Original Article

The effects of everolimus on tuberous sclerosis complex–associated renal angiomyolipoma: a preliminary report

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Running title: Everolimus and TSC–associated renal AML

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ABSTRACT

Aim: Tuberous sclerosis complex (TSC) presents with multisystem benign neoplasm induced by dysregulation of the mammalian target of rapamycin pathway. This study aimed to examine the effects of oral everolimus with either at 2.5 or 5.0 mg daily on the treatment of TSC-associated renal angiomyolipoma (AML).

Methods: Between July 2012 and August 2015, patients with TSC-associated renal AML were selected for everolimus therapy protocol. An oral everolimus starting dose at 2.5 mg was administered daily, and was gradually increased the dose to 5.0 mg daily. All patients were evaluated using magnetic resonance imaging or computed tomography scanning at baseline, 12, 24, and 36 months after the start of treatment for measuring the changes of renal AML mass volume.

Results: Eight patients were finally enrolled for analysis in this study. Everolimus treatment had the statistically significant effect on the renal AML volume reduction during follow-up ($P < 0.05$). Renal AML mass volume reduction rates were 10.5-45.3% in four patients with everolimus 2.5 mg and 40.7-73.1% in four patients with everolimus 5.0 mg daily; the difference was statistically significant between the two groups ($P < 0.05$). Longitudinal follow-up for response to everolimus showed volume reduction rates to be around

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10.5-73.1% in the initial 6-24 months after everolimus treatment, which remained stable during follow-up up to 36 months.

Conclusion: The results suggest that an oral everolimus is effective and provides a non-invasive way to treat TSC-associated renal AML, and patients are likely to require maintenance therapy to continue to derive benefit.

KEY WORDS:

everolimus, renal angiomyolipomas, mass volume reduction, tuberous sclerosis complex, the mammalian target of rapamycin.

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INTRODUCTION

Tuberous sclerosis complex (TSC) presents with multisystem benign neoplasm induced by dysregulation of the mammalian target of rapamycin (mTOR) pathway.[1] The common phenotype is seizures in childhood and the characteristic feature of intracranial lesions prompts the clinicians to make a diagnosis.[2-4] However, some may be asymptomatic in childhood until renal angiomyolipoma (AML) and chest lymphangioleiomyomatosis associated symptoms occur.[5] With the development of diagnostic tools, the 2012 International TSC consensus conference recommended patients undergo regular surveillance regardless of age and lesion size, to minimize potentially comorbidities.[6]

Renal AML in TSC is usually asymptomatic but may progress during adulthood. In a previous study assessing mortality associated with TSC, renal problems (renal failure or tumoral complications, retroperitoneal haemorrhage, and metastases of renal cell carcinoma) in TSC were the second leading cause of premature death after severe intellectual disability.[7] Disease mortality is associated with aneurysms and haemorrhage-prone renal AML and worsening outcomes are associated with progressive increases in size.[8] Thus, current guidelines recommend magnetic resonance imaging (MRI) of the abdomen to assess the progression of renal AML and renal cystic disease every 1-3 years throughout the patient's lifetime.[6,9]

For patients with renal AML presenting with acute haemorrhage, embolization followed by corticosteroids is the first-line therapy and nephrectomy is avoided as far as possible. Moreover, selective embolization or kidney-sparing resection is acceptable as a second-line therapy for asymptomatic renal AML. Recently, for asymptomatic renal AML measuring larger than 3 cm in diameter, treatment with mTOR inhibitors is the recommended first-line therapy.[6,9]

