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Original Article

# Long-Term Efficacy and Safety of Mammalian Target of Rapamycin Inhibitor Treatment for Subependymal Giant Cell Astrocytoma in Tuberous Sclerosis

Lena-Luise Becker, MD [a], Karen Agricola, MSN, FNP [b, c], David M. Ritter, MD, PhD [b, c], Marguerite M. Caré, MD [d, e], Darcy A. Krueger, MD, PhD [b, c], David Neal Franz, MD [b, c, *]

[a] *Department of Pediatric Neurology, Center for Chronically Sick Children, Institute of Cell Biology and Neurobiology, Charité—Universitätsmedizin Berlin, Berlin, Germany*
[b] *Division of Neurology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio*
[c] *Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, Ohio*
[d] *Department of Radiology and Medical Imaging, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio*
[e] *Department of Radiology, University of Cincinnati College of Medicine, Cincinnati, Ohio*

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ABSTRACT

*Background:* Subependymal giant cell astrocytoma (SEGA) is a low-grade astrocytoma occurring in approximately 5%-20% of persons affected with tuberous sclerosis complex (TSC). Therapy options include surgical resection or medical treatment with mammalian target of rapamycin (mTOR) inhibitors ([mTORis] everolimus or sirolimus) to prevent further growth and neurosurgical interventions due to life-threatening hydrocephalus. Short-term follow-up of mTORi treatment has been described in earlier studies; however, data on long-term efficacy and safety are limited.
*Methods:* Patients from SEGA studies at Cincinnati Children's Hospital Medical Center with ongoing care at our center and consented to ongoing retrospective records collection were included in the study. Data was compiled from medical records.
*Results:* Our cohort consisted of 25 patients with TSC. All but one were currently receiving mTORi treatment with either sirolimus (N = 4, 20%) or everolimus (N = 20, 80%) at the time of evaluation. Median mTORi treatment duration was 15.5 years (range: 9.8-16.8 years, interquartile range: 0.9 years). One patient underwent SEGA resection after starting mTORi. None required cerebrospinal fluid diversion. In 19 patients (76%), SEGA was stable or decreased over time. In six patients, it increased slightly from pretreatment baseline. Ongoing adverse drug reactions (ADR) at their last visit were reported in the medical record of eight patients (33%) with intermittent aphthous ulcers being the most frequent (N = 7). Long-term ADRs were observed in 17 patients (68%), including hypercholesterolemia (N = 10), diabetes mellitus type 2 (N = 6), prediabetes (N = 1), hypertension (N = 6), and osteoporosis (N = 3).
*Conclusions:* Long-term TSC-associated SEGA treatment with mTORi is safe, effective and well tolerated. Knowledge of adverse reactions is important for successful long-term therapy.


## Introduction

Tuberous sclerosis complex (TSC) is a rare autosomal dominant disease caused by identifiable mutations in either the *TSC1* (hamartin) or *TSC2* (tuberin) gene in 85% of patients.[1] The proteins hamartin and tuberin form a dimer that works through Rheb (Ras homolog enhanced in brain) to limit the activation of the mammalian target of rapamycin (mTOR) complex 1.[2] Pathogenic loss-of-function variants in either *TSC1* or *TSC2* thereby produce a constitutive upregulation of mTOR causing abnormal cellular

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* Communications should be addressed to: Dr. Franz; Neurology/MLC 2015, 3333 Burnet Avenue, Cincinnati, OH 45229.

*E-mail address:* david.franz@cchmc.org (D.N. Franz).

