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Original article

Short-term safety of mTOR inhibitors in infants and very young children with tuberous sclerosis complex (TSC): Multicentre clinical experience

Darcy A. Krueger [a,], Jamie K. Capal [a], Paolo Curatolo [b], Orrin Devinsky [c], Kevin Ess [d], Michal Tzadok [e], Mary K. Koenig [f], Vinodh Narayanan [g], Federico Ramos [h], Sergiusz Jozwiak [i], Petrus de Vries [j], Anna C. Jansen [k], Michael Wong [l], David Mowat [m], John Lawson [m], Stephanie Bruns [a], David Neal Franz [a], On behalf of the TSCure Research Group [1]*

[a] *Department of Neurology, University of Cincinnati, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA*

[b] *Child Neurology and Psychiatry Unit, Systems Medicine Department, University Hospital Tor Vergata, Rome, Italy*

[c] *Langone Medical Center, New York University, New York, New York, USA*

[d] *Monroe Carell Jr Children's Hospital, Vanderbilt University Medical Center, Nashville, TN, USA*

[e] *Pediatric Neurology Unit, Edmond and Lilly Safra Children Hospital, Chaim Sheba Medical Center, Sackler School of Medicine, Tel Aviv University, Tel HaShomer, Tel Aviv, Israel*

[f] *University of Texas at Houston, Houston, TX, USA*

[g] *Arizona Pediatric Neurology and Neurogenetics Associates, Phoenix, AZ, USA*

[h] *Department of Neurology, Sant Joan de Deu Hospital, Barcelona, Spain*

[i] *Department of Pediatric Neurology, Warsaw Medical University, Poland and Department of Neurology and Epileptology, The Children's Memorial Health Institute, Warsaw, Poland*

[j] *Division of Child & Adolescent Psychiatry, University of Cape Town, Cape Town, South Africa*

[k] *Pediatric Neurology Unit, UZ Brussel, Brussels, Belgium*

[l] *Washington University St. Louis, St. Louis, MO, USA*

[m] *Sydney Children's Hospital, Sydney, Australia*

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ABSTRACT

Objective: To evaluate the safety of mTOR inhibitors (sirolimus or everolimus) in infants and very young children with tuberous sclerosis complex (TSC) under two years of age.

Methods: Study design was retrospective to capture medical record data from 52 international TSC Centres who initiated treatment with sirolimus or everolimus in TSC children before the age of two years. Data collection included demographic and clinical information including reason(s) for initiating treatment with mTOR inhibitors, treatment duration, dosing, and corresponding serum trough levels, response to treatment, and adverse events (AE).

* *Corresponding author*. Division of Pediatric Neurology, Cincinnati Children's Hospital Medical Center, 3333 Burnet Ave., ML #2015, Cincinnati, OH, 45040, USA. Fax: +1 (513) 636 1888.

E-mail address: krueger_darcy@cchmc.org (D.A. Krueger).

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Results: 19 of 52 (37%) TSC Centres reported treatment of at least one child with TSC under the age of two years with everolimus or sirolimus. Treatment-related data were provided for 45 patients meeting inclusion criteria. Everolimus was utilised 87% of the time, compared to 24% for sirolimus (5 subjects, 11%, were treated separately with both). Refractory epilepsy (45%) was the most common primary reason for initiating treatment and treatment was initiated on average at 11.6 ± 7.6 months of age. At least one AE, suspected or definitely treatment-related, occurred in 35 of 45 (78%) treated subjects. Most AEs were mild (Grade 1) or moderate (Grade 2) in severity and most commonly related to infections. Severe AE (Grade 3) was reported in 7 subjects (20%) and no life-threatening AE (Grade 4) or death/disability (Grade 5) was reported. Treatment was discontinued due to an AE in 9 of 45 (20%).

Conclusions: Everolimus, and to a lesser extent sirolimus, are increasingly being used to treat TSC infants and very young children for multiple TSC-associated clinical indications. While AEs were common, most were not severe and did not prevent continued treatment in the majority of this younger population.