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Recently, studies demonstrate that mTOR inhibitors such as sirolimus are effective in reducing renal AML mass volume in patients with TSC and have an acceptable and tolerable safety profile.[10] Everolimus has been approved for the treatment of patients with TSC-associated AML who did not require immediate surgery. The improved pharmacokinetic profile of everolimus over sirolimus makes it an attractive, non-invasive option for patients. The Everolimus for Angiomyolipoma Associated with Tuberous Sclerosis Complex or Sporadic Lymphangioleiomyomatosis (EXIST-2) trial has confirmed the effectiveness of everolimus in reducing TSC-associated AML volume.[11] An oral everolimus 10 mg daily resulted in a significant reduction in the sum of all target renal AML volumes ≥50% relative to baseline, and most adverse events reported were mild to moderate in severity.[11] This study aimed to examine the effectiveness and its contingent effects of low cost and less adverse events of low dose of everolimus in comparison to high dose used in EXIST-2 trial in reducing TSC-associated renal AML mass volume.

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METHODS

Patient selection and study design

From July 2012 to August 2015, patients with TSC followed regularly at the Integrated Clinics for TSC at the Chung Shan Medical University Hospital (CSMUH) were prospectively selected for everolimus treatment protocol. Everolimus now carries Taiwan Ministry of Health and Welfare approval for treatment of patients with subependymal giant cell astrocytomas (SEGA) and renal AML in the setting of TSC. This prospective study was to examine the possible effect of low dose of everolimus (2.5 mg or 5.0 mg daily) in comparison to high dose used in EXIST-2 trail in reducing TSC-associated AML mass volume. The hospital's Institutional Review Board of CSMUH approved this study (CSMUH No. CS12245) and the study was performed according to the Declaration of Helsinki. All participants provided informed consent.

The patients underwent a systematic evaluation with a medical history review, including recording of cutaneous manifestations, MRI of the brain, chest computed tomography (CT), and abdomen CT/MRI. All patients had been diagnosed with TSC according to the 2012 International TSC Consensus Conference Guidelines,[12] and patients with probable TSC were excluded. A starting dose of everolimus 2.5 mg daily was chosen as a means of providing adequate exposure to all patients based on the dose proportionality of all enrolments. Dose titration to everolimus 5.0 mg daily was determined clinically and based on tolerance to everolimus-related adverse effects. The patients were evaluated using abdomen MRI or CT scanning at baseline, 12, 24, and 36 months after the start of treatment. A renal AML was defined as an identifiable lesion within the renal parenchyma of the TSC patients. Baseline renal AML mass volumes were calculated for dominant renal lesions according to the volume equation = $(4/3 \times \pi \times a \times b \times c)$, with a, b, and c, representing the respective three-dimensional radii (cm) of the lesions in question; each volume was treated as an

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ellipsoid. The total mass volumes were calculated as the sum of each section (all in-section volumes). AML mass reduction rate was determined using the equation = $(V_{Pre\text{-}Therapy}-V_{Post\text{-}Therapy})/V_{Pre\text{-}Therapy} \times 100\%$ ($V_{Pre\text{-}Therapy}$ represented for pre-everolimus therapy tumour volume, $V_{Post\text{-}Therapy}$ represented for post- everolimus therapy tumour volume).

CT scan examination

CT scan examinations were performed using a 40-slice scanner (Brilliance 40, Philips, Israel) or a 320-slice scanner (Toshiba Aquilion ONE, Toshiba Medical Systems, Otawara, Japan) with an X-ray tube potential of 80-120 kVp. The tube current was set according to the patient's size or body weight to obtain an acceptable radiographic optical density and patient dose. The projection data of the initial CT product was reconstructed into axial and coronal images with slice thickness of 3.0-4.0 mm.

MRI examination

MRI examinations were performed with 1.5-Tesla scanner (Magnetom Sonata, Siemens Medical Solutions, Erlangen, Germany, or Signa Horizon Echospeed, General Electric Medical Systems, Milwaukee, WI, USA) equipped with high-performance 3-axis gradient systems. The scan protocol included T1-weighted, T2-weighted, fluid attenuation inversion recovery and diffusion-weighted imaging. Contrast-enhanced MR imaging was used if indicated. Typically, T1-weighted images were used for volume assessment. Imaging parameters for this sequence were as follows: TR/TE = 400-500/13 msec, 4.0 or 5.0 mm slice thickness, field of view = 170-230 mm.

