Temsirolimus, an Inhibitor of Mammalian Target of Rapamycin

Brian I. Rini

Temsirolimus (Torisel, Wyeth Pharmaceuticals) is an inhibitor of mammalian target of rapamycin (mTOR), a molecule implicated in multiple tumor-promoting intracellular signaling pathways. Temsirolimus recently became the first Food and Drug Administration–approved mTOR-targeted agent based on a phase III trial showing an overall survival advantage over IFN-α monotherapy in advanced renal cell carcinoma (RCC) patients with multiple adverse risk features (1).

Temsirolimus is a soluble ester of rapamycin, a natural product that was initially developed as an antifungal drug and then as an immunosuppressive agent, with antitumor activity noted more than 20 years ago. Rapamycin (sirolimus, Rapamune) was isolated from the soil bacteria Streptomyces hygroscopicus found on Rapa Nui (commonly known as Easter Island) in the South Pacific in 1975, but its development for cancer therapies was not prioritized. The immunosuppressant effects of rapamycin were pursued and resulted in Food and Drug Administration approval in 1999 for prevention of renal allo-.

mTOR, also known as rapamycin-associated protein, rapamycin target, or sirolimus effector protein, is a molecule implicated in multiple tumor-promoting intracellular signaling pathways (Fig. 1). mTOR is a 289-kDa serine/threonine-specific kinase with highly conserved structure. It exists in cytoplasm in a complex with three peptides: regulatory-associated protein of mTOR (raptor), mLST8, and GβL. Regulation of mTOR pathway activation is mediated through a series of complex signaling interactions linking growth factor receptor signaling and other cell stimuli, phosphatidylinositol 3-kinase activation, and activation of the Akt/protein kinase B pathway. Two distinct pathways have effects on cell cycle regulation downstream of mTOR. mTOR phosphorylates and activates p70 S6 kinase, leading to enhanced translation of certain ribosomal proteins and elongation factors. This process leads, among other effects, to the production of hypoxia-inducible factor-1α, which regulates the transcription of genes that stimulate cell growth and angiogenesis, including VEGF. Indeed, it has been shown that mTOR inhibition leads to RCC cell line and xenograft tumor growth inhibition, and this effect is mediated by hypoxia-inducible factor (3). The second major mTOR effect is on the 4E-binding protein-1 and eukaryotic initiation factor-4 subunit E complex. Activated mTOR phosphorylates 4E-binding protein-1, promoting dissociation of this complex and allowing eukaryotic initiation factor-4 subunit E to stimulate an increase in the translation of mRNAs that encode cell cycle regulators, such as c-myc, cyclin D1, and ornithine decarboxylase. mTOR inhibition results in a block of 4E-binding protein-1 phosphorylation, sequestration of eukaryotic initiation factor-4 subunit E, and failure to form the complex required for translation. Inhibition of mTOR by temsirolimus requires a specific binding complex. Temsirolimus forms this complex with the FK506-binding protein and inhibits the activation of mTOR. This mechanism of action is similar for sirolimus, a temsirolimus metabolite, and both are likely responsible for inhibition of mTOR and antitumor effects after administration of temsirolimus. Temsirolimus/FK506-binding protein affects only one subpopulation of mTOR proteins, which reside in a multiprotein complex termed mTORC1. An additional complex, mTORC2, holds mTOR in a form that cannot be inhibited by temsirolimus and could have downstream signaling implications despite temsirolimus administration. The biology of the mTOR signaling pathway and relevance to cancer has been extensively reviewed (4, 5). Abnormalities in various components of the mTOR pathway have been described for a wide variety of sporadic malignancies. In addition, mutations that disrupt the tumor suppressor complex and other pathways associated with inherited hamartoma syndromes also result in unregulated activation of mTOR (6).

