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Clinical report

Compassionate use of Everolimus for refractory epilepsy in a patient with *MTOR* mosaic mutation

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Key Words: mTOR, mosaic mTOR mutation, everolimus, seizures, therapy in rare diseases

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SUMMARY

The *MTOR* gene encodes the mechanistic target of rapamycin (mTOR), which is a core component of the PI3K-AKT-mTOR signaling pathway. Postzygotic *MTOR* variants result in various mosaic phenotypes, referred to in OMIM as Smith-Kingsmore syndrome or focal cortical dysplasia. We report here the case of a patient, with an *MTOR* mosaic gain-of-function variant (p.Glu2419Lys) in the DNA of 41% skin cells, who received compassionate off-label treatment with everolimus for refractory epilepsy. This 12-year-old-girl presented with psychomotor regression, intractable seizures, hypopigmentation along Blaschko's lines (hypomelanosis of Ito), asymmetric regional body overgrowth, and ocular anomalies, as well as left cerebral hemispheric hypertrophy with some focal underlying migration disorders. In response to the patient's increasingly frequent epileptic seizures, everolimus was initiated (after approval from the hospital ethics committee) at 5 mg/day and progressively increased to 12.5 mg/day. After 5 months of close monitoring (including neuropsychological and electroencephalographic assessment), no decrease in seizure frequency was observed. Though the physiopathological rationale was good, no significant clinical response was noticed under everolimus treatment. A clinical trial would be needed to draw conclusions, but, because the phenotype is extremely rare, it would certainly need to be conducted on an international scale.

Word count: 194

INTRODUCTION

The *MTOR* gene encodes the mechanistic target of rapamycin (mTOR), a highly conserved serine-threonine kinase and core component of the MTORC1 and MTORC2 complexes. The PI3K-AKT-mTOR signaling pathway is essential for cell growth, protein synthesis, autophagy, and cytoskeletal dynamics (1–3). The term “mTORopathies” has been used to characterize the clinical syndromes in which mTOR regulation is altered (4). Dysregulation, such as hyperactivation of the mTOR pathway, can lead to various phenotypes. These include cortical development malformations that result in neurological abnormalities, including drug-resistant seizures (5). In this context, the inhibition of mTOR signaling with rapamycin has become an increasingly common therapeutic approach in patients with epilepsy (6).

The most extensively reported use of mTOR inhibitors for the control of intractable seizures associated with mTORopathies is for tuberous sclerosis complex (TSC) (OMIM #191100 and OMIM #613254). EXIST-3, a recent, double-blind, randomized controlled trial of everolimus in TSC, reported that 40% of patients in the high-exposure group (everolimus target trough 9–15 ng/ml) showed a clinical response (i.e. defined as >50% reduction in seizure frequency from baseline) compared with 28% of patients in the low-exposure group (target trough 3–7 ng/ml), and only 15% in the placebo group (7). Because studies have demonstrated that mTOR inhibitors can be used with partial success in TSC-related seizures, additional trials have been conducted in patients with other mTORopathies that have common physiopathological mechanisms (i.e. upregulation of the mTOR signaling pathway) (8,9). For instance, one study that explored the effects of rapamycin in polyhydramnios, megalencephaly, and symptomatic epilepsy (PMSE) syndrome reported a major decrease in the frequency of seizures (i.e. during the 12 months of treatment, four of five patients were seizure-free and the fifth had only one seizure) (8). Recently, a case report described the efficacy of rapamycin for intractable seizures in an infant diagnosed with

hemimegalencephaly and a mosaic *MTOR* mutation present in 16% of reads, found following deep-sequencing of brain tissue after surgery. While awaiting hemispherectomy, rapamycin treatment led to >50% reduction in seizures and the total seizure burden after 1 week of treatment (9). However, there is still limited clinical data concerning the efficacy of mTOR inhibitors in non-TSC epilepsy with *MTOR* mutation (4).

