Tuberous sclerosis complex (TSC) is a genetic autosomal dominant disorder characterized by benign tumor-like lesions, called hamartomas, in multiple organ systems, including the brain, skin, heart, kidneys, and lung. These hamartomas cause a diverse set of clinical problems based on their location and often result in epilepsy, learning difficulties, and behavioral problems. TSC is caused by mutations within the TSC1 or TSC2 genes that inactivate the genes’ tumor-suppressive function and drive hamartomatous cell growth. In normal cells, TSC1 and TSC2 integrate growth signals and nutrient inputs to downregulate signaling to mammalian target of rapamycin (mTOR), an evolutionarily conserved serine-threonine kinase that controls cell growth and cell survival. The molecular connection between TSC and mTOR led to the clinical use of allosteric mTOR inhibitors (sirolimus and everolimus) for the treatment of TSC. Everolimus is approved for subependymal giant cell astrocytomas and renal angiomyolipomas in patients with TSC. Sirolimus, though not approved for TSC, has undergone considerable investigation to treat various aspects of the disease. Everolimus and sirolimus selectively inhibit mTOR signaling with similar molecular mechanisms, but with distinct clinical profiles. This review differentiates mTOR inhibitors in TSC while describing the molecular mechanisms, pathogenic mutations, and clinical trial outcomes for managing TSC.

Keywords: everolimus, mammalian target of rapamycin, sirolimus, tuberous sclerosis complex.
disease. Everolimus and sirolimus selectively inhibit mTOR signaling with similar molecular mechanisms, yet with quite distinct clinical profiles. This review differentiates mTOR inhibitors in TSC while describing the molecular mechanisms, pathogenic mutations, and clinical trial outcomes in TSC.

**Genetic and Molecular Basis**

To understand why mTOR inhibitors have gained prominence in TSC treatment, it is essential to appreciate the disorder’s underlying genetic and molecular mechanisms and how mTOR plays a central role in disease pathogenesis. Initial studies involving multigenerational families demonstrated locus heterogeneity in TSC with linkage to 9q34 (TSC1) and 16p13.3 (TSC2), as well as loss of heterozygosity in hamartomas. Multiple groups have meticulously mapped the pathogenic mutations to better understand the effects of the diverse TSC1 and TSC2 missense mutations and in-frame insertions or deletions on TSC1–TSC2 activity (Fig. 1). The ratio of TSC2:TSC1 mutations has been reported to be 3.4:1, and the TSC2 gene has a higher mutation frequency per nucleotide compared with TSC1. A majority of mutations of TSC1 (99%) and TSC2 (75%) consist of single base-pair deletions or insertions and point mutations that cause premature termination codons downstream in the open-reading frame, thus generating a truncated or partial protein product causing complete inactivation of the gene or nonterminating missense mutations. In rare instances, although equally important, mutations can result in defective splicing that causes the disease. The extensive diversity and functional consequences of each mutation, combined with location and timing of acquired second hit mutations, have an important impact on the observed variability of clinical disease symptoms and range of organ involvement. Importantly, the majority of TSC patients harbor a TSC2 mutation that is associated with more severe clinical features. Patients with phenotypes with no mutation identified are generally less severe than those with TSC1 or TSC2 mutations. This potential relationship between mutational status and clinical severity underscores the need to better understand TSC1 and TSC2 pathogenic mutations for optimal clinical management of the disease.

Critical functions of the TSC-mTOR pathway are nutrient-, growth factor-, and energy-sensing. Multiple upstream inputs from growth factors and energy converge on the mTOR signaling cascade (Fig. 2). Mammalian TOR forms 2 distinct multiprotein complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2), that are differentiated by their interaction partners (raptor [mTORC1] versus rictor/SIN1 [mTORC2]), substrate selectivity, and sensitivity to rapamycin and its analogs. In a normal cellular context, mTORC1 negatively regulates catabolic processes (such as autophagy) and activates anabolic processes (such as protein synthesis). In cells with constitutive mTORC1 activation, such as in TSC, the anabolic processes dominate over the catabolic processes, disrupt the normal balance, and give a cell-growth advantage over surrounding cells. The TSC1/TSC2 complex exerts control of the mTOR pathway by functioning as a GTPase-activating protein toward Ras homolog enriched in brain, a direct and positive regulator of mTORC1. In patients with TSC1 or TSC2 mutations, this functional complex is disrupted, leading to constitutive mTORC1 activation and the formation of hamartoma lesions.

