Yves Pirson

Nephrology, Université Catholique de Louvain, Bruxelles, Belgium

Correspondence and offprint requests to: Yves Pirson; E-mail: yves.pirson@uclouvain.be

In a pioneering study published in 2008, Bissler et al. showed that sirolimus, an immunosuppressive drug that acts by inhibiting the mammalian target of rapamycin (mTORi) and is currently used in organ transplantation and cancer treatment, was able to decrease the volume of renal angiomyolipomas (AMLs) in patients with tuberous sclerosis complex (TSC) [1]. Over the last year, three other independent trials conducted by other teams in similar patients have confirmed and extended this remarkable step forward [2-4]. Together with other works demonstrating a comparable efficacy of sirolimus or everolimus on the neurological [5], pulmonary [6] and cutaneous [7] manifestations of TSC, these papers establish mTORi as the first medical treatment of this so far incurable disorder.

Since mTORi is, however, a treatment with a concerning safety profile and since not all patients with TSC-associated AMLs require intervention, the time has come for nephrologists to examine the pros and cons of this new treatment and try to draw some insights into the current management of their patients.

Before scrutinizing the above-mentioned trials on the treatment of TSC-associated AMLs with sirolimus, the main clinical and genetic features of TSC are briefly reviewed and the rationale for treatment with mTORi is summarized. Then, after weighing the burden of mTORi side-effects, current indications are discussed mentioning the remaining uncertainties and unanswered questions.

TSC is an autosomal-dominant disorder characterized by the development of benign tumours (hamartomas) in multiple organs. The term tuberous describes the potato-like appearance of brain lesions observed by the French neurologist Bourneville in the late 1880s [8, 9]. The clinical diagnostic criteria have been well defined [10]. The disorder has a birth rate of 1 in 6000 [11]. The main organs involved are the brain, skin, lungs and kidneys, with a wide diversity of symptoms and severity across patients.

About 85% of children and adolescents with TSC have neurological manifestations including epilepsy, cognitive impairment and behavioural problems, whereas a subset of affected adults have no signs of cerebral manifestations and have a normal mental status [8, 12]. Brain lesions mainly consist of cortical tubers, subependymal nodules and subependymal giant-cell astrocytomas, whose growth is a fearful complication.

Skin manifestations, present in 90% of patients, include hypomelanotic macules, shagreen patches, ungual fibromas and facial angiofibromas typically involving malar eminences and nasal labial folds.

Lung involvement, also called lymphangioleiomyomatosis (LAM), is characterized by proliferation of smooth-muscle cells and cystic changes within the parenchyma. Affecting almost exclusively women and present in about one-third of them, it manifests, in a minority of them, by dyspnoea or pneumothorax and may lead to respiratory insufficiency.

Renal AMLs occur in up to 80% of patients with TSC and represent the most common cause of TSC-related morbidity in adults [9]. AMLs manifest as multiple and bilateral tumours composed of blood vessels, smooth-muscle and fat cells [13]. Because vasculature is abnormal with frequent aneurysm development, spontaneous haemorrhage, sometimes life-threatening, may occur. The risk factors for AML bleeding are the size of AML (>4 cm), the size of aneurysm(s) (>0.5 cm) and the growth of AMLs, as assessed by serial measurements [14, 15].

In addition to AMLs, kidney lesions include cysts (occurring in 30% of patients and mimicking autosomal-dominant polycystic kidney disease (ADPKD) in 3% of patients with TSC) and carcinoma (occurring in 3% of patients with TSC) [13].

A few patients experience renal failure resulting from kidney parenchymal loss from AMLs growth and/or surgery/embolization; kidney transplantation may then be considered [16].

The only treatment of AML has hitherto been surgery—as nephron sparing as possible—or embolization. The identification of the two genes in which mutations were found in TSC patients was the starting point in the deciphering of the...
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pathophysiological pathways linking gene mutation and hamartoma formation, culminating with the discovery of the role of the mTOR cascade, and opening the way for drug intervention.