1. Introduction

The clinical manifestations of Tuberous Sclerosis Complex (TSC) are diverse, varying in organ system affected, timing of onset, and severity.[1] Brain tubers and subependymal nodules (SEN) are nearly universally present in brains of affected individuals at birth. Also common are cardiac, skin and kidney lesions.[2] The pathological lesions of TSC are called hamartomas and are largely benign in that the potential for malignancy is very low. However, depending on location, timing, composition, and progression, secondary complications can be life-threatening as normal organ structure and function is disrupted.[3–6] For example, cardiac rhabdomyomas can obstruct ventricular outflow and cause cardiac failure during infancy. In the brain, subependymal giant cell astrocytomas (SEGA) may arise and due to proximity near the Foramen of Monro can cause acute or chronic obstructive hydrocephalus. Epilepsy is another very common neurological manifestation of TSC, often presenting in the first year of life as infantile spasms or focal seizures that can become refractory to currently available treatments and interventions.[7] Autism spectrum disorder, intellectual disabilities, and other TSC-associated neuropsychiatric manifestations are also very common and are seen in up to 90% of individuals with TSC.[2,8] Renal cysts or angiomyolipomas can accumulate to replace normal kidney, leading to renal insufficiency and failure.[9,10]

Identification of a set of clinical characteristics has been the mainstay for diagnosis of TSC, but a genetic diagnosis can also be made when a pathological mutation is identified in *TSC1* or *TSC2*, the causative genes for TSC.[1] TSC1 and TSC2 function within a protein regulatory complex to limit activity of a key intracellular signaling pathway mediated by the mechanistic target of rapamycin (mTOR) kinase, which is essential for normal cell growth, differentiation, metabolism, function, and survival.[11] Small molecule inhibitors of mTOR, such as rapamycin (sirolimus) and everolimus, were first reported to be effective for treatment of SEGA in 2005, and everolimus is now approved both in the US and EU for the treatment of SEGA, angiomyolipoma, and epilepsy.[12–17] In addition, sirolimus was approved in the US for the treatment of lymphangioleiomyomatosis (LAM) in 2015.[18]

Human clinical trials with everolimus or sirolimus that led to regulatory approvals in TSC have demonstrated safety and efficacy in older children and adults.[12–14,16–18] In all studies, treatment was initiated well after clinical progression or symptoms were manifest rather than early in the disease course such as infancy when many lesions and clinical symptoms first arise. Children below the age of 2 years are noticeably excluded. Isolated case reports of early treatment do exist,[19] but no systematic study has to date collated and analysed treatment data for very young children with TSC. Given that it is this very population that may be targeted for prevention studies against epilepsy, SEGA, and other neurological manifestations of TSC that present in the first years of life, establishment of a basic safety profile for mTOR inhibitors in this population is required. Here we report the treatment utilization and cumulative safety data of children with TSC under the age of two years treated with sirolimus or everolimus for TSC-associated clinical indications.

2. Materials and methods

There currently are no prospective studies to evaluate mTOR inhibitor treatment in infants and young children with TSC under the age of two years. We therefore used a retrospective design to capture data from medical records of TSC Centres, both US and internationally, who treat children in this age range. To be included, subjects had to have a clinical or genetic diagnosis of TSC and had to have been initiated on treatment with either sirolimus or everolimus before the age of two years. Data collection included all treatment-related data up to the cut-off date, March 1, 2017. All data were collected in a de-identified fashion and entered onto a standardised case report form that included demographic

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information (age, gender, race), *TSC1/TSC2* genotype, presence or absence of TSC-associated phenotypic features, reason(s) for treatment with an mTOR inhibitor, treatment dosing and duration, response to treatment, and any AE reported. Where serum trough levels were available, this also was included in the analysis.

The primary aim of this study was to create a safety profile for mTOR inhibitor treatment in infants and very young children with TSC under the age of 24 months and capture current clinical utilization practises. This data could be used as a starting point for future clinical trials targeting a prospective population. The statistical analysis largely consists of summary and descriptive measures (i.e., group percentage, means ± standard deviation). For the limited comparisons between subgroups of the analysis cohort, statistical significance was determined using student t-test and Mann–Whitney Rank Sum test, depending on distribution. Fisher's Exact test was used for categorical comparisons. All analyses were performed using SigmaPlot 13.0 (Systat Software, Inc.; San Jose, CA) and GraphPad Prism 7 (GraphPad Software Inc.; San Diego, CA).