**Subependymal Giant Cell Astrocytomas**

SEGAs are the most commonly found brain tumor in TSC and usually arise near the caudothalamic groove proximal to the foramen of Monro in children and young adults. It is important to note that SEGAs have been reported throughout the CNS, and congenital SEGAs are being increasingly identified through prenatal ultrasounds, fetal MRI, or routine imaging obtained during the perinatal period. SEGAs arise from SENs, but not all SENs grow to become SEGAs. SENs, which are present at birth, exist in 80% of individuals with TSC, but the overall prevalence of SEGAs in TSC is estimated at only 5%–15%. The distinction between SENs and SEGAs is not always clear, because both are considered benign lesions with little or no malignancy potential, and there are no essential histological or molecular

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**Fig. 1.** Structural features of TSC1 and TSC2. The TSC1 gene is encoded by 21 exons and 1164 amino acids, whereas the TSC2 gene is encoded by 41 exons and is 1807 amino acids in length. The TSC1 and TSC2 gene products form a complex through defined interaction domains that inhibit the GTPase activity of Ras homolog enriched in brain that normally activates mTOR and cell growth. TSC1 and TSC2 contain several important regulatory phosphorylation sites indicated, along with kinase responsible. The arrows and amino acid positions indicate mutations identified in patients with TSC1 and TSC2 mutations.
differences between them. Officially, SEGAs are classified as low-grade, World Health Organization grade I astrocytic neoplasms, although a mixed neuronal-glial classification would likely be more accurate as tumors almost universally stain positive for both neuronal and glial cell markers. Grossly, tumors are typically homogeneous and appear with mild atypia consisting of spindle cells, gemistocytic-like cells, and occasional ganglion-like cells. Microcalcification and gross calcification are common, but rosettes, endothelial proliferation, mitoses, inflammation, and necrosis can also be present. The hallmarks of SEGA, however, are large giant cells with prominent nuclei, microtubules, abundant rough endoplasmic reticulum, free ribosomes indicative of neuronal differentiation, and bundles of intermediate filaments indicative of glial differentiation.

Prior to the advent of modern imaging, SEGA classification was limited to lesions associated with hydrocephalus arising from obstruction of cerebrospinal fluid passage through the foramen of Monro. Although this clinical definition remains appropriate, SEGA now includes tumors with characteristic radiological features on CT or MRI, such as well-circumscribed tumors arising in typical subependymal locations, associated ventricular enlargement, or evidence of clear growth over time with repeated imaging (Fig. 3). SEGA diameter can range from <1 cm to >10 cm, leading to disfavor in using a minimal size as the criterion to distinguish between SEN and SEGA. Similarly, intravenous contrast uptake and calcification ranging from none to complete are typical and not useful for differentiating one from the other.

Most SEGAs are slow-growing, typically <0.5 cm/year in the longest dimension. Early symptoms of hydrocephalus may be subtle and gradually progressive, such as headaches, nausea, vomiting, ataxia, visual disturbances, increased seizures, irritability, or other mental status changes. If unaddressed or acutely obstructing, patients may present instead with lethargy or obtundation progressing rapidly to death. For best long-term survival and neurological outcome, evidence supports earlier intervention prior to emergence of clinical symptoms. In 2012, the International TSC Consensus Group recommended imaging surveillance every 1–3 years in asymptomatic patients until a minimum age of 20 and before clinical symptoms limit intervention options. Patients with SEGA require imaging more frequently, while treatment options are considered or treatment response is monitored. Historically, surgical excision has been the main SEGA treatment option and offers potential for definitive treatment. However, complete excision is not assured, and a comprehensive analysis reported a 15% SEGA recurrence rate and a 25% serious complication or injury rate. Modern technological advances, neurosurgeon expertise, operation timing, and individual patient presentation favor better outcomes at individual centers. For example, intraoperative MRI and adoption of minimally invasive techniques significantly lower morbidity risk. Despite such advances, a claims database review of TSC patients who underwent SEGA surgery between 2000 and 2009 revealed that nearly 13% required repeat surgery within the first year due to primary tumor recurrence, development of new SEGA, shunt placement or revision, or to treat complications, including bleeding, empyema, and abscesses. In this context that mTOR inhibitors, such as sirolimus and everolimus, evolved as a medical alternative to surgery for the treatment of SEGA.