**mTORi AS A HOPE**

Since the identification of the TSC1 and TSC2 genes [17, 18], more than 300 allelic variants of TSC1 and more than 1000 of TSC2 have been reported. The mutation detection rate is ~90% [8]. Mutations in TSC2 are more common and are associated with a more severe phenotype than those in TSC1 [8]. A contiguous deletion in TSC2 and PKD1 accounts for the subset of patients with a severe cystic phenotype often leading to renal failure.

Both TSC1 and TSC2 act as tumour suppressor genes: the inactivation of both alleles of TSC1 or TSC2 is thus required for AML development (in addition to germlinal mutation, a second, somatic mutation occurs later, resulting in a complete loss of function). Interestingly, in women with LAM, identical somatic mutations of TSC2 have been identified in abnormal lung and kidney cells, but not in normal cells, suggesting that LAM and AML cells have the same origin. This led to the hypothesis that LAM results from ‘benign metastasis’ of AML cells to the lung [13]. This should be kept in mind when medical treatment of AML is considered in patients with or are at risk for LAM (see below).

After the discovery of TSC1 and TSC2 and their encoded proteins (hamartin and tuberin, respectively), the two proteins were found to form an intracellular complex interacting with a variety of other proteins [8]. Of crucial importance, this complex was found to physiologically inhibit a serine-threonine-kinase, known as mTOR, which has a central role in the control of cell growth and proliferation (Figure 1A) [13]. A complete loss of hamartin or tuberin allows for an unregulated activation of mTOR with subsequent stimulation of cell growth and proliferation (Figure 1B).

The available mTORi drugs, such as sirolimus (or rapamycin) and everolimus, were therefore quickly identified as potential therapeutic agents, able to restore the physiological inhibition of the pathway (Figure 1C). In rat and mouse models of TSC, inhibition of mTOR by sirolimus effectively reduced cell proliferation and tumour size [19, 20]. Together with a few case reports showing regression of astrocytoma [21] and AML [22] under sirolimus treatment, these experimental data prompted the launch of clinical trials with mTORi in TSC patients.

**mTORi AS AN EFFECTIVE TREATMENT OF AML**

The Cincinnati team demonstrated for the first time the efficacy of mTORi on AML. In 20 patients with TSC and/or LAM, sirolimus was given at an initial dose adjusted to achieve a blood drug level between 1 and 5 ng/mL. If the total AMLs volume had not decreased by 10% of the baseline value at 2 months, the target blood drug level was increased to 5–10 ng/mL; if this threshold was not yet reached at 4 months, the level was further increased to 10–15 ng/mL. After 12 months of treatment, the mean AML volume was 53% of the baseline value [1]. In 17 patients with only TSC, Cabrera et al. later showed that, with a dose of sirolimus adjusted on a fixed target drug level of 4–8 ng/mL, the volume of the largest AML was reduced to a mean of 66% at 12 months [4]. In their trial including 16 patients with TSC and/or LAM, Davies et al. started with a sirolimus dose adjusted to achieve a blood level between 3 and 6 ng/mL and, if the largest diameter of target lesions was not reduced by 10% at 2 months, the dose was increased to reach a (maximum) level between 6 and 10 ng/mL. At 12 months, the mean sum of the longest diameters of all target AMLs (calculated from individual figures provided in the paper) had decreased by 39% [2]. In the last study, conducted by Dabora et al., 36 patients with TSC (with or without LAM) received sirolimus at an initial dose adjusted on a blood level of 3–9 ng/mL, with a further increase at 4 months to a target level of 9–15 ng/mL if the mean size of targeted AMLs (as the sum of the largest diameters) had not decreased by 30%. At 12 months, the mean decrease in the total AMLs size was 30% [3].