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growth and proliferation. In 10%-15% of patients no mutation is identified using conventional genetic testing.[1] Clinically this leads to the characteristic benign tumors and hamartomas of TSC, affecting multiple organ systems including the brain, kidneys, and skin.[2] In the brain, subependymal giant cell astrocytomas (SEGAs) are present in 5%-20% of patients with TSC, being almost exclusively present in patients with *TSC2* variants.[1,3,4] Although the definition of a SEGA is not consistent, in general a SEGA is defined when a growth >1 cm is documented.[5,6] SEGAs frequently arise near the foramen of Monro and are thereby associated with a high risk of cerebrospinal fluid obstruction and hydrocephalus.[7] SEGAs do not regress spontaneously but exhibit progressive growth over time.[3,7] Therefore, treatments options included early neurosurgical resection in nearly all patients and/or cerebrospinal fluid diversion including ventriculoperitoneal (VP) shunt placement.[1,8,9] mTOR inhibitor (mTORi) treatment with everolimus has been approved for SEGAs in patients for whom surgical resection is not thought to be curative. mTORi treatment has demonstrated a reduction in SEGA volume in multiple studies.[3,10]

Besides SEGAs, other neurological phenotypes are associated with TSC.[1] Epilepsy is present in approximately 63%-84% patients with TSC with onset mostly <2 years (TOSCA registry: 78%) and can be treated with mTORis.[1,11] TSC-associated neuropsychiatric disorder (TAND) is present in >90% of patients and includes a spectrum of symptoms including intellectual disability, autism, learning disabilities, and psychiatric disorders among others.[1,11,12] Catastrophic hydrocephalus from SEGAs can worsen these neurological phenotypes, and there are little data about how treatment of these TSC symptoms interact with long-term mTORi treatment.

Short-term adverse drug reactions (ADRs) with mTORi treatment are well known and include aphthous ulcers, stomatitis, fever, upper respiratory infections, and dyslipidemia/hypercholesterolemia.[8,13] Data exist regarding the long-term use of mTORis in transplant and oncology patients. However, long-term effects (>10 years) of mTORis in patients with TSC have not been reported.

We conducted a retrospective study on patients with TSC from two initial mTORi treatment studies for SEGA from our TSC center[3,14] providing long-term efficacy and safety data of mTORi treatment for SEGA in TSC.

## Methods

We performed a retrospective study of all patients who participated in clinical trials of mTORi for SEGA at our center with a minimum follow-up of 10 years. These studies were completed and results published previously.[3,5] All patients received magnetic resonance imaging (MRI) to monitor SEGA size every 0.5-3 years depending on SEGA progression. Progression of SEGA was analyzed by the neuroradiologist at Cincinnati Children's Hospital Medical Center (CCHMC). Two of five from the Franz et al. trial and 23 of 28 from the Krueger et al., 2010, trial were still under care at CCHMC. One study patient passed away in 2012 and was, therefore, excluded from this study. The cause of death was identified as sudden unexplained death in epilepsy and judged not to be related to mTORi treatment or SEGA progression. The other patients were lost to follow-up and therefore not included. Data were compiled from medical records. Drug levels measured <1 ng/mL in three patients were used in further calculations as 1 ng/mL. Statistical analysis was performed with R version 4.2.1 using the packages *psych, crosstable, dplyr,* and *resphape2*. The retrospective study data were collected under a repository that was approved by the Institutional Review Board (CCHMC IRB 2012-2317). Written informed consent/assent was obtained from the patient or guardian before data collection in the repository.

## Results

### Cohort

The cohort consisted of 25 patients with clinically diagnosed TSC (male: n = 13 of 25, 52%; female: n = 12 of 25, 48%) with a current age at last visit of a median of 26.0 years (range: 20.0 to 49.0 years, interquartile range [IQR]: 10.3 years; Table 1). In 14 of 25 patients (56%), a variant in *TSC1* (n = 2 of 25, 8%) or *TSC2* (n = 12 of 25, 48%) was identified. In the other 12 patients, no genetic testing was performed. All patients showed other features of TSC at their last visit (Table 1).