Fig. 2. Nutrient, growth factor, and energy sensing impinge on the mTOR pathway. Both TSC1 and TSC2 are major pathway components in the mTOR signaling cascade. TSC is caused by mutations in either the TSC1 or TSC2 genes that interact in a protein-protein signaling complex to negatively regulate cell growth. Nutrients, growth factor, and energy status signal to the rapamycin-sensitive TORC1, while TORC2 is known to fully activate Akt.
Discovery and Development of Rapamycin and Rapamycin Analogs

Rapamycin is a natural product isolated from soil bacterium extracts found on Easter Island, which is also known by its native name, Rapa Nui. In 1964, a Canadian scientific expedition traveled to Easter Island to gather plant and soil samples from which they isolated and purified an active metabolite with antibiotic properties known as rapamycin from the bacterium Streptomyces hygroscopicus. It was demonstrated that rapamycin contained strong antifungal activity. Two years after its isolation and characterization, rapamycin was found to have cytostatic activity in immune cells, and 5 years later in human tumor cells, including medulloblastoma and glioma. Rapamycin also was determined to be a potent immunosuppressive agent effective in preventing allograft rejection. Antiproliferative effects in yeast and lymphocytes led to rapamycin studies on childhood rhabdomyosarcoma tumor cell lines and adult lung cancer cells. Subsequently, the race began to test rapamycin (sirolimus) and develop new analogs (temsirolimus, everolimus, and ridaforolimus) to pilot in tumor models and clinical trials. These first-generation small molecule inhibitors of mTOR, consisting of rapamycin and rapamycin derivatives, can be collectively referred to as rapamycin analogs.

The mechanism of rapamycin started to be uncovered using Saccharomyces cerevisiae, where it bound the immunophilin FK506 binding protein (FKBP) and arrested yeast in the G1 phase of the cell cycle. Importantly, 2 genes—target of rapamycin 1 and 2 (TOR1 and TOR2)—were identified and appeared to interact in a complex with rapamycin. In mammalian cells, TOR exists as a single 289 kDa isoform (mTOR) that specifically binds to FKBP12. The ternary crystal structure was solved in 1996, revealing how rapamycin mediates FKBP12 dimerization with mTOR, which then blocks access to the mTOR kinase active site located in a deep cleft and hydrophobic pocket behind the FKBP12–rapamycin binding domain. Thus, rapamycin does not directly bind to the mTOR protein; rather, it is highly selective binding of rapamycin to FKBP12 and subsequent association of the FKBP12–rapamycin complex with mTORC1 that conveys highly sensitive and targeted mTORC1 inhibition.

Rapamycin (Sirolimus) and Its Analogs

Sirolimus was the first pharmacological agent in this class of mTORC1 inhibitors to be developed and approved (1999) by the FDA to prevent graft rejection in kidney transplant patients. Other rapamycin analogs have been approved subsequent to sirolimus, including temsirolimus (CCI-779) for advanced renal cell carcinoma and everolimus (RAD001) for prevention of solid organ transplant rejection and treatment of breast, renal, and neuroendocrine tumors. Another analog, AP23573 or MK-8669 (ridaforolimus), is in advanced stages of clinical development; however, it is not yet approved for any specific clinical indication. Only everolimus has been approved for the management of TSC disease manifestations, including SEGAs and renal angiomyolipomas; however, sirolimus has been shown in various case reports and multiple prospective clinical trials to benefit TSC patients.