Comparing the mean percentage decrease in AML volume or size reveals an apparently lower decrement in Davies’ and Dabora’s patients (Table 1). The explanation very likely lies in the end-point definition: the two latter authors used indeed a one-dimensional measurement (sum of the longest diameters of all evaluable AMLs), whereas the two others used the volume (generated by a software program) of either all evaluable AMLs (Bissler’s trial) or only the largest one (Cabrera’s trial), which more than doubles the effect on diameter. That Cabrera et al. obtained the best result—despite a drug trough level rather lower than in the other trials—could have been affected by an additional methodological particularity, that is, the measurement of the largest AML only. The largest tumour could actually be that with the highest proliferative activity and, hence, with the highest sensitivity to the effect of the drug.

Whatever the explanation, the overall results of these four trials using sirolimus are impressive. The preliminary data from the EXamining everolimus In a Study of TSC 2 (EXIST-2) trial (NCT00790400), assessing the effect of everolimus, another mTORi, in 118 patients with TSC and/or LAM and ≥1 AML with longest diameter ≥3 cm are in the same line. The proportion of patients with a ≥30% reduction rate in the sum of volumes of all target AMLs relative to baseline reached 76 and 80% at weeks 12 and 24, respectively, in the everolimus group compared with 6 and 3%, respectively, in the placebo group [23].

The timing of response is also of interest. As seen in the EXIST-2 trial, most of the effect is obtained within the first 6 (and even the first 3) months with a subsequent stabilization of the tumour, in the majority of patients, over the next 6 months.

Finally, as expected for a drug palliating a constitutionally defective pathway, its effect vanishes after withdrawal [1, 3]—with further regrowth of AML—thus, implying the need for a lifelong treatment.
It is, therefore, essential to balance the benefits of the treatment against its side-effects, which, in this case, are potentially numerous.

**mTORi AS A TREATMENT WITH A CONCERNING SAFETY PROFILE**

We know from our transplantation practice that the use of mTORi may be limited by a number of side-effects, some of which are serious and thus of concern for a lifetime treatment. Several of them are likely related to a generalized antiproliferative activity.

Mouth ulcers occur frequently. This side-effect is dose-related and is usually easily managed by topical corticosteroids and dose reduction [24]. Mild lower-limb oedema is also frequent and usually acceptable. As previously shown in transplanted patients, mTORi impair wound healing [25]. This should be kept in mind in the management of patients under mTORi in whom a surgery is planned.

**Table 1. Effect of sirolimus treatment on AML size/volume in four open-label trials**

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td><strong>Patients</strong></td>
<td>n = 20</td>
<td>n = 16</td>
<td>n = 36</td>
<td>n = 17</td>
</tr>
<tr>
<td></td>
<td>6: TSC only</td>
<td>7: TSC only</td>
<td>15: TSC only</td>
<td>all TSC only</td>
</tr>
<tr>
<td></td>
<td>8: TSC + LAM</td>
<td>3: TSC + LAM</td>
<td>21: TSC + LAM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6: sporadic LAM</td>
<td>6: sporadic LAM</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Inclusion criterion</strong></td>
<td>≥1 AML ≥1 cm</td>
<td>≥1 AML ≥2 cm</td>
<td>≥1 AML ≥2 cm</td>
<td>≥1 AML &gt;2 cm</td>
</tr>
<tr>
<td><strong>Maintenance sirolimus troughlevel (ng/mL)</strong></td>
<td>1–5 in 1</td>
<td>3–6 in 12</td>
<td>3–15</td>
<td>4–8</td>
</tr>
<tr>
<td></td>
<td>10–15 in 19</td>
<td>6–10 in 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>End point</strong></td>
<td>Total AMLs volume (MRI)</td>
<td>Total AMLs size(^a) (MRI)</td>
<td>Total AMLs size(^a) (MRI)</td>
<td>Volume of the largest AML (MRI)</td>
</tr>
<tr>
<td></td>
<td>47% in volume</td>
<td>39% in size</td>
<td>30% in size</td>
<td>66% in volume</td>
</tr>
</tbody>
</table>

\(^a\)As defined by the sum of the longest diameters of all target AMLs.
Dyslipidaemia (hypercholesterolaemia and hypertriglyceridaemia) is a well-known drawback of mTORi, implicating that a fraction of patients will need additional treatment with a lipid-lowering drug.