Ethical approval, including provision to waive individual informed consent, was obtained from the Institutional Review Board (IRB) at Cincinnati Children's Hospital Medical Center (CCHMC) prior to initiation of study procedures. Any additional local ethical and IRB policies and procedures also were observed when applicable.

3. Results

3.1. Patient characteristics

Data requests were made to 66 international TSC Centres. Of those, 43 were located in North America, 17 in Europe, three in the Middle East, two in Australia, and one in Africa. Among 52 responding TSC Centres, 19 (37%) treated at least one TSC child under age two years with either sirolimus or everolimus (9 in the United States, 8 International). Not all clinics reporting treatment of TSC patients in this age range, however, were able to provide patient-specific prescribing and safety data due to local ethics requirements regarding patient data under this protocol. In total, clinical data for 45 patients meeting inclusion criteria were available for analysis, the majority of which were from the US both for everolimus (n = 34, 85%) and sirolimus (n = 8, 72%). (Table 1). Cincinnati Children's Hospital, which first reported mTOR treatment for TSC patients in 2005,[15] was the largest contributor of patient data (n = 24, 53%).

Patients were more likely to be male (n = 30, 66%), white non-Hispanic (n = 33, 73%), and with mutations in the *TSC2* gene (n = 31 of 35 with genetic testing, 88%). Since most clinical trials in TSC involved everolimus rather than sirolimus and regulatory approval in most countries is limited to everolimus, most subjects had been treated with everolimus (n = 39, 87%). Sirolimus was used to treat 11 subjects (24%), including five subjects (11%) that had been previously treated with everolimus. At time of data cut-off, 22 of 39 subjects (56%) were still being treated with everolimus and 6 of 11 (55%) were still being treated with sirolimus.

Table 1 – Patient characteristics.

	Everolimus (n = 39*)	Sirolimus (n = 11*)
Age at Treatment Initiation (months)	16.1±7.2	11.7±7.8
Duration of Treatment (months)	27.3±24.7	15.9±19.1
Country of Birth		
Australia	2	0
Israel	0	2
Italy	2	0
Spain	1	0
United States	34	8
Venezuela	0	1
Sex		
Male	26	6
Female	13	5
Race		
White, Non-Hispanic	22	10
White, Hispanic	3	1
Black	0	0
Asian	5	0
Pacific Islander	0	0
Other	4	0
Genotype		
TSC1 mutation	2	1
TSC2 mutation	28	6
No mutation identified	1	1
No testing performed	8	3
Primary Reason for Treatment		
Epilepsy	19	4
SEGA	16	4
Rhabdomyoma	2	1
Other Hamartoma	2	2

Five subjects treated separately with both everolimus and sirolimus are included in both reporting groups.

3.2. Reason for treatment

Epilepsy was the most common primary reason to initiate treatment with everolimus or sirolimus, accounting for 20 (45%) of subjects overall (Table 1). SEGA (n = 18, 39%), rhabdomyomas (n = 3, 7%), and other hamartomas (n = 2, 4%) were the remaining reasons clinicians started treatment before the age of two years. It is common for multiple manifestations to be present at early ages in TSC.[20,21] Not surprising than that in several instances multiple aspects of TSC were reported as additional, or secondary, consideration for treatment with mTOR inhibitors in this age group. SEGA was reported as a secondary consideration for treatment in five (11%), developmental concerns in three (7%), epilepsy in two (5%), and angiomyolipoma in one (2%).