Here, we describe the case of a patient with a mosaic *MTOR* mutation associated with hypomelanosis of Ito (HMI), asymmetric regional body overgrowth and left cerebral hemispheric hypertrophy with some focal underlying migration disorders who received compassionate off-label treatment with everolimus for intractable seizures and psychomotor regression.

PATIENT AND METHODS

Case report

The patient was a 12-year-old female with a clinical diagnosis of severe syndromic HMI. She was the third and only affected child of non-consanguineous parents. Since birth, she had linear hypomelanosis along Blaschko's lines (Figure 1A) combined with left corporal hemihypertrophy, hemimegalencephaly and hypotonia with spina bifida occulta. Her developmental milestones were delayed, with the ability to sit acquired only at the age of four, a severe language delay and autistic-like behavior. Retinitis pigmentosa was found on eye fundus examination. Peripheral hypothyroidism was diagnosed at age five, and she was treated with L-thyroxine. A percutaneous endoscopic gastrostomy was done at age six because the patient was experiencing frequent food inhalation and impaired esophageal motility. At seven years of age, peripheral premature puberty was diagnosed.

The patient started experiencing tonic-clonic seizures at three years of age. She was initially treated with sodium valproate, later in combination with clobazam and topiramate. Her

epilepsy was well controlled up to the age of 8 years, at which point the frequency of seizures intensified to approximately 40 a day. No other antiepileptic drugs, alone or in combination were able to control, even partially, the seizures (see Supplementary table 1). A 3-month ketogenic diet also failed to decrease the frequency of the seizures.

At ten years of age, the epilepsy remained highly intractable and polymorphic, combining atypical absences, focal seizures with secondary generalization, apparently generalized tonic-clonic seizures, and both convulsive and non-convulsive episodes. Her neurological status deteriorated, she stopped speaking, she developed urinary and fecal incontinence, and she could not walk (i.e. she used a wheelchair for ambulation). Brain magnetic resonance imaging (MRI) showed focal cerebral hypertrophy involving the whole right hemisphere with focal enlargement of the left caudate nucleus with subsequent distortion of the inter-hemispheric fissure (white arrows). Sub-ventricular heterotopia facing the external border of the left occipital horn as well as a slight band of grey matter heterotopia within the left occipital white matter were present. These latter features were suggestive of underlying migration disorders.

Standard karyotype and array-based comparative genomic hybridization on blood- and skin-derived DNA samples were normal. In-depth whole exome sequencing performed on hypopigmented skin found a mosaic *de novo* missense change of *MTOR* (c.7255G>A, p.Glu2419Lys [RefSeq accession number NM_004958.3]) in 21 of 74 reads (28%), found in the COSMIC database. Analysis of dermal fibroblasts showed upregulation of mTOR signaling, with increased phosphorylation of AKT and p70 ribosomal protein S6 kinase (data not shown).

Everolimus treatment rationale and monitoring

Considering the limited therapeutic alternatives, we hypothesized that everolimus could be a therapeutic option, as observed in studies of TSC patients with intractable seizures (7,10).

Moreover, our patient's activating *MTOR* mutation (p.Glu2419Lys) had previously been shown to be rapamycin-sensitive *in vitro* and *in vivo* (11,12).

Following approval by the Ethics Committee of Dijon University Hospital, we initiated off-label everolimus therapy. Low-dose treatment was initiated (5 mg) and progressively increased to obtain blood levels between 5 and 15 µg/L, as previously reported in TSC (10). Clinical examination by a neuropsychiatrist, laboratory tests (i.e. blood count, uremia, creatinemia, proteinuria, glycemia, cholesterolemia) and blood levels of everolimus were performed before treatment initiation, two weeks and one month later, and every following month to assess compliance and tolerance.

Treatment efficacy was assessed as follows: regular seizure count by the parents, 24-hour video-electroencephalography (VEEG) monitoring (before treatment initiation and every month), and Tremblay and Nisonger neuropsychological scales used before and at the end of treatment.