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Statistical Analysis

Non-parametric data were expressed as medians and ranges. Wilcoxon signed-rank test, Mann-Whitney *U* test, or Friedman test was used to compare the statistical significance of differences of continuous variables. Statistical significance was set at $P < 0.05$. All statistical analyses were conducted using SPSS for Windows, Version 14.0 (SPSS Inc., Chicago, IL).

RESULTS

Twelve patients were treated with everolimus for TSC-associated comorbidities in the study. Of them, four patients were withdrawn from the study, including hesitation to everolimus-related adverse events in three and chest tightness in one. They all consequently discontinued everolimus. The timing of withdrawal was within the phase of initiation and no follow-up imaging was performed in these patients. Finally, one male and seven female aged 14 to 68 years who had completed follow-up in this study were enrolled for analysis. The main indications for everolimus therapy included large SEGAs, dyspnoeic lymphangioleiomyomatosis, and large renal AMLs with/without bleeding tendency (Table 1). All of the eight patients showed cutaneous features and intracranial lesion. In neuropsychiatric aspects, seven of them had history of seizures and five had normal intelligence with good medication compliance. Genotyping identified three patients with mutations to *TSC1* and four with mutations to *TSC2*; and genetic mutation was not identified in one patient.

Four patients received oral everolimus 2.5 mg daily and 4 received 5.0 mg daily. All patients were responders to everolimus, with statistically significant volume reduction after treatment (Table 2). The average of renal AML volume reduction rates ranged from 10.5% to 73.1%. Adverse events presented by the patients included stomatitis/oral ulcers (n = 5, 62.5%), acne-like skin lesions (n = 2, 25%), and hyperlipidaemia (n = 2, 25%), which were

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well tolerated by the patients, and no other severe complications occurred through the follow-up period (Table 2).

All of the patients treated with everolimus either at 2.5 or 5.0 mg daily had a statistically significant volume reduction (cm^3) between before (median 251.9, range 1.9-3677.3) and after (median 96.3, range 1.3-3292.7) treatment ($P < 0.05$). The volume reduction rates were 10.5-45.3% in four patients with everolimus at 2.5 mg daily and 40.7-73.1% in other four patients with everolimus at 5.0 mg daily. There was statistically significant difference in the volume reduction rates between the two groups ($P < 0.05$; Fig. 1). The effect of everolimus on AML volume reduction over time is shown in Fig. 2. The difference of AML volume reduction in the study patients was statistically significant between at baseline and after 12 months of treatment ($P < 0.05$). However, there were no significant differences of AML volume reduction between at 12 months and at 24 months and between at 24 months and at 36 months of treatment, respectively (each $P > 0.05$). The maximal AML volume reduction was achieved at 12 months of treatment. AML volume reduction seemed to not change significantly after 12 months of treatment and it remained stable during follow-up up to 36 months. The treatment and follow-up of case 8 was illustrated (Fig. 2 and 3). AML mass volume rapidly reduced to 55.5% and 59.3%, respectively, at 6 and 12 months of everolimus 5.0 mg, and the mass volume remained stable during 36 months of follow-up.

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DISCUSSION

The main goal in the treatment of TSC-associated renal AML is to prevent their progressive enlargement, thereby ameliorating the risk of future bleeding, impaired renal function and mortality. Our results highlight the efficacy of everolimus for medical treatment in patients with TSC-associated renal AML, resulting in significant volume reduction at different dosage. The current study shows that the volume reduction occurred soon after everolimus therapy for the initial 6 to 24 months, and was consistent during 36-month follow-up period. Although all patients presented with side effects, these were tolerable. Minimizing TSC-renal AML-associated comorbidities and complications by medical therapy are crucial for TSC patients. Our results provide information regarding acceptable dose-dependent response and tolerable side effects for TSC-associated renal AML to everolimus therapy, and provide the clinicians with another choice when following up their TSC patients.