3.3. Dosing

Treatment was initiated at 11.6 ± 7.6 months. Both medications were started as early as the first month of life (Table 1). Eighteen (40%) were started during the first year, including 13 before the age of six months, and the remainder during year two (n = 27, 60%). On average everolimus was started earlier than sirolimus (11.7 ± 7.8 vs. 16.1 ± 7.2 months). Everolimus treatment duration was longer as well compared to sirolimus

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(mean 27.3 ± 24.7 vs. 15.8 ± 19.1 months). The average starting dose of everolimus was 1.05 ± 0.58 mg/m^2/day. Peak dosing was 2.26 ± 2.05 mg/m^2/day. Measured serum trough levels (N = 51) were available from 18 subjects and averaged 6.0 ± 4.8 ng/ml (range 1.0–26.5 ng/ml). Sirolimus dosing on average was much less than everolimus (likely due to significant longer half-life of sirolimus compared to everolimus).[11] The average sirolimus starting dose was 0.40 ± 0.28 mg/m^2/day and peak sirolimus dose was 0.65 ± 0.28 mg/m^2/day. Six separate measured serum trough levels were available from two subjects and averaged 7.6 ± 6.6 ng/ml (range 3.2–20.3 ng/ml).

3.4. *Outcomes and safety profile*

At least one adverse event (AE), suspected or definitely treatment-related, was reported for 35 of 45 (78%) treated subjects (Table 2). Those treated with everolimus (n = 38, 84%) reported more AE than sirolimus (n = 28, 63%), but this was not statistically significant (p = 0.197). Most AEs were mild (Grade 1) or moderate (Grade 2) in severity. Seven subjects (20%) reported severe (Grade 3) AEs and none reported a life-threatening AE (Grade 4) or death/disability (Grade 5). The most common AEs were related to infections: URI, sinusitis, otitis, gastroenteritis, or other infections occurred in 20–69%, and four cases (9%) were rated Grade 3. Aphthous ulcers and stomatitis occurred in 18 (40%) with one case rated Grade 3. By comparison, significant laboratory abnormalities were rare. Elevated cholesterol, the most common, was reported in six (14%), of which one case was rated with a severity of Grade 3 (hyperlipidemia in patient treated with everolimus and ketogenic diet simultaneously). Five subjects (11%) reported a serious AE (SAE). In three cases, this was due to hospitalization for infectious causes (severe upper respiratory infections or pneumonia). Another was hospitalised for vomiting and seizures, and the last was due to severe anaemia requiring blood transfusion. At time of data cut-off, all SAEs and all but four AEs had resolved. In most instances, AEs and SAEs were managed by temporarily suspending treatment until the AE or SAE resolved, at which time treatment was resumed at the same or a lower dose. Recurrent AEs in two instances prompted change from everolimus to sirolimus. Three others changed mTOR inhibitor due to improved availability or cost differences. As mentioned above, 17 of 39 patients treated with everolimus (44%) and 5 of 11 patients treated with sirolimus (45%) had discontinued treatment at the time of data cut-off. Of those in the everolimus group, 9 of 17 (53%) reported an AE as the reason for stopping treatment. By comparison, none of the 5 (0%) in the sirolimus group stopped because of an AE. Drug availability, cost, or lack of perceived benefit were reasons other than AE for electing to discontinue treatment.

Although our study was not designed to evaluate efficacy, we asked each clinician whether or not the primary reason for treatment had demonstrated a favorable response. In 29 of 45 subjects (64%), clinicians reported at least partial benefit compared to no improvement or favorable response in 8 of 45 subjects (18%). Response for the remaining eight subjects (18%) was uncertain or undetermined at the time of data cut-off. Fig. 1 shows a representative case of a 26-day old female treated for bilateral congenital SEGA with everolimus (1.25 mg 2x/week, corresponding to serum trough level of 3.2 ng/ml). Treatment was continued with most recent follow-up at 44 months of age at time of data cut-off. During this time, occasional interruptions in treatment occurred due to AEs (upper respiratory infections) and scheduled immunizations. Successful strategies to reduce the frequency and severity of infections improved treatment continuity, and at most recent follow-up imaging at 44 months of age demonstrated a significant reduction in volume and progressive calcification of both SEGA.

4. Discussion

Clinical trials of mTOR inhibitors to treat SEGA, angiomyolipoma, epilepsy, and LAM have consistently demonstrated efficacy and tolerability for mTOR inhibitors everolimus or sirolimus compared to placebo.[14,17,22–24] Treatment response is maintained with extended treatment without introduction of new toxicities or complications.[23,25–27] Although mTOR inhibitors are approved in many countries for the treatment of specific TSC-associated manifestations, data to evaluate

Table 2 – Adverse events.