### mTORi treatment

Everolimus (n = 23 of 25, 92%)[3] or sirolimus (n = 2 of 25, 8%)[14] treatment was started at a median age of 11.0 years (range: 3.0-34.0 years, IQR: 11.0 years). Twenty-four patients were receiving ongoing mTORi treatment with either sirolimus (n = 4 of 25, 16%) or everolimus (n = 20 of 25, 80%), resulting in a median mTORi treatment duration of 15.7 years (range: 9.8-18.8 years, IQR: 0.92 years) and a retention rate of 96%. One patient discontinued treatment due to mouth sores for three months but resumed afterward due to growth of angiomyolipomas and the SEGA. One patient discontinued treatment after 13.2 treatment-years due to worsening acne under treatment and has not restarted treatment; no SEGA progression was noted. The current median daily sirolimus dose was 1 mg/day (range: 1-2 mg/day, IQR: 0.25 mg/day) resulting in median serum levels of 3.4 ng/mL (range: 1.6-4.9 ng/mL, IQR: 2.1 ng/mL). The current median daily everolimus dose was 7.5 mg/day (range: 2.5-30 mg/day, IQR: 5.6 mg/day) resulting in median serum levels of 7.2 ng/mL (n = 16, range: 1-25.5 ng/mL, IQR: 7.6 ng/mL).

### Adverse drug reactions

Ongoing, intermittent acute ADRs were reported in eight of 25 patients (33%) with mouth sores being the most frequent in seven patients (29%). One patient reported being tired and having abdominal cramping (Table 2). There were no opportunistic infections reported. One was diagnosed with skin melanoma *in situ* nine years after initiation of mTORis with no recurrence after surgical excision. Chronic ADRs were observed in 17 of 25 patients (68%) and none in eight of 25 patients (32%). The most frequent chronic ADR was hypercholesterolemia/hyperlipidemia reported in 10 of 25 patients (40%) with a first diagnosis after a median mTORi treatment of 8.7 years (range: 2-16 years, IQR: 6.0 years), with nine (38%) taking statin medications. In six of 25 patients (24%), diabetes mellitus type 2 (DM) was present with a first diagnosis after a median mTORi treatment of 11.3 years (range: 1.5-14.8 years, IQR: 9.7 years) and needing insulin treatment in two of 25 patients (8%), metformin in three of 25 patients (12%), and management on carbohydrate-restricted diet in one patient. In one patient, prediabetes was present. Osteoporosis was present in three of 25 patients (12%) with a first diagnosis after a median mTORi treatment of 12.1 years (range: 11.3-14.2 years, IQR: 1.4 years) with all being treated with alendronate. Hypertension was present in six of 25 patients (24%) with a first diagnosis after a median mTORi treatment of 9.0 years (range: 1.3 to 12.7 years, IQR: 4.2 years) with four patients (16%) taking medication. The median body mass index (BMI) ($kg/m^2$) was 25 $kg/m^2$ (range: 16.1-41.0 $kg/m^2$, IQR: 7.9 $kg/m^2$) with 12 of 25 patients (48%) having normal BMI, one patient (4%) being underweight, five of 25 patients (20%) being overweight, and seven of 25 patients (28%) being obese. There was no

**TABLE 1.**
Cohort Characteristics

| Cohort | N = 25 |
|---|---|
| Gender | |
| Male | 13 (52%) |
| Female | 12 (48%) |
| Race | |
| White | 23 (92%) |
| Black | 2 (8%) |
| Current age | 26 years (20-49, 11) |
| Age mTORi start | 11 years (3-34, 11) |
| Ongoing mTORi treatment | |
| Everolimus | 20 (80%) |
| Sirolimus | 4 (16%) |
| No | 1 (4%) |
| Total duration mTORi | 15.6 years (9.8-16.8, 0.9) |
| Current organ involvement | |
| Brain | |
| SEGA | 25 (100%) |
| Size increase | 6 (24%) |
| Size stable/decrease | 19 (76%) |
| Nodules | 25 (100%) |
| Tuber | 25 (100%) |
| History of epilepsy diagnoses | 25 (100%) |
| Ongoing seizures | 9 (36%) |
| Kidney | |
| Angiomyolipomas | 18 (72%) |
| Cysts | 12 (48%) |
| Renal insufficiency | |
| Grade I | 17 (68%) |
| Grade III | 1 (4%) |
| Surgery | |
| Embolization | 2 (8%) |
| Partial nephrectomy | 1 (4%) |
| Heart | |
| History of rhabdomyomas | 9 (36%) |
| With arrhythmias/AV block | 2 (8%) |
| With heart insufficiency | 2 (8%) |
| Lung | |
| LAM | 3 (12%) |
| MMPH | 1 (4%) |
| Single nodule | 1 (4%) |
| TAND | |
| Overall | 23 (92%) |
| Low average IQ | 3 (12%) |
| Intellectual disability | 20 (83%) |
| Autism | 7 (28%) |
| OCD | 8 (32%) |
| Anxiety/agitation | 11 (46%) |
| PTSD | 1 (4%) |
| Depression | 2 (8%) |
| Sleeping problems | 3 (12%) |