TSC-associated renal AML is likely to grow, making these patients susceptible to comorbidities like spontaneous life-threatening haemorrhage. A retrospective analysis of patients with TSC showed that the risk of haemorrhage increases as renal AML enlarges, and a significant relationship has been noted between lesion size, aneurysm formation, and haemorrhagic rupture.[13,14] To determine the relationship between growth rate and size of renal AML, Tsai et al.[15] retrospectively analyzed patients with TSC, and reported the correlation between rate of growth of renal AML and age. It showed that the frequency of cysts/renal AMLs increased with age from 45.4% in preschool age (<6 years), 75.0% in school age (6-18 years), 85.7% in adulthood (18-30 years), to 100% in patients aged >30 years. Renal comorbidities increased from 0% in preschool age, 10% school age, 21.4% in adulthood, to 70% in patients aged >30 years. Furthermore, the size of renal AML was correlated with patient age, and growth rate tended to be greater in younger patients. Renal AML exhibits continued growth throughout the life of a patient with TSC, and as renal AML increases in

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size, patients should be continuously monitored. Therefore, serial growth is a risk factor for hemorrhagic events.

The most novel strategy for treating renal AML tumour burden in patients with TSC involves the use of targeted therapeutics focused on inhibition of the mTOR pathway, with the goal of halting further tumour progression and promoting regression of existing tumours.[16] Also known as rapamycin, sirolimus was initially developed as an immunosuppressive agent for use in solid organs transplantation. Sirolimus was the first mTOR inhibitor studied for the management of renal AML in the setting of TSC, and showed the significant volume reduction.[17] Everolimus is an mTOR inhibitor derived from sirolimus and effective anti-proliferative agent. Some studies demonstrated that everolimus was effective in reducing the volume of TSC-associated renal AML.[18] Both sirolimus and everolimus have been demonstrated to be effective for TSC-associated renal AML but with significant adverse effects in most patients.[19] For sirolimus, a two-year trial showed that rapamycin 1 mg daily was relatively effective and less aggressive, and was an available option for the management of TSC-associated renal AML.[20] Although data for sirolimus and everolimus are available to support the efficacy and safety in the treatment of patients with other TSC-associated comorbidities, everolimus currently carries U.S. Food and Drug Administration approval for treatment of adult patients with TSC-associated renal AML and paediatric and adult patients with TSC-associated SEGA.[21]

The Response Evaluation Criteria in Solid Tumours (RECIST) defines partial response (PR) as at least a 50% reduction in mass volume.[22] In the extension study of EXIST-2 trial, everolimus, initiated at 10 mg daily, achieved 44.2% and 55.3% PR at 12 and 24 months, respectively.[23] Our study revealed that all patients treated with everolimus responded to either at 2.5 mg or 5.0 mg daily. When stratified by dosage based on RECIST studies' defining, 3 of 4 patients in the 5.0 mg group achieved PR, whereas all patients of 2.5 mg group failed to

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achieve PR. The everolimus 5.0 mg group showed significant decrease in volume reduction ranging from 40.7% to 73.1% after the start of everolimus, which remained stable for 12 to 36 months of follow-up. This finding potentially implies that the cut-off dosage for successful treatment of TSC-associated renal AML is around 5.0 mg to achieve PR. Moreover, this finding suggests that the shrinkage effect of the drug is at its maximum at the beginning of treatment, probably owing to its anti-angiogenic effect. While no beneficial effect was observed after one year, some authors reported that by withdrawing the treatment, the renal AML volume increased again.[24-26] Therefore, it seems reasonable to assume that TSC patients with a large renal AML should receive mTOR inhibitors for lifetime.