The Tremblay scale (TS) contains several items to evaluate global development (i.e. total score out of 404 points) in terms of skills and abilities. A higher score for each item and for the overall score indicates a more competent individual. The Nisonger scale explores the behavioral components of children with intellectual disabilities aged 3 to 16 years. Higher scores indicate more severe behavioral disorders. The USQOLCE (13) was also used before and at the end of treatment to evaluate quality of life.

RESULTS

Treatment was initiated at 5 mg/day and further increased to 12.5 mg/day. The aim was to obtain a blood dosage between 5 and 15 µg/L (Figure 2B). The patient experienced a few mild adverse events (diarrhea, alopecia and acne) (Figure 2C). Some adverse events were only noted for few days, whereas asthenia was constant during all the everolimus period treatment.

Blood count normalized at the everolimus end period. After five months of treatment, the number of seizures increased (120 seizures/month and 130 seizures/month at baseline and after 5 months of treatment respectively) (Figure 2A) and the duration of the seizures gradually increased. At the first month of everolimus treatment, seizures duration varying between 30 seconds and 5 minutes, whereas after two months of everolimus treatment, 60% of seizures lasted between 90 seconds and 5 minutes and some seizures lasted more than 5 minutes. The initial 24-hour EEG showed diffuse abnormal background activity (during both arousal and sleep) with diffuse multifocal spikes, slow waves and theta activity elements in the left fronto-central and inferior cerebral region. The results of the EEGs performed during treatment were similar to pretreatment EEG. Of note, besides lacosamide and lamotrigine, no other medication was given during the period of everolimus treatment, and no change in dosage of these medications has been made. Only amoxicilline and salbutamol were given during 7 days for a suspicion of pneumonia at the end of the third month of everolimus treatment.

Clinically, various types of seizures were observed (myoclonic seizures, brief tonic contractions and tonic-clonic seizures). The TS reflected a decline in global cognitive function (TS total score of 167/407 and 121/407 at baseline and 6 months respectively) with a deterioration in sensory abilities, communication, autonomy and cognitive and social development items. However, there was a modest improvement in motor and information-collecting skills (TS score of 22/76 and 16/32 vs. 28/76 and 20/32 before and after everolimus treatment, respectively). The Nisonger scale showed a mild deterioration for the item “difficulty to keep calm or adapted behavior” (9/18 before everolimus vs. 13/18 after everolimus) while the other items remained stable (global behavioral score of 63/210 before everolimus vs. 60/210 after everolimus). Quality of life remained stable (8/10 before and after everolimus in USQOLCE). Following a multidisciplinary meeting and discussion with the

parents, everolimus was definitively withdrawn after five and a half months of treatment.

After stopping the medication, alopecia and acne disappeared progressively in several months.

Asthenia persisted after the everolimus treatment but behavioral improvements were noted, with a more smiling attitude for example.

DISCUSSION

In this case of intractable seizures with a mosaic *MTOR* mutation, everolimus treatment showed no beneficial effect, even though the rapamycin-sensitivity of the activating *MTOR* mutation of the patient was confirmed *in vitro* and *in vivo* (11,12). Indeed, sensitivity to rapamycine of yeast cells carrying the p.Glu2419Lys have been demonstrated *in vitro* (11), and later Wagle et al demonstrated sensitivity to everolimus in a patient with an advanced solid tumor carrying the same variant in his tumor DNA (12). The literature also provided support for this type of treatment, including previous trials using mTor inhibitors, first in animal models (14), and then in humans with TSC (4,7,10), another disease with increased mTOR activity, and other mTORopathies (8,9).

There are several factors that may explain the failure of everolimus in this case. Firstly, the severity of the epilepsy may have contributed to the failure of this umpteenth anti-epileptic treatment despite the specificity of this mTOR inhibitor (15). Secondly, the mosaic form of the mutation may have led to a difference in efficacy compared with the results of trials conducted on TSC patients. However, the previous study that reported a 50% decrease in seizure frequency in a context of hemimegalencephaly with intractable epilepsy and a mosaic *MTOR* mutation only obtained a molecular diagnosis after surgery, and rapamycin treatment was given before surgery (9). Finally, in our case, the cerebral imaging pattern was more suggestive of a focal cerebral hypertrophy involving the left hemisphere with underlying migration disorders than a true hemimegalencephaly, which, on a pathological point of view,

represents a disorder of precursor cell phenotype characterized by “balloon cells” leading to both diffuse cortical dysplasia and extensive white matter changes within the involved hemisphere (16).