Abbreviations:
AML = Angiomyolipoma
AV = Atrioventricular
IQ = Intelligence quotient
IQR = Interquartile range
LAM = Lymphangioleiomyomatosis
MMPH = Multifocal multinodular pneumocyte hyperplasia
mTORi = Mammalian target of rapamycin inhibitor
OCD = Obsessive-compulsive disorder
PTSD = Post-traumatic stress disorder
SEGA = Subependymal giant cell astrocytoma
TAND = Tuberous sclerosis complex–associated neuropsychiatric disorder
Absolute and relative frequencies are shown in absolute number (percentage). Descriptive statistics are shown in median years (range: minimum-maximum years, IQR years).

correlation between an increased BMI (overweight/obese) and the occurrence of chronic ADR (chi-square test: $P = 0.411$).

### Long-term efficacy on SEGA

The short-term effect of mTORi treatment in SEGA has been reported in previous studies.[3,14] Long-term representative MRI follow-up images are shown in Figure. All patients had a current SEGA on their last MRI. Before mTORi treatment, two of 25 (8%) patients had VP shunt surgery to treat hydrocephalus due to SEGA obstruction and three patients had SEGA reduction surgery. In the long-term follow-up, one patient (P11) with an increase in SEGA volume leading to increased lateral ventricles underwent two elective debulking procedures 12 years after mTORi and 14 years after mTORi initiation (Fig Y). Laser interstitial thermal therapy was utilized; the patient tolerated both procedures without complication or difficulty. Before initiation of mTORi, this patient had SEGA resection for acute hydrocephalus, which subsequently led to the initial diagnosis of TSC.

Surgical pathology was available in two patients: one had a pilocytic astrocytoma before mTORi treatment and the other (P11) had a typical SEGA. In five of 25 patients (21%), the SEGA increased slightly, and in one patient, significantly (4%). In the other 19 of 25 patients (76%), the SEGA was either stable or decreased further over time. Of the six patients with increased SEGA size, two were taking sirolimus (P8: 1 mg/d, last level 4.2 ng/mL, P15: 1 mg/d, last level: 1.6 ng/mL) and three were taking everolimus (P11: 5 mg/d, last level: 3.8 ng/mL; P16: 2.5 mg/d, last level: <1 ng/mL; P23: 7.5 mg/d, last level: 16.1 ng/mL). Owing to low number in both groups, no statistical analysis was performed.

**TABLE 2.**
Adverse Drug Reactions

| | | |
|---|---|---|
| Acute ADRs | None | 16 (67%) |
| | Mouth sores | 7 (29%) |
| | Tiredness | 1 (4%) |
| | Acne | 1 (4%) |
| | Abdominal cramping | 1 (4%) |
| Chronic ADRs | None | 8 (32%) |
| | Hypercholesterolemia/hyperlipidemia | 10 (40%) |
| | Diabetes mellitus type 2 | 6 (24%) |
| | Prediabetes | 1 (4%) |
| | Osteoporosis* | 3 (13%) |
| | Hypertension† | 6 (24%) |

Abbreviations:
ADR = Adverse drug reaction
BMI = Body mass index
mTORi = Mammalian target of rapamycin inhibitor
* All patients were taking antiseizure medication in parallel (P2: vigabatrin and lacosamide; P14: eslicarbazepine and cenobamate; P21: cannabidiol, felbamate, and zonisamide); therefore no clear association to the mTORi can be made.
† In all but two patients (P11: 20 years, BMI 20.6 kg/m$^2$; 23 years, BMI 24.2 kg/m$^2$) with hypertension, a coexisting renal disorder was present; therefore no clear association to the mTORi can be made.