These analogs all share a central macrolide chemical structure, yet differ in the functional groups added at C40 (Fig. 4). Everolimus and ridaforolimus are hydroxyethyl ester and dimethylphosphinate derivatives, respectively, of sirolimus that are biochemically active without modification. In contrast, temsirolimus is a prodrug that requires removal of the dihydroxymethyl propionic acid ester group at C40 after administration, becoming sirolimus in its active form. The shared macrolide structure among the analogs permits an interaction with FKBP12, the mechanism by which these allosteric molecules selectively inhibit mTORC1 over mTORC2.

Although relatively minor, the differences at C40 have important clinical implications. First, bioavailability and half-life are significantly different among the compounds (Table 1). Sirolimus and everolimus are taken orally each day. Comparative pharmacokinetics suggest that everolimus is more readily absorbed and exhibits greater oral bioavailability compared with sirolimus due to selective intestinal cell efflux.
for which sirolimus alone is a substrate.\textsuperscript{6,67,68} Temsirolimus is formulated for weekly intravenous administration, bypassing this system altogether. The relative hydrophobicity of sirolimus does have its own benefits because it is readily absorbed through the skin\textsuperscript{69} and is used in custom topical preparations to treat TSC-related facial angiofibromas.\textsuperscript{11} The various rapamycin analogs differ in hepatic metabolism, in which everolimus is 2.7-fold lower than sirolimus.\textsuperscript{70} Nonetheless, sirolimus systemic clearance is half that of everolimus,\textsuperscript{67,71} giving everolimus faster steady state levels after initiation and faster elimination after discontinuation. On a pharmacodynamic level, mTORC1 inhibition appears comparable at physiologic dosing ranges, but tissue- and organelle-specific differences have been observed. For example, there is evidence that everolimus, but not sirolimus, distributes to brain mitochondria and stimulates mitochondrial oxidation in the brain.\textsuperscript{72}

More recently, second-generation pharmacological mTOR inhibitors have been developed. In contrast to the rapamycin analogs, these molecules are direct kinase inhibitors that do not target FKBP12, and instead inhibit both mTORC1 and mTORC2 by directly blocking the ATP catalytic site. There are >20 catalytic mTOR inhibitors; however, these compounds vary in sensitivity and selectivity for other intracellular protein kinases. These agents are useful to differentiate TORC1- versus TORC2-mediated intracellular signaling, and important differences are reported compared with rapamycin in downstream target inhibition, feedback loops, and protein translation control. These newer agents are potent inhibitors of cellular proliferation and therefore could have therapeutic benefit. Second-generation mTOR inhibitors are in either preclinical or early clinical development for oncology indications, but to

### Table 1. Clinical pharmacology of sirolimus, everolimus, temsirolimus, and ridaforolimus

<table>
<thead>
<tr>
<th></th>
<th>Sirolimus\textsuperscript{68}</th>
<th>Everolimus\textsuperscript{6,67}</th>
<th>Temsirolimus\textsuperscript{97}</th>
<th>Ridaforolimus\textsuperscript{63,98,99}</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Commercial names</strong></td>
<td>Rapamune\textsuperscript{8}, rapamycin</td>
<td>Afinitor\textsuperscript{8}, RAD001, SDZ-RAD, Votubia\textsuperscript{8}, Certican\textsuperscript{8}</td>
<td>CCI-779, Torisel\textsuperscript{8}</td>
<td>AP23573, MK-8669, deforolimus</td>
</tr>
<tr>
<td><strong>Mechanism of action</strong></td>
<td>Inhibition of PI3K–TSC–mTOR pathway, resulting in modulation of cellular metabolism, growth, proliferation, and angiogenesis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Molecular weight</strong></td>
<td>914.2 g/mol</td>
<td>958.2 g/mol</td>
<td>1030.3 g/mol</td>
<td>990.2 g/mol</td>
</tr>
<tr>
<td><strong>Route of administration</strong></td>
<td>Once daily by mouth</td>
<td>Once daily by mouth</td>
<td>Intravenous infusion once per week</td>
<td></td>
</tr>
<tr>
<td><strong>Protein binding</strong></td>
<td>92%</td>
<td>75%</td>
<td>$\approx$85%</td>
<td>$\approx$94%</td>
</tr>
<tr>
<td><strong>Bioavailability</strong></td>
<td>Solution: 14% Tablet: 18%</td>
<td>Tablet: 20%</td>
<td>Injection: 100%</td>
<td>Tablet: 16%</td>
</tr>
<tr>
<td><strong>Terminal half-life</strong></td>
<td>46–78 h</td>
<td>26–30 h</td>
<td>9–27 h</td>
<td>$\approx$30–75 h</td>
</tr>
<tr>
<td><strong>Solubility</strong></td>
<td>Water insoluble</td>
<td>Water insoluble</td>
<td>Water insoluble, alcohol soluble</td>
<td>Water insoluble</td>
</tr>
<tr>
<td><strong>Main drug-drug interactions</strong></td>
<td>CYP3A4, P-glycoprotein</td>
<td>CYP3A4, CYP3A5, CYP2C8</td>
<td>CYP3A4</td>
<td>CYP3A4, P-glycoprotein</td>
</tr>
<tr>
<td><strong>Elimination</strong></td>
<td>Feces (91%), urine (2%)</td>
<td>Feces ($&gt;$90%), urine (2%)</td>
<td>Feces (82%), urine (5%)</td>
<td>Feces (88%), urine (2%)</td>
</tr>
</tbody>
</table>
date none have completed phase III clinical trials or received regulatory approval for human use. Clinical development for TSC has yet to occur, and it will be important to demonstrate that dual targeting of TORC1/TORC2 provides superior efficacy without introducing significant increases in toxicity.

