TSC guidelines from Europe recommend that vagus nerve stimulation (VNS) – a procedure that involves stimulating the vagus nerve with electrical impulses – be considered in combination with the KD or in cases where the KD is not acceptable.[17] Responder rates (⩾50% reductions in seizures) have been reported in

50%–92% of TSC patients, although these results are from retrospective studies with a small population of patients.[117–120] Seizure freedom was rarely reported. VNS may also have a positive impact on level of functioning,[118] adaptive behaviours and QoL, particularly in patients who had the implantation in childhood.[120] Of note, while VNS is used for a wide range of drug-resistant epilepsies, TSC has been associated with a better response to VNS.[121,122]

Surgery

International, European and United Kingdom (UK) guidelines recommend that epilepsy surgery be considered in medically refractory TSC patients, with early intervention increasing the probability of seizure-freedom.[16–18] Patients need to be carefully selected following a risk–benefit assessment requiring extensive pre-surgical evaluations by a team of epilepsy surgery experts (epileptologists, neurosurgeons, neuroradiologists and neuropsychologists).[123] While studies have demonstrated the long-term benefits of epilepsy surgery in a significant proportion of selected TSC patients,[124–126] it has traditionally been underutilised due to the potential risk of severe complications including infection and neurocognitive side effects. These complications in the modern era are rare because of the extensive pre-surgical evaluations to determine eligible patients[127,128]; however, the multifocal nature of epileptogenic tubers and/or their location in deep brain structures excludes many patients. However, novel techniques are being developed, with the potential to expand the number of eligible patients, while reducing the risk of complications.[129,130] For example, magnetic resonance imaging (MRI)-guided laser interstitial thermal therapy (MRg-LITT) is a minimally invasive technique that uses heat emitted from a laser device, while the MRI enables real-time accurate monitoring of the thermal ablation process. Initial experiences of using MRg-LITT to identify and treat cortical tubers responsible for clinical seizures in TSC patients have reported seizure freedom in two out of three,[131] and three out of seven TSC patients.[132] Improvements in neuropsychiatric symptoms were also reported.[132] In addition, Hooten et al. reported a novel method that involved a frameless stereotaxy with the aim of expanding MRg-LITT to younger patients, with a successful application of this technique in a 6-month-old infant with TSC.[133]

Potential future therapies

There are currently a handful of therapies in various phases of clinical and preclinical development for the treatment of TSC-associated seizures.

Ganaxolone is a positive allosteric modulator of gamma aminobutyric acid A (GABA-A) receptors that is being developed for various rare genetic epilepsy syndromes and treatment of status epilepticus. A phase III trial has recently met its primary endpoint in CDKL5 deficiency disorder, with a significant reduction in median 28-day major motor seizure frequency compared with placebo.[134] The results of its phase II trial in TSC are anticipated to be reported in mid-2021.[135]

Soticlestat (TAK-935/OV935) is a highly selective inhibitor of the enzyme cholesterol 24-hydroxylase (CH24H) that is being developed for various developmental and epileptic encephalopathies (DEEs). Its key phase II study, ELEKTRA, met its primary endpoint in reducing seizure frequency in children with DS and LGS.[136] Soticlestat may have application in TSC patients, although it is not clear if trials in this specific population are planned.

Similarly, the clinical development of fenfluramine (FFA) has to date focussed mainly on the treatment of seizures associated with DS and LGS, with approval for the treatment of seizures associated with DS in the US and the EU in mid and late 2020, respectively.[137] A phase II 'basket' clinical trial in multiple rare epilepsies including TSC is planned for 2021.[138]

Also of interest is a phase I/II clinical trial (STOP2) that started in September 2020 to evaluate the mTOR inhibitor sirolimus for the prevention or delay of seizure onset in TSC infants, although the estimated primary completion is not due for a while yet (September 2022).[139]