Previous studies demonstrated that everolimus 10 mg daily significantly shrank the mass volume of AML.[11] In the initial aim of the current study, we began an oral everolimus at 2.5 mg and gradually titrated the dose to 5 mg daily based on tolerability. Some participants complained of the adverse events after titrated dose and hesitated to take the higher dose due to treatment intolerance. We observed that everolimus at 2.5 or 5.0 mg daily might significantly shrink the mass volume of TSC-associated renal AML, and everolimus at 5.0 mg produced more mass volume reduction than at 2.5 mg. Nevertheless, everolimus-associated comorbidities were observed in the patients on either 2.5 or 5.0 mg daily, and increased in severity for the higher dose group. Previous studies initiated at 10 mg daily reported that 54% of its study cohort achieved at least 50% reduction in AML volume.[22,23] In the present study, we found that the effect on the volume reduction rates of the everolimus 5.0 mg group ranged from 40.7 to 73.1% (mean 64.1%), similar to previous larger cohorts studies. The most commonly reported everolimus-related adverse reactions were stomatitis/oral ulcers. Although the occurrence of adverse events were noted after the start of treatment in this study, these were relatively minor in comparison to high dose used in previous reports.[11,23,27] Therefore, we suggest that the use of minimal dose to achieve the goal

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of therapy may benefit patients owing to less adverse events and low costs.

The limitations of this study were at a single centre, small sample size and variable follow-up durations. Everolimus was expensive and was paid by our enrolled patients themselves, which resulted in the small enrolled cases number in the current study. The number of reported cases presented herein may be too small to make a firm conclusion. The lack of a placebo group to compare the mass volume changes during study period could be considered as a major limitation. However, given the magnitude of the effects of everolimus therapy in this preliminary report, maintenance of an untreated arm would have been unethical.

Further prospective studies with larger cohorts and a longer follow-up period are required to address the minimal dosage of mTOR inhibitors that is effective to prevent TSC-associated renal AML growth after the initial reduction, the durability of responses, the duration of maintaining treatment and impact of toxicity from chronic therapy.

In conclusion, the study shows that all patients with TSC-associated renal AML responded to oral everolimus therapy, either at 2.5 or 5.0 mg daily. Additionally, the mass volume reduction remains stable during the 36 months of follow-up. The results of the study suggest that the responses in TSC-associated renal AML, treated with everolimus, are likely to be durable, and patients may require maintenance therapy to continue to derive benefit. Therefore, oral everolimus should be considered as a non-invasive way for management of patients with TSC-associated renal AML.

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Table 1 Baseline demographics and cutaneous features, neurologic comorbidities and genotypes of patients with TSC-associated AML, *n* = 8.

TSC, tuberous sclerosis complex; AML, angiomyolipoma; TAND, TSC-associated neuropsychiatric disorder; FA, facial adenoma; HP, hypomelanic patch; NU, nail unguinal; SP, shagreen patch; SEN, subependymal nodule; SEGA, subependymal giant cell astrocytoma; MR, mental retardation; CPS, complex partial seizure; GTCS, generalized tonic-clonic seizure; IS, infantile spasm; LAM, lymphangioleiomyomatosis.

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Case	Age (Year)	Sex	Cutaneous Features	Neurologic comorbidity			Genotype	Main causes for everolimus
				Brain lesions	TAND	Epilepsy		
1	14	M	FA, HP	Cortical tuber/SEN/SEGA	MR	CPS	*TSC2*	Large SEGA
2	20	F	FA, HP, NU, SP	Cortical tuber/SEN/SEGA	Normal	CPS, remission	*TSC2*	AML with previous partial nephrectomy
3	22	F	FA, HP, SP	SEGA/hydrocephalus (operation)	Normal	IS, CPS, remission	Not done	Large SEGA
4	26	F	FA, HP, NU, SP	SEN/SEGA/hydrocephalus (shunting)	MR	Refractory epilepsy	*TSC2*	Large SEGA, huge AML
5	27	F	FA, HP, NU, SP	Cortical tuber/SEN	Normal	GTCS,CPS, Remission	*TSC1*	Huge AML
6	31	F	FA, HP, NU, SP	SEN	MR	Epilepsy, remission	*TSC1*	LAM-associated pneumothorax
7	39	F	FA, HP, NU, SP	Cortical tuber/SEN/SEGA	Normal	IS, CPS, remission	*TSC2*	Huge AML
8	44	F	FA, HP, NU	Cortical tuber/SEN	Normal	None	*TSC1*	Huge AML, LAM-associated dyspnoea