Gonadal effects are less well-known, since they were only rarely assessed. There are, however, a few reports from transplant centres which clearly show that sirolimus treatment is associated with a significantly reduced total sperm count, a decreased proportion of motile spermatozoa and a pattern of hypergonadotrophic hypogonadism, strongly suggesting gonadal toxicity [26, 27]. Along the same line, menstrual-cycle disturbances were reported in 52% of 18 ADPKD patients enrolled in the Swiss sirolimus trial [28]. This largely underestimated that toxicity of mTORi would warrant further evaluation and should be taken into consideration in young patients embarking on treatment.

Acute interstitial pneumonitis is a rare but serious and potentially life-threatening side-effect of sirolimus. The clinician should quickly evoke this complication in front of a patient presenting with fever, dyspnoea and cough [24] since it is usually reversible upon the drug withdrawal.

Most vexing for the nephrologist is the occurrence of mTORi-induced proteinuria. That sirolimus may cause or worsen proteinuria has been unequivocally shown in transplanted patients [24, 29]. In the Swiss trial evaluating the effect of sirolimus on ADPKD patients, Serra noticed that, after 18 months of treatment, albuminuria was 138% of that in the control group [30]. The mechanism of proteinuria likely includes both a glomerular and a tubular component. Several experimental data support a direct toxic effect of mTORi on podocyte function and structure. Both in cultured human podocytes and in kidney graft biopsies, the slit diaphragm-associated proteins (such as nephrin and podocin) expression is reduced under mTORi treatment, in a clearly dose-dependent manner [31]. In mice carrying a podocyte-selective knockout of the Mtor gene as well as in cultured human podocytes treated with sirolimus, podocytes accumulated autophagosomes, suggesting that inhibition of mTOR disrupts the autophagic pathway in podocytes resulting in toxicity to the cell [32]. In cultured human proximal tubular epithelial cells, mTORi decrease albumin endocytosis and downregulate cubilin and megalin expression; interestingly, these effects were reversed by the use of ramipril or losartan, strongly suggesting that this effect is exerted via an angiotensin-dependent pathway [33]. In the clinical setting, there is a paucity of data on the effect of angiotensin blockers on mTORi-induced proteinuria, and their interpretation is made difficult by the usual concomitant tapering of mTORi dose [34]. Not unexpectedly, microalbuminuria developed in 30% of sirolimus-treated TSC patients reported by Dabora et al. [3]; more worrying, this culminated in a full-blown nephrotic syndrome in one of Cabrera’s patients, reversible after sirolimus withdrawal [4]. Chronic kidney toxicity is thus a concern from a long-term treatment perspective. In practice, screening mTORi-treated patients for albuminuria is warranted.

It should also be remembered that like any immunosuppressant, sirolimus may increase susceptibility to infections. Fortunately enough, the data of the available trials in TSC patients do not point to a higher incidence of serious infections.

Most of the above described side-effects are dose-related. Among the four trials performed in patients with AML-associated TSC, stomatitis, for example, was reported in only 30% of the patients reported by Cabrera’s who used the lowest mean dose of sirolimus, versus 37–85% in the three other series using a higher dose [1–4]. As a final consideration, an abnormal kidney function is a recognized risk factor for the occurrence of side-effects, especially proteinuria [35].

**Towards a Careful Use of mTORi in TSC-Associated AMLs**

Weighing the remarkable effect of mTORi on the growth of AML and their significant potential toxicity, in which TSC patients should the nephrologist consider today this treatment as a valuable option?

First of all, it should be remembered that TSC is a systemic disease: any patient subjected to such a treatment should be evaluated by a multidisciplinary team (nephrologist, urologist, neurologist, pneumologist and dermatologist) and undergo a comprehensive work-up including cerebral, thoracic and abdominal magnetic resonance imaging (MRI) and skin lesion photographs. Management at an experienced centre for TSC is highly recommended, with the inclusion of the patient data in the ongoing international TSC Registry.