	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	**Total (Any Grade)**	SAE	Ongoing
Ulcers/Stomatitis	2	11	1	0	0	**14**	0	0
URI/Sinusitis/Otitis	0	22	2	0	0	**27**	3	0
Gastroenteritis	0	6	1	0	0	**7**	0	0
Other infections	0	9	1	0	0	**10**	0	0
Rash	0	10	0	0	0	**10**	0	1
Fever	0	7	0	0	0	**7**	0	0
Vomiting/Feeding	0	1	0	0	0	**2**	1	0
Behaviour Change	0	2	0	0	0	**2**	0	0
Abnormal Complete Blood Count (CBC)	1	1	1	0	0	**4**	1	0
Abnormal Basic Metabolic Panel (BMP)	0	0	0	0	0	**0**	0	–
Elevated Cholesterol	3	1	1	0	0	**5**	0	3
Abnormal Hepatic Function (LFT)	0	0	0	0	0	**0**	0	–
Abnormal Urinalysis (UA)	0	1	0	0	0	**1**	0	0
Other AE or Laboratory Abnormality	0	0	0	0	0	**0**	0	–
TOTAL	**6**	**71**	**7**	**0**	**0**	**84**	**5**	**4**

Bold is solely to distinguish tabular data (non-bold) from summary total data (bold).

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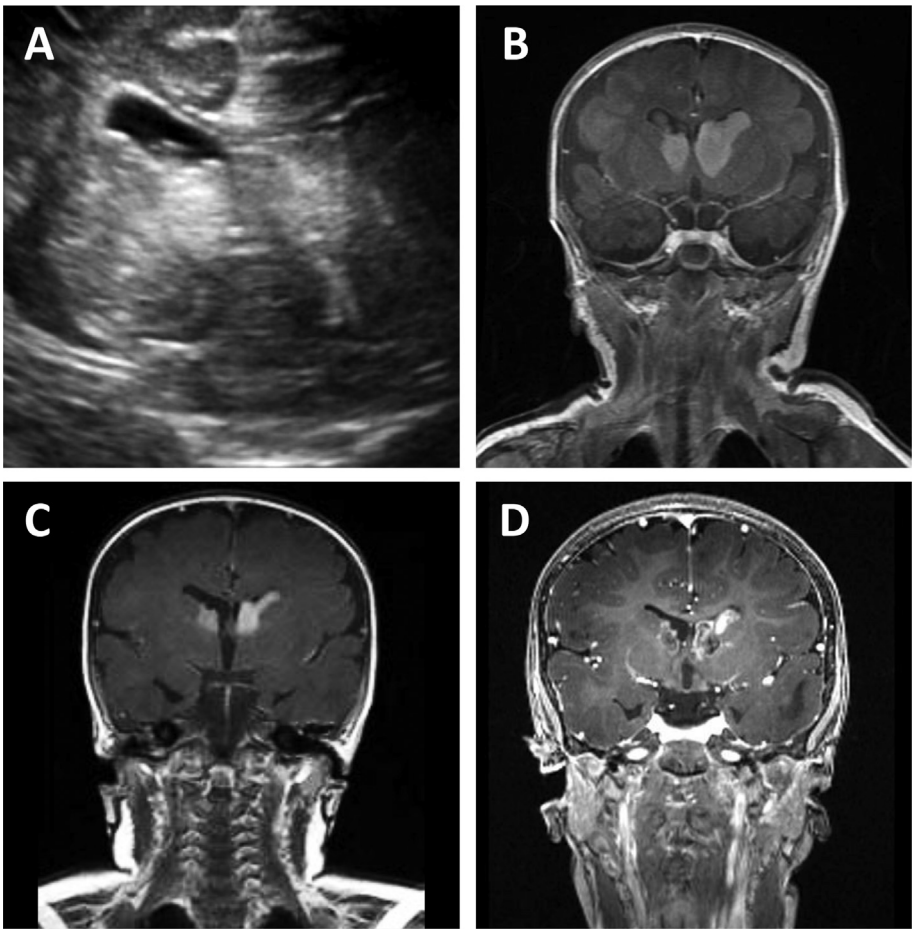