In conclusion, the inhibition of the mTOR activation pathway with everolimus did not result in a clinical benefit in this case. The severity and advanced state of the refractory epilepsy, and the mosaic form of the *MTOR* mutation may have contributed to the failure of what appeared to be a promising treatment with a strong physiopathological rationale. This study highlights the challenges of treating heterogeneous developmental diseases. Before any formal conclusions can be made, additional case series and clinical trials will be needed to assess the efficacy of mTOR inhibitors in mTORopathies other than TSC, in particular in cases of mosaic *MTOR* mutations.

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LEGENDS TO FIGURES AND TABLES

Figure 1. Clinical manifestations and brain magnetic resonance imaging (MRI) of our case

A: Photographs of the index case show hypopigmented skin lesions following Blaschko's lines on the left part of the abdomen (a), left arm (b), and left leg (c)

B: Brain MRI at 5 (a,b,c) and 12 (d) years

Axial T1-weighted images (a,b) show focal cerebral hypertrophy involving the left hemisphere with focal enlargement of the left caudate nucleus (*) with subsequent distortion of the inter-hemispheric fissure (white arrows). Coronal T2-weighted (c) and T1-weighted inversion recovery (d) images demonstrate both sub-ventricular gray matter heterotopia (c :black arrows, d : long white arrow) as well as a slight band of grey matter of heterotopia (small white arrows) suggestive of underlying migration disorders, despite an imaging pattern non suggestive of a true hemi-megalencephaly

Figure 2. Follow-up of Everolimus treatment

A: Graphical representation of the monthly frequency of seizures for the index case according to everolimus dosage over time