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Table 2 Mass volume reduction of patients with TSC-associated AML treated with everolimus, $n = 8$.

Case	Dose (mg/day)	Trough level (ng/mL)	Body weight (Kg)	Maximum diameter (cm)	Mass volume (cm^3)		Volume reduction rate (%)	Adverse events
					Before	After		
1	2.5	3.14	80	7.5	82.3	51.6	37.2	Acne
2	2.5	Not done	54	2.1	2.3	1.3	45.3	Oral ulcers, acne
3	2.5	<1.5	55	2.3	1.9	1.3	30.3	Stomatitis, cellulitis
4	5.0	8.5	53	20.6	2951.5	793.5	73.1	Oral ulcers
5	2.5	2.4	62	24.5	3677.3	3292.7	10.5	Hyperlipidaemia
6	5.0	15.4	49	6.4	94.8	35.2	62.9	Diarrhoea
7	5.0	13.9	64	8.5	408.9	140.9	65.2	Oral ulcers
8	5.0	15.4	58	15.2	2000.1	1186.3	40.7	Oral ulcers, hypertension hyperlipidaemia, hyperglycaemia

TSC, tuberous sclerosis complex; AML, angiomyolipoma.

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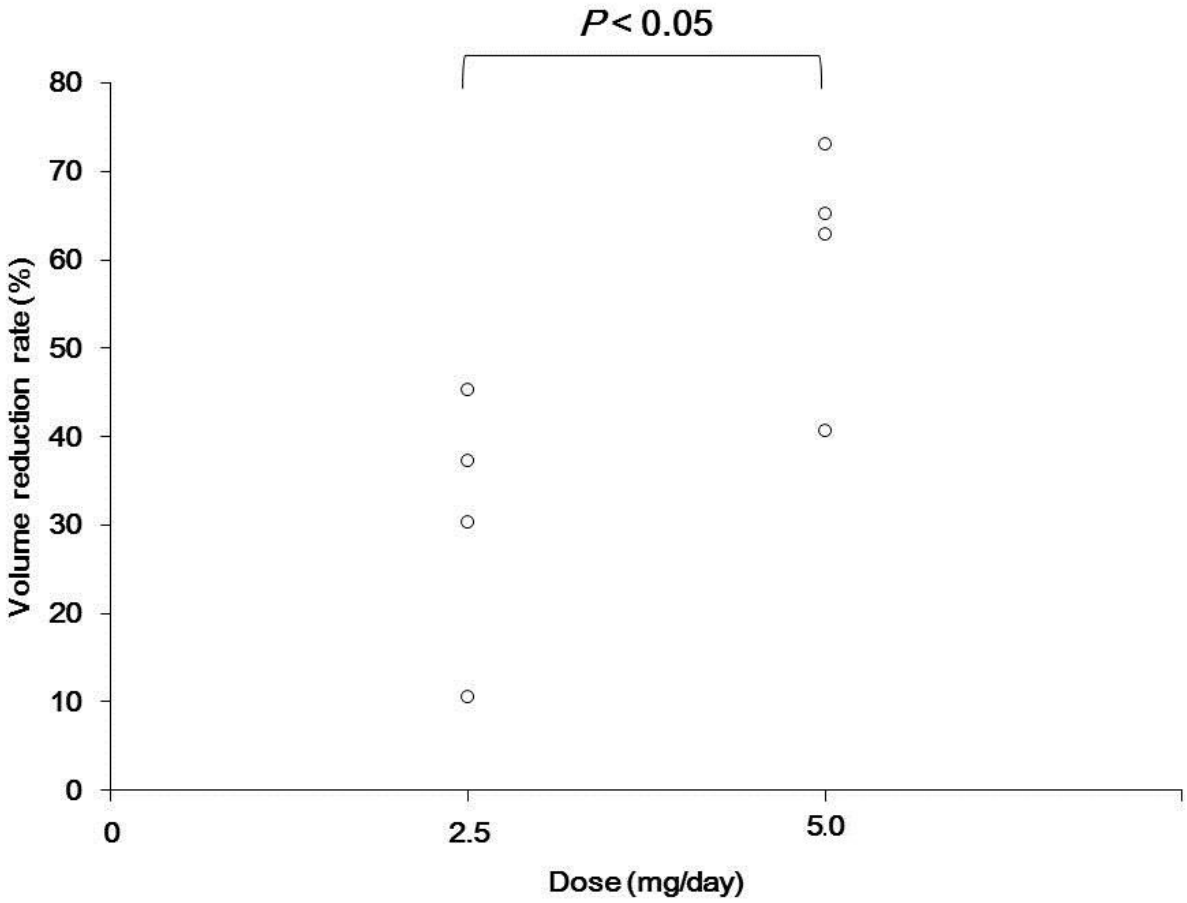