### Epilepsy

Additional neurological symptoms were seen in most patients (Table 1). Epilepsy was reported in all patients; however, only nine of 25 patients (38%) reported seizures within six months of study evaluation. Four of 25 patients (16%) reported daily seizures, two of 25 patients (8%) reported weekly seizures, and three of 25 patients (12%) had monthly seizures. Last described seizure types were heterogeneous, mostly focal (n = 3 of 25, 12%, without further information), focal without impaired awareness (motor: myoclonic: n = 2, nonmotor: sensory: n = 2, emotional: n = 1), and focal with impaired awareness (atonic: n = 1, tonic: n = 4, myoclonic: n = 2, behavior arrest: n = 9). Four had focal seizures with rapid secondary generalization to tonic-clonic seizures. In two patients, the current seizure type was described as primarily generalized tonic-clonic seizures. The median number of current antiseizure medications (ASMs) was 1 (range: 0-4, IQR: 1), with two patients not taking any ASM. Current ASMs reported were lamotrigine (n = 8 of 25, 32%), lacosamide (n = 5 of 25, 20%), eslicarbazepine (n = 3 of 25,

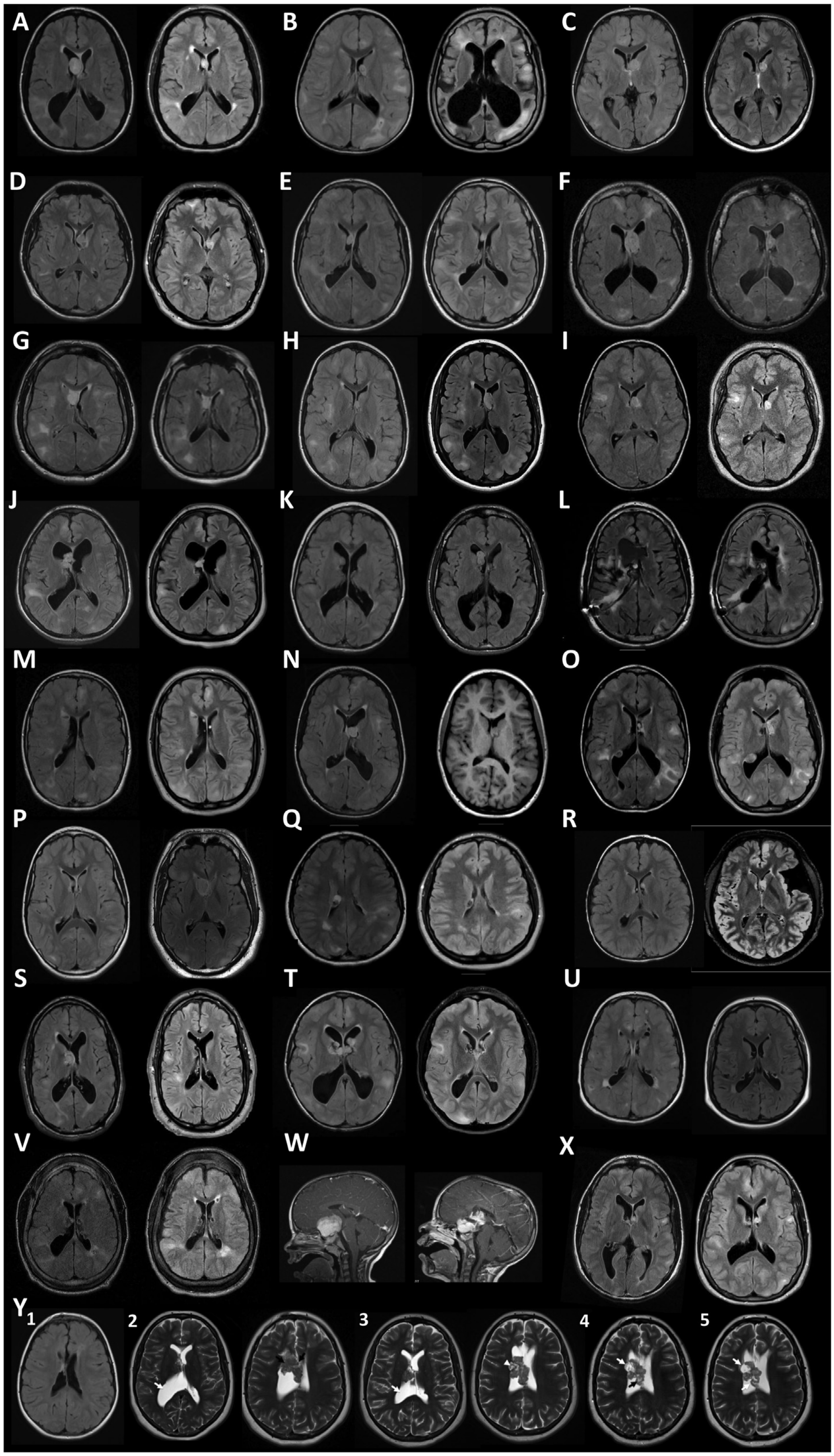


**FIGURE.** Long-term magnetic resonance imaging (MRI) follow-up. All images show a baseline MRI before treatment initiation for each patient, unless otherwise clarified, and the last follow-up MRI, all within the last two years of the last follow-up appointment. All images shown are fluid-attenuated inversion recovery (FLAIR) sequences, unless otherwise stated. MRI (A) of patient P1, (B) of patient P2 with additional severe brain atrophy, (C) of patient P3, (D) of patient P4, (E) of patient P5, (F) of patient P6, (G) of patient P7, (I) of patient P9, (J) of patient P10, (L) of patient P13 with ventriculoperitoneal (VP) shunt, (M) of patient P14, (N) of patient P15, (Q) of patient P18 (second image reformatted [RFMT] sequence), (R) of patient P19 with newly developed arachnoid cyst, (S) of patient P20, (T) of patient P21, (U) of patient P22, (V) of patient P23, and (X) of patient P25 showing a decreased or stable subependymal giant cell astrocytoma (SEGA) in size over time. MRI (H) of patient P8, (K) of patient P12, (O) of patient P16, and (P) of patient P17 showing a slight increase in the size of the SEGAs over time. (W) Sagittal T1-weighted postcontrast of patient P24 (VP shunt not in picture) showing the slight increase of the pilocytic astrocytoma.