Clinical Trials to Treat Tuberous Sclerosis Complex

Of the different mTOR inhibitors, only sirolimus and everolimus have been clinically evaluated for the management of TSC patients. The first report of sirolimus as a TSC treatment occurred in 2006, when 5 patients with SEGAs were treated for 2.5 to 20 months and demonstrated an average 55% reduction in tumor volume, negating the need for resective surgery. In 2008 a prospective, open-label clinical trial with sirolimus to treat renal angiomyolipomas began; in 2011 a randomized, double-blind, placebo-controlled clinical trial to treat LAM with or without TSC was completed. Both studies demonstrated a favorable safety and efficacy profile of sirolimus for treating specific TSC disease manifestations. Importantly, both also included follow-up observations wherein it was demonstrated that disease progression resumes if treatment is discontinued. In other words, for clinical benefit to be sustained, treatment must be maintained for prolonged periods and possibly indefinitely. It remains to be demonstrated whether there is a critical threshold, such as minimum treatment duration or magnitude of response, or a combinatory therapeutic strategy (ie, dual targeting of mTOR and autophagy) that might allow efficacy to be maintained.

More recently, rapamycin analogs have been explored in topical formulations for the management of facial angiofibromas. Sirolimus is available in a topical formulation, and individual case reports have demonstrated the effectiveness of custom preparations varying in composition and concentration. In the only prospective clinical trial reported to date, 73% of patients (n = 28) experienced reductions in facial angiofibroma. To confirm these findings, a follow-up, placebo-controlled, randomized, double-blind, phase III clinical trial evaluating different sirolimus concentrations is ongoing (NCT01526356). Despite consistent findings across various TSC studies, sirolimus is not yet approved, due largely to clinical trial study design limitations and/or lack of phase III clinical trials evaluating the same TSC manifestation. There are active efforts seeking regulatory approval for sirolimus to treat TSC disease manifestations in the United States and other countries, under alternative approval processes established for orphan drugs and rare disease populations.

Following the initial report of sirolimus to treat SEGAs, individual case reports and small case series and open-label clinical trials demonstrated similar reduction in SEGAs. However, to date everolimus is the only mTOR inhibitor to be FDA approved to treat SEGAs in TSC after 2 major clinical trials demonstrated efficacy and safety. The first was a prospective, open-label study of 28 patients who demonstrated SEGAs growth on MRI before treatment initiation. All 28 patients demonstrated a reduction in tumor volume or cessation of growth. Overall, nearly 80% of patients had SEGAs that were reduced by a third, and more than 30% of patients had SEGAs that were reduced by more than half within 6 months of treatment. Side effects were largely mild to moderate in severity, and none led to treatment discontinuation. Further analysis after continuous treatment (median of 3 y) revealed that efficacy was sustained without encountering new or additional significant side effects. The second study, a randomized, placebo-controlled, double-blind trial involving 117 patients with SEGAs from 24 different TSC centers, reported similar results: SEGAs volume was reduced more than 50% in 49% of patients treated a median of 29 months.