Figure 1. Comparison of the volume reduction rates of tuberous sclerosis complex-associated angiomyolipoma between the treatment of everolimus at 2.5 mg (n = 4) and 5.0 mg daily (n = 4).

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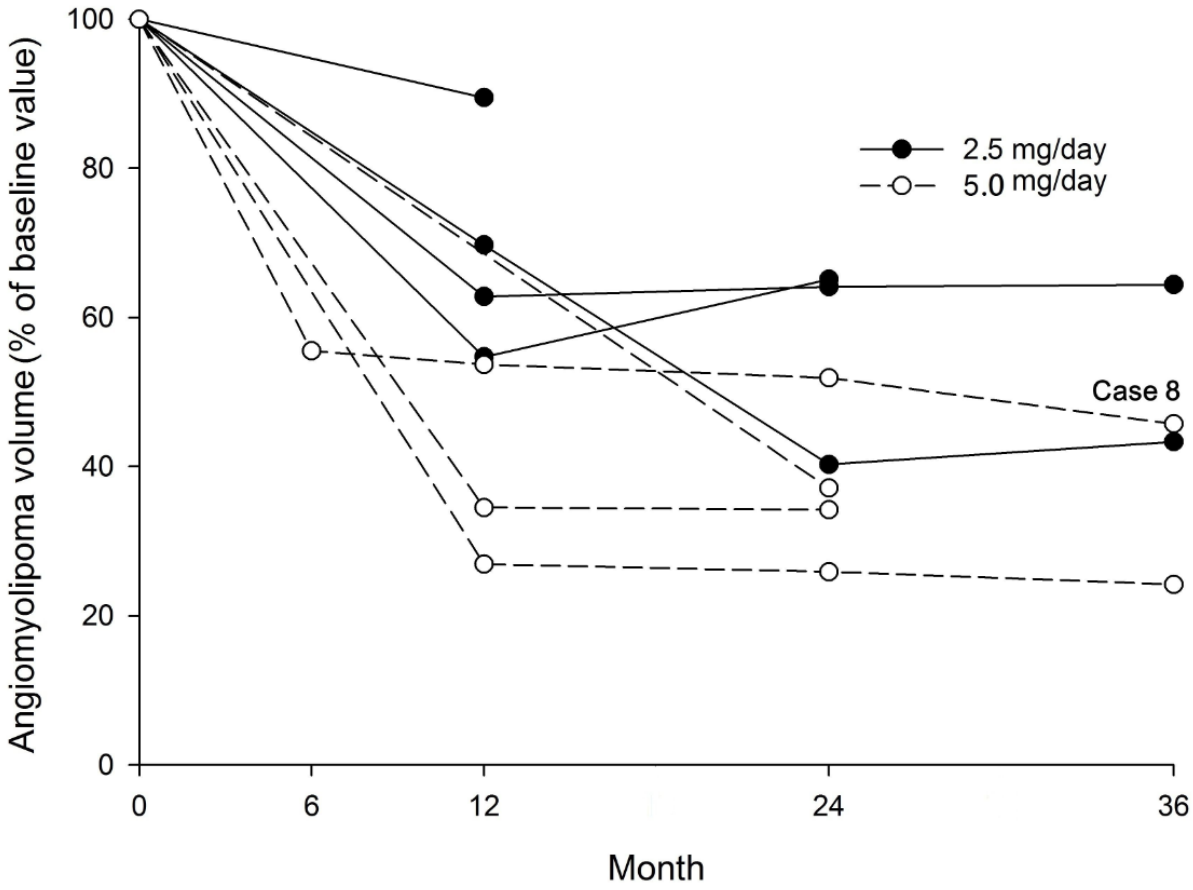