B: Change in daily dose and blood levels of everolimus

C: Summary of the adverse effects of everolimus

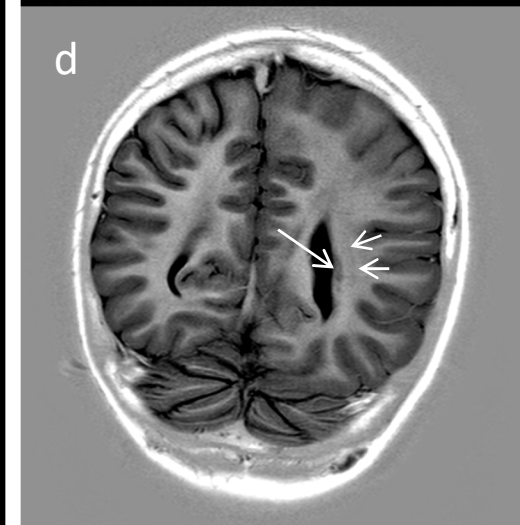
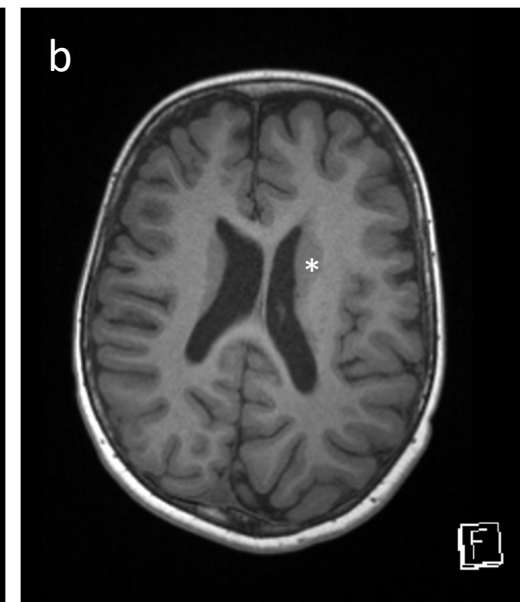
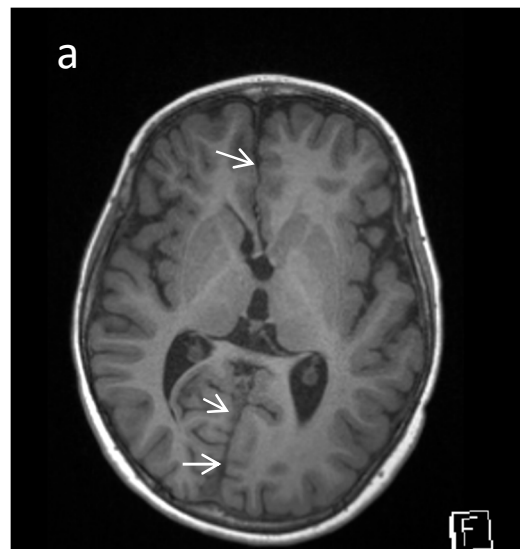
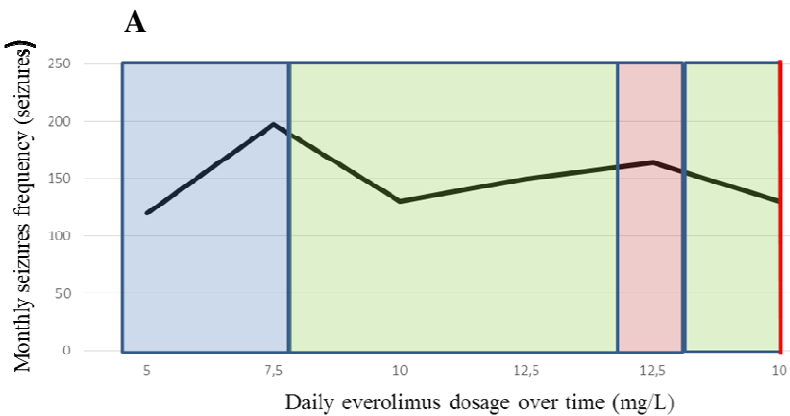
A**B**

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	Underdose blood measurement of everolimus
	Efficient blood measurement of everolimus
	Overdose blood measurement of everolimus
	Treatment Withdrawal

B

Days	0	16	26	45	60	75	90	120	127	150 ^a	165	171	180
Daily everolimus dosage (mg)	5	7.5	7.5	10	10	12.5	12.5	12.5	10	10	5	2.5	X
Everolimus blood measurements (ug/L)		3.4		5	10.4	10.1	9.2	18	14.4	11.9			

Everolimus blood concentration monitoring (target range between 5 and 15 µg/L)	
	No everolimus blood concentration measurements
	Underdose blood concentration of everolimus
	Efficient blood concentration of everolimus
	Overdose blood concentration of everolimus
a	Treatment Withdrawal

C

Days	Daily everolimus dosage (mg)	Everolimus blood levels (ug/L)	Adverse events (AE)
16	7.5	3.4	Mild asthenia (AE of grade 1 according to Common Terminology Criteria for Adverse Events (CTCAE) version 4.0)
26	7.5	No everolimus blood measurement was down	Facial acne, asthenia, hypersialorrhea, mild leukopenia and neutropenia (neutrophils at 1480 mm ³) (AE of grade 1)
45	10	5	Facial and forearms acne (AE of grade 1)
60	10	10.4	Stabilisation of leukopenia and neutropenia
75	12.5	10.1	Mild side effects including alopecia, asthenia, acne, epistaxis, and unexplained fever with rhinorrhea and diarrhea (AEs grade 1)
90	12.5	9.2	Alopecia worsening, asthenia, fluctuating behavior
120	12.5	18	Alopecia worsening but hair loss of <50% of normal for the index case (AE of grade 1)
150	10	11.9	Normalization of complete blood count

Figure 2. Follow-up of Everolimus treatment