In addition to treatment of SEGAs, mTOR inhibitors may have additional benefit for treatment of CNS-related disease manifestations. Seizure control, cognitive development, and behavior have improved with everolimus when evaluated as a secondary outcome. In 2013 the first prospective clinical trial to specifically evaluate everolimus efficacy for medically refractory epilepsy in patients with TSC was conducted. Seizure frequency was reduced by 50% or more in 12 of 20 patients, including several cases where dramatic improvement was observed in patients with prior history of failed medications, vagus nerve stimulation, or epilepsy surgery. Improvement in multiple aspects of behavior and neurocognition were also reported. It is worth noting that response to treatment was highly variable, and 3 patients experienced an unexpected increase in seizures. Complicating the clinical picture are the phase III clinical trial results for SEGAs in which seizure frequency and behavior measures as secondary endpoints failed to confirm the treatment benefit of everolimus. Multicenter, randomized, double-blind, placebo-controlled clinical trials are currently under way to specifically evaluate everolimus’ effect on primary outcome measures of seizure control (NCT01713946) and neurocognition (NCT01289912).

Sirolimus and everolimus were initially developed to treat fungal infections and cancer and to prevent organ transplant rejection. As a result, robust knowledge around dosing and treatment-related side effects existed years before these drugs were first used to treat TSC. The most frequently encountered medication-related toxicities include mouth ulcers, hypercholesterolemia, marrow suppression, infections, and metabolic effects. These toxicities are relatively well known and straightforward to manage, except when frequent or severe. The same toxicities occur in TSC patients with overall reduced frequency and severity. The exact reason for this is not known; however, a possible explanation is that these agents are monotherapies for TSC patients, whereas in other oncologic and transplant settings they are frequently combined with chemotherapy or immunosuppressant regimens. Another key difference to note, particularly for cancer treatment, is that dosing is closer to the maximum tolerated dose, while in TSC dosing strategies seek to identify the minimum effective dose, thus avoiding side effects associated with higher doses.

The effect that these molecules have on the immune system should also be noted, as it was their immunosuppressive properties that led them to be initially used in transplant medicine. Early TSC clinical trials reported infections in as many as 80%–90% of patients; however, this is misleading because the study designs recorded all infections as treatment related and the evaluation period for study participants was a minimum of 1 year. These infections were of common type and severity, and the infection rate actually decreased the longer the patient received treatment. The larger, placebo-controlled phase III clinical trials using everolimus to treat angiomyolipomas and SEGAs provide
a clearer picture of infection risk. The infection rate in these latter studies generally occurred in only 10%–20% of individuals and was nearly identical in both the treatment and placebo groups. In fact, in TSC clinical trials, most infections are reported as either mild or moderate in severity, and infections are rarely cited as the reason for discontinuing treatment.

**Clinical Decision Making: Sirolimus Versus Everolimus**

Given the abundance of supporting evidence for the efficacy of sirolimus and everolimus in treating the same TSC manifestations, which medication should be favored when initiating treatment? In preclinical studies, the 2 agents are often used interchangeably, but direct side-by-side comparisons are rarely made in the same study design. Sirolimus demonstrates slightly higher binding affinity to FKBP12 than everolimus (half-maximal inhibitory concentration of 0.4–0.9 nM vs 1.8–2.6 nM, respectively), although this difference is not likely to be relevant clinically because human serum levels range between 5 and 15 ng/mL (5.5–16.4 nM). When compared, both agents similarly inhibit cell proliferation and T-cell immunologic activity, and both are efficacious in preventing organ rejection.

To date, clinical trials directly comparing everolimus and sirolimus are lacking in oncology, transplantation, and TSC. In TSC specifically, we performed a cross-sectional analysis of patients at 2 major TSC centers treated with either sirolimus or everolimus and found that tumor reduction was comparable between the 2, generally within 1%–5% when examined by quartiles. Along these lines, we observed best responses to treatment in patients with newly identified SEGA or renal angiomyolipoma with unmistakable growth exceeding the typical 0.5 cm/year evidenced on serial imaging. Tumors with much slower growth rates or increasing degree of calcifications also respond to treatment, but the degree of response is often less robust. Analysis of existing clinical trials and postmarketing studies are needed to confirm these observations and uncover potential clinical predictors for clinicians to identify those patients more likely to respond to treatment.