Figure 2. The effect of everolimus with either at 2.5 mg or 5.0 mg daily on renal angiomyolipoma volume reduction over time. The difference of angiomyolipoma volume reduction in the study patients was statistically significant between at baseline and after 12 months of treatment ($P < 0.05$). The mass volume reduction remained stable during follow-up up to 36 months.

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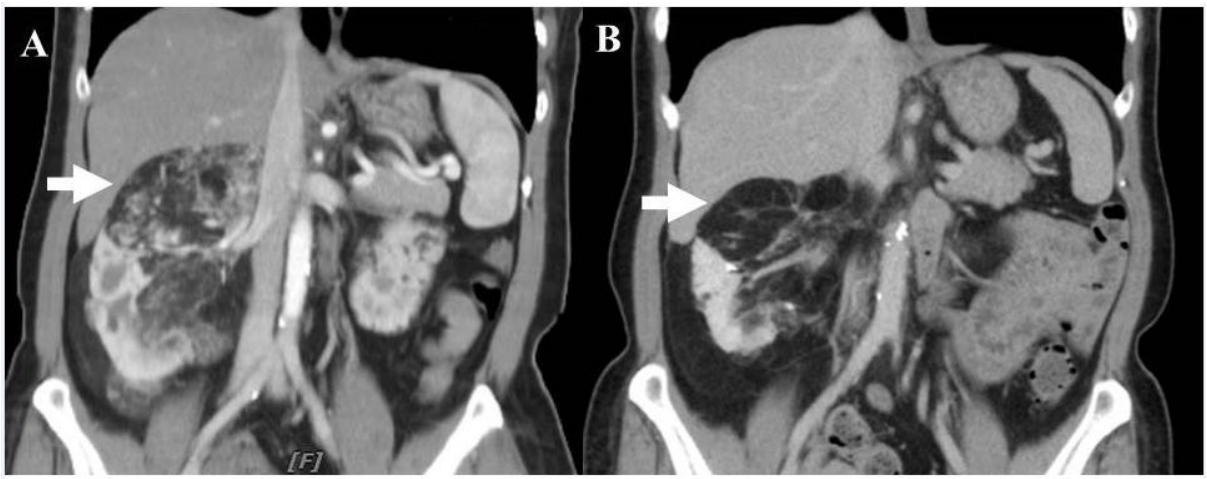

Figure 3. In case 8, angiomyolipoma mass volume rapidly reduced to 55.5% after 6 months of everolimus 5.0 mg (white arrow).

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