Fig. 1 – EVEROLIMUS TREATMENT OF TSC INFANT WITH CONGENITAL SEGA. (A) Head ultrasound performed on first day of life, demonstrating bilateral large sega. (B) Coronal T1 MRI with gadolinium demonstrating same SEGA on fifth day of life. (C) Coronal T1 MRI with gadolinium obtained at 5 months of age, demonstrating marked reduction of SEGA following everolimus treatment that was initiated on day of life 26. (D) Coronal T1 MRI with gadolinium obtained after continued treatment with everolimus, now 44 months of age. SEGA in interim have developed significant calcification and tumor volume continues to be reduced compared to baseline.

treatment safety in patients younger than age two years are almost non-existent, largely due to the study design of individual clinical trials and the natural history of many specific TSC-associated manifestations not appearing or progressing until later in life.[28] As raised in the introduction, establishing a safety profile for everolimus and sirolimus in the very young is extremely important for clinical decision making and clinical trial design moving forward for potential early and preventative treatment of infants and very young children with TSC.

As in prospective clinical trials involving older children and adults,[12,13,16–18] we found that most AE were mild (Grade 1) or moderate (Grade 2) in severity in our younger cohort. Jozwiak et al. (2016) provides the only comparable safety analysis for everolimus treatment in very young TSC patients.[29] Of 117 patients who participated in the larger randomised, placebo-controlled clinical trial for the treatment of SEGA (EXIST-1, *clinicaltrials.gov NCT00789828*),[14] 18 patients with a median age of 1.8 years were treated with everolimus under the age of three years. Median treatment duration was 31.1 months and median dose was 5.86 mg/m^2/day. All 18 reported at least one AE, and one patient discontinued treatment because of AEs. The majority of AEs were mild/moderate (grade 1 or 2), but 12 (66%) reported at least one severe (grade 3) AE and two (11%) reported at least one life-threatening (grade 4) AE. Our study provides additional evidence that most AE associated with mTOR inhibitor treatment in young children with TSC are mild or moderate in severity, but discontinuation of treatment because of AE did occur (20% of all treated patients) that is higher than the 0–5% typically observed in TSC clinical trials with mTOR inhibitors for older children.[16,17,22,30] Yet the type and severity of AE in our cohort was no different than in older children, with stomatitis and various types of common infections or infection-related symptoms most prevalent. We speculate the higher rate of discontinuation is due to parent and/or clinician discomfort with continuing treatment in the context of an AE rather than the AE itself. In other words, it isn't that the children in our cohort were sicker or more prone to AE, but rather that the parents and clinicians are less comfortable continuing treatment. Well-designed, prospective clinical trials with randomized, placebo-controlled, double-blind design in this population would provide a

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more definitive safety profile for this population and prove whether or not this is indeed the case.

It is possible that the reduced frequency of AEs in our study compared to the younger EXIST-1 cohort reported by Jozwiak et al.[29] is due to better tolerability at younger ages (i.e., <24 months versus < 36 months). However, both studies reported types and severity of AEs highly comparable to older children and adults with TSC of other clinical studies,[12,13,16–18] and alternative explanations more likely explain the observed difference. For example, the relatively small sizes of both cohorts (n = 18 and n-45) could be overestimating and/or underestimating the true frequency of AEs or SAEs, especially for those with relatively low rates of occurrence. Also, we included both sirolimus and everolimus treatment in our analysis of treatment safety of young children with TSC. If sirolimus was more easily dosed or better tolerated than everolimus, its inclusion would lower overall AE estimates compared to everolimus alone. Based on the high similarity in molecular structure between the two mTOR inhibitors and demonstrated equivalence in preclinical studies and non-TSC clinical applications,[11] this seems unlikely.