12%), oxcarbazepine (n = 2 of 25, 8%), clobazam (n = 4 of 25, 16%), clonazepam (n = 1 of 25, 4%), cannabidiol (n = 2 of 25, 8%), vigabatrin (n = 3 of 25, 12%), cenobamate (n = 3 of 25, 12%), felbamate (n = 3 of 25, 12%), gabapentin (n = 1 of 25, 4%), topiramate (n = 1 of 25, 4%), valproate (n = 1 of 25, 4%), primidone (n = 1 of 25, 4%), brivaracetam (n = 1 of 25, 4%), and zonisamide (n = 1 of 25, 4%). In three of 25 patients (12%), a vagus nerve stimulator had been implanted. One patient's battery was dead and never replaced.

*TSC-associated neuropsychiatric disorder*

It has been questioned whether mTORi therapy, particularly longstanding treatment, may have benefits for developmental disability or cognitive impairment. This fact was not evaluated prospectively in this patient group. However, a history of TSC-associated neuropsychiatric disorder (TAND) was present in all but two patients. Intellectual disability of various degrees was the most common, being reported in 18 of 25 patients (72%; not specified: n = 1 of 25, 4%; mild: n = 6 of 25, 24%; moderate: n = 4 of 25, 16%; profound: n = 2 of 25, 8%; severe: n = 5 of 25, 20%). Standardized testing was available in four of 25 patients (16%): Wechsler Adult Intelligence Scale, Fourth Edition, in P4 with an intelligence quotient (IQ) of 83, P5 with an IQ of 67, P9 with an IQ of 86, P16 with an IQ of 46, and P21 with an IQ of 49. The highest educational degree was no degree in 11 of 25 patients (46%), high school in nine of 25 patients (38%), and college in two of 25 patients (8%). No information was available in three patients. Two patients were still in college, one in high school, 10 of 22 patients (45%) were employed, and 12 of 22 were not employed (55%). In one patient, the employment status was not specified and no information was available.