In the absence of head-to-head clinical trials, selection of a specific mTOR inhibitor in TSC has generally followed the best published evidence to date for the specific disease manifestation. Sirolimus is generally favored for TSC-LAM because there have been no published major clinical trials in TSC-LAM that have used everolimus, although a multicenter trial has recently been completed. Considering that only sirolimus is currently formulated for topical application, it is generally used for the management of facial angiofibromas. Decision making for management of renal angiomyolipoma and SEGA is less clear. Multiple clinical trials have been published that used either agent with similar efficacy and tolerability in TSC patients, but clinical trials using everolimus typically have involved greater numbers of subjects and include several studies with a randomized, double-blind, placebo-controlled design. Furthermore, long-term treatment studies have exclusively focused on everolimus, and the patients have been treated continuously for 2–7 years. These long-term studies have not revealed any new toxicities different than those observed in the initial short-term treatment studies spanning 0.5 to 2 years. Rather, prolonged treatment appears to be associated with improved tolerability, with fewer reported adverse events overall. In the absence of such direct comparisons, the more robust clinical trial experience with everolimus combined with regulatory approvals by the FDA and the European Medicines Agency provide the most compelling reason favoring everolimus over sirolimus to treat SEGA and other TSC disease manifestations at this time.

**Future Directions and Conclusions**

Successful development of sirolimus and everolimus for TSC has stimulated intense interest in improving therapeutic options for patients with TSC and other similar disorders. Two major strategies have emerged. First, all published evidence indicates that treatment must be sustained, perhaps indefinitely, for durable treatment effect. A management paradigm in which treatment could be reduced or discontinued over time while benefit is maintained would be preferable to lifelong therapy. One explanation for the loss of sustained treatment effect could be the unintended upregulation of competing cell survival mechanisms, such as autophagy. Exposure to rapamycin analogs induce autophagy, and combination treatment with mTOR inhibitors and anti-autophagy agents sustains treatment response in vitro. Multiple human clinical studies that combine autophagy and mTOR inhibitors are under way, including a phase I study of hydroxychloroquine and sirolimus in women with TSC-LAM (NCT01687179).

The second major strategy is to expand on the treatment progress found in TSC patients for other disorders with similar pathologic and/or disease manifestations. For example, patients with phosphatase and tensin homolog (PTEN) hamartoma tumor syndromes, such as Cowden syndrome and Bannayan-Riley-Ruvalcaba syndrome, develop hamartomas and exhibit high rates of autism, developmental delay, epilepsy, and intellectual disability. PTEN, similar to TSC1/TSC2, acts as a tumor suppressor by negatively regulating phosphatidylinositol-3 kinase (PI3K) signaling upstream of TSC1/TSC2. Mammalian TOR inhibitors have been shown to reverse disease characteristics in PTEN animal models, and isolated case reports suggest similar benefits may be possible in humans. Additional proliferative and overgrowth syndromes, including large vascular and lymphatic malformations, have also responded well to mTOR inhibitors.

Meanwhile, investigation with mTOR inhibitors continues in TSC. Initial studies focused on reduction of hamartomatous lesions of the brain, lung, kidney, and skin. Current studies are focused on the safety and efficacy of these agents when used to treat additional TSC-associated disease manifestations with greater prevalence and morbidity—including epilepsy, cognitive impairments, and autism—that affect 50%–80% of these patients. In addition, strategies to prevent or reduce disease severity are being actively pursued, including preventing SEN from transforming to SEGA. Perhaps using these agents as disease modifiers represents overzealous enthusiasm; however, when we consider the remarkable progress made so far—from the discovery of rapamycin on Rapa Nui to the successful repurposing of this antifungal, immunosuppressive, antigrowth agent in TSC—prospects for their future therapeutic applications appear vast.
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References