Basic research is still hugely important for future translational directions in TSC. For example, reductions in neuronal ciliation have recently been implicated in the pathogenesis of TSC, and inhibitors of heat shock protein 90 (Hsp90) were identified as being able to interfere with this action, which could have relevance for future therapeutics.[140]

Of course, in light of TSC being a monogenic disease, gene therapy represents the 'holy grail' for

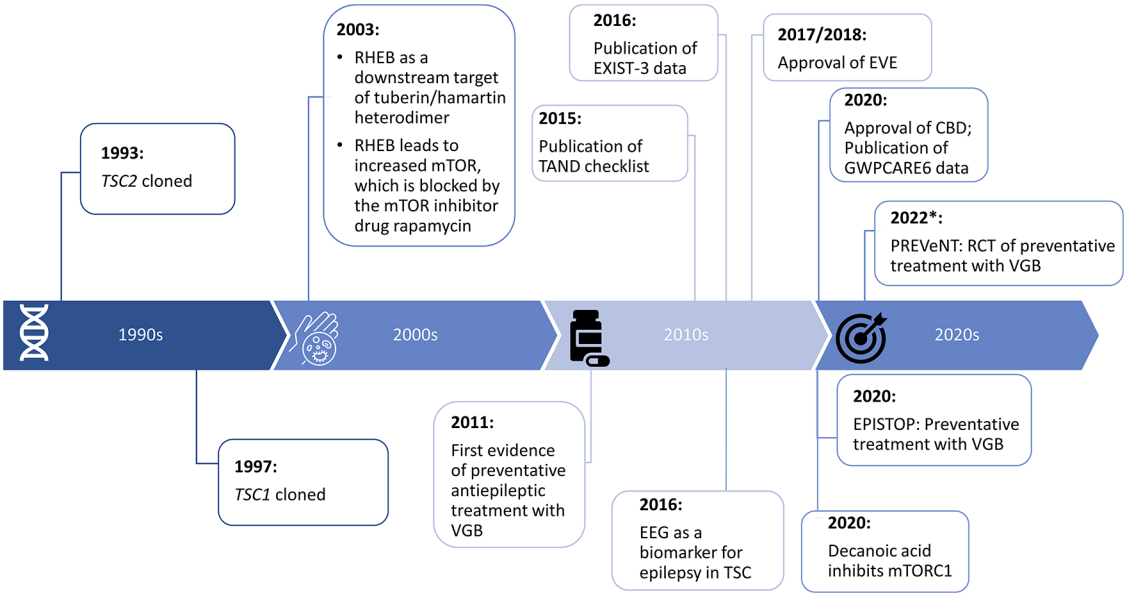

Figure 4. Key milestones towards precision medicine for the amelioration and prevention of TSC-associated epilepsy and TAND. *PREVeNT; Estimated primary completion date of May 2022, and study completion date of December 2022.[64]

CBD, cannabidiol; EVE, everolimus; RHEB, Ras homolog enriched in brain; mTOR, mammalian/mechanistic target of rapamycin; RCT, randomised controlled trial; TAND, TSC-associated neuropsychiatric disorders; TSC, tuberous sclerosis complex; VGB, vigabatrin.

patients, but with progress to date being confined to animal models,[141] its application is still experimental with many challenges remaining.

Conclusions

While there has been an awareness surrounding TSC for over 200 years, the genes responsible for this syndrome were not identified until the 1990s (Figure 4) – a breakthrough that furthered the understanding of the mechanisms of the action of the respective proteins. Seminal studies in the early 2000s showed that RHEB was a downstream target of the tuberin/hamartin heterodimer and an upstream regulator of mTOR-mediated signalling,[142] work that continues to be further refined and explored to this day. These discoveries have now been translated into establishing targeted treatments, culminating in the approval of the mTOR inhibitor EVE in 2017/2018. In addition, the newly approved CBD and the already established KD/MCT diet may also have a role in the mTOR pathway, in addition to other putative mechanisms. It is now hoped that the 2020s and beyond will see the benefits of precision treatments consolidated and, in particular, progress investigating their utility for preventing or modifying epileptogenesis may be seen (Figure 4).