Other TAND manifestations noted were autism in seven of 25 patients (28%), obsessive-compulsive disorder in eight of 25 patients (32%), anxiety/agitation in 11 of 25 patients (44%), post-traumatic stress disorder in one patient, and depression in two of 25 patients (8%). Sleep difficulties were reported in three of 25 patients (12%). Fifteen of 25 patients (60%) were receiving medication for TAND with a median of 2 medications (range: 1-4, IQR: 3). Medications included escitalopram/citalopram (n = 6 of 25, 24%), duloxetine (n = 2 of 25, 8%), desvenlafaxine (n = 1 of 25, 4%), quetiapine (n = 1 of 25, 4%), risperidone (n = 4 of 25, 16%), trazodone (n = 1 of 25, 4%), amitriptyline (n = 1 of 25, 4%), chlorpromazine (n = 1 of 25, 4%), clonazepam (n = 1 of 25, 4%), fluoxetine (n = 2), and guanfacine (n = 1 of 25, 4%).

## Discussion

SEGA is a potentially life-threatening low-grade astrocytoma brain tumor that occurs primarily in TSC. Treatment has classically involved surgical resection, which invariably leads to tumor recurrence, if gross total resection cannot be achieved. Since regulatory approval in 2010, mTORis are increasingly used as first-line treatment, as opposed to surgical resection.[8] However, the long-term efficacy and tolerability of mTORi in TSC has not been previously reported. In this retrospective review we show that treatment with mTORi has been effective and tolerated with a median mTORi treatment duration of 15.6 years.

In this cohort, mTORi treatment for SEGA had a durable effect on SEGA growth with only one patient undergoing surgical debulking twice and no patients needing VP shunt after treatment initiation. In five other patients, a transient increase in SEGA size was noted, but in no case did this require a neurosurgical intervention. These data are consistent with prior reports of follow-up from the placebo controlled randomized EXIST-1 trial showing a sustained reduction in SEGA volume after four years of therapy.[15] In recent years, mTORi treatment has also been shown to be successful in the acute setting of an obstructive hydrocephalus, preventing VP shunt implantation.[16,17]

The long-term safety data (i.e., > 10 years) of mTORi treatment are limited to patients after transplant surgery and therefore not representing the genetic background of this disease-modifying therapy in TSC. Over time the incidence of acute ADR decreased in both frequency and severity compared with those reported in the trials. However, throughout mTORi treatment, patients remain subject to acute ADRs such as aphthous ulcers and upper respiratory infections. For example, mouth sores were reported in 29% of patients, like previous shorter-term studies.[15] This finding gives rise to the suggestion that even if many acute ADRs decrease, mouth sores are a persistent ADR that comes and goes, requiring intervention.

No patients experienced opportunistic infections or lymphoproliferative disorders. One patient developed a localized melanoma, which was resected without difficulty or recurrence. It is unknown if this was causally associated with the mTORi exposure. Overall, 67% of patients developed chronic ADRs. Hyperlipidemia and hypercholesterolemia were the most common, occurring in 42% of patients, with all but one under treatment with statins. In the EXIST-1 trial, hypercholesterolemia was only present in 11%.[3,15] In a Dutch five-year follow-up study on mTORi treatment in 151 patients with TSC, only 15.5% were on lipid-lowering medication.[3,15,18] Because the median mTORi treatment at first diagnosis in our cohort was 8.7 years, this increased frequency could reflect the longer follow-up interval of our study. Additionally, the cotreatment with other medications (e.g., ASMs or antipsychotic medications) may also play a role in the frequency.

DM was present in 28% of patients. Previous studies have shown an increased incidence of DM of 4% in patients with TSC taking mTORi compared with 0.6% in those not receiving mTORi.[19] Another study reported a 2.5% incidence of DM after five-year exposure to mTORi.[18] Similar to hypercholesterolemia, the median time to onset of DM was 11.3 years after initiation of mTORi treatment, perhaps explaining why this was not observed in earlier trials.