Temsirolimus showed antitumor effects across a wide variety of tumor histotypes in preclinical models, particularly those with defective PTEN (7–9). An initial dose-escalation phase I trial administered temsirolimus i.v. weekly at doses ranging from 7.5 to 220 mg/m² (10). Although thrombocytopenia was dose limiting and reversible maculopapular rash and stomatitis were observed, the formal definition of a maximum tolerated dose was not met. In addition, objective partial and minimal responses were observed at lower dose levels. Based on these observations and pharmacokinetic data indicating comparable area under the curve variability between body surface area–normalized and flat dosing, subsequent phase II testing investigated one or more of the following dose levels: 25, 75, and/or 250 mg i.v. weekly. Phase II trials were conducted in glioblastoma multiforme (11, 12), with well-documented PTEN alterations, mantle cell lymphoma (13), with potential mTOR-mediated cyclin D1 regulation, as well as melanoma (14), neuroendocrine tumors (15), breast cancer (16), and lung cancer (17). Modest single-agent activity was observed in these trials of pretreated patients, most notably in mantle cell lymphoma and breast cancer (Table 1). No evidence of a dose-response relationship was evident in any trial, and higher dose levels generally resulted in greater toxicity.
Temsirolimus was also tested in treatment-refractory, metastatic RCC in a phase II trial that randomized 111 patients to 25, 75, or 250 mg i.v. weekly (18). The overall response rate was 7%, with an additional 26% of patients showing minor responses (Table 1). Retrospective assignment of risk criteria to patients in this study identified a poor-prognosis group with three or more adverse risk features as established for metastatic RCC patients receiving IFN-based initial systemic therapy (19). These risk factors consisted of Karnofsky performance status <80%, lactate dehydrogenase >1.5 × laboratory upper limit of normal, hemoglobin < laboratory lower limit of normal, serum calcium corrected for albumin >10 mg/dL, and time from first diagnosis of RCC to start of therapy of <1 year. Temsirolimus-treated patients in this poor-prognosis group had a median overall survival of 8.2 months compared with 4.9 months for historical control IFN-α–treated patients (19). No evidence of a dose response was seen in this trial.

A subsequent randomized phase III trial was conducted in patients with metastatic RCC and three or more adverse risk features as defined by existing prognostic schema [the five noted above plus a sixth factor of three or more metastatic sites identified as prognostic in a separate analysis (20)]. A total of 626 patients were randomized with equal probability to 25 mg temsirolimus i.v. weekly (n = 209) versus 18 million units IFN-α thrice weekly (n = 207) versus 15 mg temsirolimus i.v. weekly + 6 million units IFN-α thrice weekly (n = 210).
A separate phase I trial had established the safety maximum tolerated dose of combination therapy with temsirolimus (21). The primary study end point of the phase III study was overall survival and the study was powered to compare each of the temsirolimus-containing arms with the IFN-α arm. Patients treated with temsirolimus had a statistically longer overall survival than IFN-α monotherapy patients (10.9 versus 7.3 months; \( P = 0.0069 \); Table 1; ref. 1). There was also a progression-free survival benefit from temsirolimus monotherapy versus IFN (median, 3.8 versus 1.9 months; \( P < 0.0001 \)). The objective response rate was 9% in the temsirolimus monotherapy arm. There was no survival advantage to the combination therapy arm over IFN-α monotherapy, perhaps due to the lower dose of temsirolimus delivered, which may have been inadequate for mTOR inhibition. These data lead to Food and Drug Administration approval of temsirolimus for advanced RCC on May 31, 2007. Results from these studies validated mTOR as a relevant therapeutic target in RCC, at least in the subset of patients with multiple adverse risk features. Indeed, the study population was truly poor risk, with a third of patients without prior nephrectomy and 80% with a Karnofsky performance status \( \leq 70\% \). Recent subset analysis from this trial supports a hypothesis of the greatest benefit of temsirolimus in the poorest-risk patients and in patients with non–clear cell histologies (22). These results must be interpreted cautiously given small numbers and the retrospective nature but may identify the clinical phenotype associated with response to this agent. The tumor biology of poor-risk and/or non–clear cell RCC patients that may account for a greater effect of mTOR inhibition is unclear at present.

Toxicity of this agent is best evaluated from the prospective randomized RCC trial. Grade 3 adverse events occurred in 67% of the temsirolimus monotherapy group (lower than the other two treatment arms). The most frequently occurring temsirolimus-related grade 3 or 4 hematologic toxicities included anemia and thrombocytopenia. Hypercholesterolemia, hyperlipidemia and hyperglycemia were also more common in the temsirolimus arm, reflecting inhibition of mTOR-mediated lipid and glucose metabolism, and generally manageable with dietary or medical management. The most frequently occurring grade 3 adverse events in the temsirolimus arm were asthenia (11%), anemia (20%), and dyspnea (9%). Dyspnea may be due in part