Our approach attempted to capture real-world, clinical experience when infant treatment at any TSC clinic remains rare. In the absence of any prospective studies of this nature, our results represent a valuable synthesis of clinical cases from multiple TSC Centres—primarily from the US (n = 33, 67%) but also additional experience from patients treated at centres outside the US (n = 17, 33%). Although TSC exists as a genetic disorder affecting individuals worldwide, clinics dedicated to treatment of TSC patients in the US far outnumber those outside the US. A disproportionate number of our cases were from a single treating center (Cincinnati Children's Hospital), further increasing the possibility of significant reporting bias and limiting the generalizability of our results as there could be country and/or centre-specific treatment decision making and AE management. This skew likely results from multiple reasons and under the current circumstances is difficult to eliminate. For example, US prescribing laws and payment programs, compared to many other countries, likely provide greater access to treatment for patients with non-approved indications or age ranges. In addition, several clinics were able to report having prescribed treatment in this age group but local ethics requirements prevented sharing of patient-specific treatment and safety data under this protocol.

The retrospective design of this study, however, reveals an even greater limitation of our study design. The data collection methods used may be significantly subject to recall bias, variations in patient reporting, and variable clinical documentation at individual centres. Ascertainment of AE in the very young may also be much more difficult for parents and clinicians. Children under age two years with TSC have very limited vocabularies, limiting their ability to communicate and report symptoms such as pain. In TSC many infants may also have had neurodevelopmental delays that might further have impaired their ability to communicate less obvious AEs. Only prospective clinical trials with uniform reporting procedures and documentation from larger TSC cohorts under age 2 years will provide an accurate safety profile of everolimus and sirolimus.

Despite the relative lack of safety experience in this younger population, the number of clinicians treating very young children with TSC for both approved and unapproved indications, is growing, resulting in recently published clinical recommendations.[31] Single case reports and small patient series independently demonstrate increasing treatment of very young patients with TSC, including infants, in clinical practise with both everolimus and sirolimus.[19,32–42] In literature, early utilization focused on treating congenital cardiac rhabdomyomas, but other uses include SEGA, lymphangiectasia, and segmental lymphedoema. Since reports with mTOR inhibitors in TSC infants has predominantly focused on cardiac rhabdomyomas, which are reported to occur in 60–75% of TSC infants,[3,43] we found it somewhat surprising that treatment for rhabdomyomas in our cohort was relatively infrequent. Congenital SEGA and early intractable epilepsy were the most common reasons for initiating treatment in the current study. We speculate this is not only because most cardiac rhabdomyomas are asymptomatic and may resolve spontaneously without treatment or intervention,[3] but that the majority of TSC clinicians which reported infant treatment in our study had previously participated in the large multicentre TSC clinical trials for SEGA and epilepsy and hence have greater familiarity with mTOR inhibitors for these indications in older children and less hesitation to extend treatment to younger patients.

Epilepsy affects more than 80% of individuals diagnosed with TSC, with onset in the vast majority before the first birthday.[7,44] Epilepsy is closely associated with poorer neurodevelopmental outcomes and TSC-associated neuropsychiatric disorders,[45] with similar onset very early in life.[44,46–50] Focusing on these highly penetrant, potentially devastating aspects of TSC with mTOR inhibitors would more directly target the underlying molecular defects responsible but would by necessity require earlier treatment than currently approved indications. Previous clinical studies have suggested early, even presymptomatic treatment of seizures with vigabatrin in TSC patients can reduce the frequency of medically refractory epilepsy and preserve normal neurodevelopmental trajectory.[47,50] Furthermore, preclinical studies indicate that early treatment with mTOR inhibitors has antiepileptogenic actions in preventing epilepsy and other neurological features of mouse models of TSC.[51,52] We found that mTOR inhibitor treatment in this population is reasonably well tolerated and suggests that it could safe or reasonable to use in infants in a future prevention study.

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All authors must provide a statement of competing interests, even if the authors have no competing interests to declare (i.e., conflicts of interest: none).

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Author contributions

Study Concept and Design: DAK, JKC, PC, SJ, PdV, ACJ, MW ET, DM, DNF, JKC, PV, AJ, MW, JAL, SR.

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Data Analysis and Interpretation of Data: DAK, JKC, SB.

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Conflict of interest

None declared.

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