The approval of EVE represents an important milestone for the treatment of TSC-associated seizures (Figure 4), especially given the recent data showing its longer-term efficacy, with new responders emerging over time, while 50% of patients experienced sustained responses over 2 years of treatment[75]; this is in contrast to VGB, which appears to lose efficacy within a year of treatment initiation.[56] In addition, EVE has shown efficacy across a range of TSC manifestations including SEGA, renal AML, skin lesions and cardiac rhabdomyoma, bringing the idea of having a single multi-system treatment for TSC closer.[67] However, the jury is still out on the benefits to TAND outcomes, which may require earlier treatment with EVE.

CBD is also a welcome addition to the treatment armamentarium. Evidence suggests that CBD has multiple cellular targets,[143] and while further studies are required to elucidate the key mechanisms through which it exerts its anti-seizure properties, including its potential role in the mTOR pathway, it has clearly demonstrated efficacy in a proportion of TSC patients with significant reductions in TSC-associated seizure compared with placebo, and ⩾50% responder rates of 53%–61% through 48 weeks of treatment in the OLE (Figure 3, Table 2).

It is also of interest that the KD/ MCT diet, with its long-established application in the treatment of retractable epilepsies, may also inhibit mTOR pathway signalling, suggesting that the KD/MCT diet could be a useful early disease-modifying treatment. There is now overwhelming evidence regarding the feasibility and effectiveness of the KD in infants as young as 3 weeks old[116,144,145]; a recent meta-analysis in infants less than 2 years of age with drug-resistant epilepsy reported ⩾50% responder rates of 59%, while 33% of infants attained seizure freedom.[146] A limitation of the KD, however, especially in older children and adults, is that it is difficult to maintain; the recent evidence that decanoic acid reduces mTORC1 activity in model systems, including astrocytes derived from TSC patients, suggests that a more sustainable diet rich in decanoic acid may be able to produce similar results to the KD, with better compliance.[109]

The EPISTOP trial should hopefully pave the way for evaluating targeted treatments that address the underlying pathophysiology of TSC in a preventative capacity, potentially including EVE and CBD. To date, EVE and CBD have been evaluated only in a conventional setting, while it is certainly valid to hypothesise that prophylactic treatment with these treatments, especially EVE, will be even more favourable than VGB due to the targeted mechanism of action, strengthened by data from some animal studies showing the benefits of early treatment with mTOR inhibitors.[147] However, evidence from TSC mouse models has shown that prenatal treatment with mTOR inhibitors may have concerning side-effects including negatively impacting development and neurological symptoms including learning and memory tasks, together with poor birth weight/weight gain.[148,149] Given the potential wide-ranging consequences of inhibiting mTOR signalling during foetal development, caution is needed in designing preventative studies using mTOR inhibitors to take into account the safety implications.[23,150,151] On the other hand, a case report has described less than optimal outcomes from early treatment with VGB and EVE that may not have been early enough.[152] Indeed, the jury is still out as to whether the results from EPISTOP can be replicated easily in a real-world situation with regard to pin-pointing what the authors called 'the point of no return' on the EEG.

Overall, it may also be important to further refine the window of opportunity, as the time between detecting epileptiform EEG activity and the onset of seizures postnatally may be minimal but prenatal treatment with mTOR inhibitors may have safety concerns; identifying additional biomarkers may be useful in this respect. Further research is also needed into elucidating the fundamental molecular mechanisms in TSC, as well as the mechanisms of epileptogenesis, which may translate into identifying new disease-modifying treatments. Overall, due to advances in understanding the molecular genetics and pathophysiology, TSC represents a prototypic clinical disorder for studying epileptogenesis and the impact of precision medicine.

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Author contributions

Both authors reviewed the literature, drafted the manuscript, generated the figures, and assume full responsibility for the final publication.

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Supplemental material

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