If a patient presenting with AML also harbours subependymal giant cell astrocytoma (SEGA) or LAM, this extra-renal involvement can be a good reason to initiate mTORi treatment. On the basis of a Phase-II trial with everolimus, this drug has been approved by both Food and Drug Administration and European Medicine Agency for the treatment of TSC-associated SEGA requiring intervention but not amenable to surgery; noteworthy, this is the only indication currently approved by these agencies. Knowing the gloomy prognosis of advanced LAM, giving mTORi treatment to patients suffering from symptomatic or worsening LAM also seems worthwhile. What about the TSC patient in whom kidney AML is the only clinically relevant involvement?

Since the growth of the tumour is an important determinant of the treatment strategy, serial measurement is recommended, if possible by MRI at yearly intervals (or every 6 months for a fast growing AML). For small (<3 cm) and stable AMLs, watchful waiting remains today reasonable. For larger and fast growing AML, intervention is justified, essentially to prevent bleeding. As detailed above, in addition to the size and growth rate, the presence of aneurysms has to be considered in the decision to intervene [36]. Historically, the only treatment modality was surgery. Nowadays, prophylactic subselective embolization has superseded surgical techniques. Even in emergency cases, prompt embolization is used.

However, any surgery or embolization leads to the loss of healthy nephrons. In addition, nearly 90% of patients undergoing embolization suffer from a so-called post-embolization syndrome including fever, vomiting and pain. Recently, the use of radiofrequency ablation has been proposed as an...
alternative technique, potentially more nephron-sparing and better tolerated [37]. Moving forwards mTORi has become a novel option, able to spare more nephrons and, above all, to avoid further AML growth. Though their place is yet to be determined, available data has led to the consideration of their use as first-line treatment in patients with remaining AML who previously underwent nephrectomy or embolization as well as in those with fast growing multiple AMLs. Moreover, when assessing indication of medical treatment, the benefit effect on other organs (including skin) should not be neglected. At the present time, an individualized approach is recommended.

The next question is how to initiate treatment, and then, how to continue it in the perspective of a lifetime therapy. In an effort to determine proper dosing, Bissler et al. examined in 30 patients two different daily doses (5 or 10 mg) and three different weekly doses (30, 50 or 70 mg) of everolimus during 2 years. Interestingly, both weekly and daily dosing resulted in a similar 50% reduction in AML volume, raising the possibility that lower doses and different dosing schedules may be a valuable option in the long term [38].

Of course, treated patients have to be carefully followed with a particular attention to the potential toxicity of mTORi.

Since the schedule of administration is yet to be defined, inclusion of new patients in future trials designed to determine this is warmly encouraged. Among the many unanswered questions for future studies are the effect of mTORi on the vascular component of AML, the growth of kidney cysts and the prevention of carcinoma.

Scientists, clinicians, patients’ associations and pharmaceutical companies have to be congratulated to have been able to promptly join their forces and provide in a relatively short time the concerned families with the first effective medical treatment of TSC, allowing us to leave the era of contemplation to that of (a thoughtful) action. The dawn is breaking.

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CONFLICT OF INTEREST STATEMENT

None declared.

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Recent insights into C3 glomerulopathy

Thomas D. Barbour,
Matthew C. Pickering
and H. Terence Cook*

*Correspondence and offprint requests to: H. Terence Cook; E-mail: t.h.cook@imperial.ac.uk

Centre for Complement & Inflammation Research (CCIR), Division of Immunology and Inflammation, Department of Medicine,
Imperial College London, London W12 0NN, UK

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dense deposit disease (DDD), C3 glomerulonephritis (C3GN) and CFHR5 nephropathy. These disorders share the key historical feature of isolated complement C3 deposits in the glomerulus. A common aetiology involving dysregulation of the alternative pathway (AP) of complement has been elucidated in the past decade, with genetic defects and/or autoantibodies able to be identified in a proportion of patients. We review the