Not previously described with mTORi treatment is osteoporosis present in 13% of patients. Osteoporosis is not a common feature of TSC, and there is no recommendation to monitor bone density in TSC.[1] Although not known to be an ADR in studies originally, osteoporosis in this cohort was seen after a median mTORi duration of 12.1 years and thereby not detected by previous shorter studies. However, all three patients with osteoporosis had taken additional ASMs that are known to impact bone density[20]; therefore no definite association can be made. Monitoring bone density in patients with TSC on mTORis and ASMs should be considered due to the risk of osteoporosis.

(Y) MRIs of patient P11. (1) MRI FLAIR sequence before treatment initiation at age four years. (2) Eleven years after mammalian target of rapamycin (mTOR) inhibitor (mTORi) initiation (age: 16 years), axial T2-weighted images demonstrate (left) asymmetric enlargement of the right lateral ventricle (white arrow) secondary to narrowing of the right foramen of Monro by a (right) large, right intraventricular mass consistent with a SEGA (black arrows). Therefore, 12 years after mTORi initiation laser interstitial thermal therapy (LITT) was performed. (3) Axial T2-weighted images obtained six months following LITT demonstrate a (left) decrease in size in the right lateral ventricle (white arrow) and (right) new area of hyperintensity at the tumor ablation site (white arrowhead). Owing to further SEGA growths, a year after the first LITT, a second LITT was performed. (4) Axial T2-weighted image three months following a second LITT demonstrates the initial area of laser ablation (white arrow), as well as a new, additional ablation site (black arrow). (5) Axial T2-weighted images at almost two years after second ablation demonstrates evolution of post-treatment changes with a reduction in the size of the original SEGA (white arrows).

Hypertension was present in 24% of patients; however, in all but two patients (P11: 20 years, BMI 20.6 kg/m$^2$; P16: 23 years, BMI 24.2 kg/m$^2$) significant AMLs were present. Therefore no clear association with the mTORi treatment can be made. Larger studies with patients without coexisting renal disorders need to evaluate if hypertension is an ADR or an underlying predisposition that might be aggravated. Overall, most chronic ADRs were manageable with medication or lifestyle changes and only led to a permanent discontinuation in one patient.

In this cohort, other neurological manifestations of TSC were present. Although drug-resistant seizures are common in TSC, after prolonged treatment with mTORi most patients with epilepsy were only on one or less typical ASMs and only nine had a seizure within the six months of their last visit. Future long-term studies of mTORi will be beneficial to understand if prolonged exposure to mTORis improves seizure control. Focus on TAND has increased since the initial clinical trials. Unfortunately, no standardized tests before treatment with mTORis are available and there was no standardized tracking of TAND in this cohort. Regardless, comparison of this cohort with future cohorts that started treatment at earlier ages may be of benefit to understand if there is an optimum time or duration of mTORi treatment for TAND.

Limitations of this study include the small cohort size and a retrospective design without a control group. Therefore statistical analysis of patient subgroups regarding the risk of specific ADRs was not possible.

Our data suggest long-term efficacy and safety of mTORi treatment in SEGAs while preventing SEGA surgery and hydrocephalus. With long-term mTORi treatment, monitoring lipid levels, glucose metabolism, bone density, and blood pressure to ensure early detection and treatment of possible ADRs is important, especially in those with other risk factors including TSC-associated kidney disease and cotreatment with other medications with the same risk factors. Larger studies and longer follow-up periods are needed to distinguish the rate of ADRs with long-term mTORi treatment and if certain patient groups are at higher risk for ADRs and possibly prevent them from occurring.

## Data sharing statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Patient consent statement

Written informed consent/assent was obtained from the patient and/or their legal guardian.

## Author contributions

D.M.R. and D.N.F. were involved in conceptualization. L.-L.B., D.M.R., and K.A. were involved in investigation, collection of patient data, and validation. L.-L.B. and D.N.F. were involved in writing the original draft. L.-L.B., D.L.F., D.A.K., K.A., D.M.R., and M.M.C. were involved in the review and editing process. M.M.C. assisted with preparation of images. D.N.F. and D.M.R. were involved in supervision and project administration.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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