<table>
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<th>Disease</th>
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<td>RCC (1)</td>
<td>Randomized phase III trial: temsirolimus monotherapy vs IFN-α vs combination</td>
<td>ORR: 9% ( (n = 626) ) PFS: 3.7 mo ( (vs 1.9 mo for IFN-α monotherapy) ) OS: 10.9 mo ( (vs. 7.3 mo for IFN-α monotherapy) )</td>
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<td>RCC (18)</td>
<td>Randomized phase II: 25, 75, or 250 mg i.v. weekly in treatment-refractory patients ( (n = 111) )</td>
<td>ORR: 7% ( (n = 111) ) PFS: 5.8 mo ( (n = 111) )</td>
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<td>Mantle cell lymphoma (13)</td>
<td>Phase II in relapsed/refractory patients: 250 mg i.v. weekly ( (n = 35) )</td>
<td>ORR: 38% ( (n = 35) ) PFS: 6.5 mo Duration of response: 6.9 mo</td>
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<td>Breast cancer (16)</td>
<td>Randomized phase II: 75 or 250 mg i.v. weekly in treatment-refractory patients ( (1-2 prior regimens; n = 109) )</td>
<td>ORR: 9% ( (n = 109) ) PFS: 12 wk Duration of response: 6 mo</td>
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<td>Extensive-stage small cell lung cancer (17)</td>
<td>Randomized phase II: 25 or 250 mg i.v. weekly in stable/responding patients after induction chemotherapy ( (n = 87) )</td>
<td>ORR: 1.2% ( (n = 87) ) PFS: 2.2 mo</td>
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<td>Melanoma (14)</td>
<td>Phase II trial of 25 mg i.v. weekly in treatment-refractory patients ( (1-2 prior biotherapy/chemotherapy regimens; n = 33) )</td>
<td>ORR: 3% ( (n = 33) ) PFS: 10 wk</td>
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<td>Neuroendocrine carcinoma (15)</td>
<td>Phase II trial of 25 mg i.v. weekly ( (0-4 prior regimens; n = 37) )</td>
<td>ORR: 5%; tumor burden reduction in 57% of patients PFS: 6 mo</td>
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<tr>
<td>Glioblastoma (12)</td>
<td>Phase II trial of 25 mg i.v. weekly ( (0-1 prior regimens; n = 65) )</td>
<td>ORR: 0%; 36% of patients with regression* PFS: 2.3 mo</td>
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<tr>
<td>Glioblastoma (11)</td>
<td>Phase II trial of 250 mg i.v. weekly ( (radiation-refractory and 0-3 prior chemotherapy regimens; n = 43) )</td>
<td>ORR: 5% PFS: 9 wk</td>
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Abbreviations: ORR, objective response rate; PFS, progression-free survival; OS, overall survival.

*Regression defined as unequivocal reduction in size of contrast enhancement or decrease in mass effect.
to an underlying treatment-induced pneumonitis. Pulmonary toxicity with sirolimus has been reported in the transplant population as patchy or diffuse infiltrates accompanied by dyspnea/dry cough and either a restrictive pattern or reduced diffusion capacity for carbon monoxide on pulmonary function testing (23). The phase III RCC trial reported six patients (5%) with nonspecific pneumonitis not related to dose. One retrospective review of 22 patients with endometrial cancer or neuroendocrine tumors enrolled on phase II trials of 25 mg temsirolimus i.v. weekly identified 8 patients (36%) with radiographic changes on routine computed tomography scans (either ground glass opacification or parenchymal consolidation) consistent with possible drug-induced pneumonitis (24). Dyspnea and dry cough were observed in the 50% of patients who had clinical symptoms. No specific treatment was given, and temsirolimus treatment was continued in four patients without reported worsening of pulmonary abnormalities. The exact incidence of radiographic changes consistent with pneumonitis, percentage of patients with clinically relevant symptoms, and the mechanism of this toxicity is poorly defined at present. Immunosuppression is an additional potential toxicity of temsirolimus given the known immunosuppressive effects of sirolimus. The phase III RCC trial, however, showed no significant difference in the incidence of neutropenia, lymphopenia, or infection versus the IFN control arm (1). In addition, a limited analysis of lymphocyte subsets and proliferative responses to nonspecific antigens did not show any significant effect in temsirolimus-treated patients on a phase I trial (25). The weekly schedule of temsirolimus, in contrast to the daily schedule of sirolimus, may have implications for effect of immune function. Additional experience in a greater number of patients and with longer-term treatment is required to fully characterize the effect of temsirolimus on immune variables and clinically relevant immune-mediated toxicity.

As with any targeted agent, the presence of a viable target, measurement of target inhibition, and defining the molecular phenotype of susceptible versus resistant tumors are critical goals to allow for rational drug development. Some initial efforts in this regard relevant to the mTOR pathway and temsirolimus have been made. Evidence of mTOR pathway activation has been investigated through immunohistochemical staining of paraffin-embedded RCC samples with demonstration of increased expression of phosphorylated Akt, mTOR, and S6 in the majority of samples (26–28). Target inhibition has been shown after temsirolimus administration by decreased p70 S6 kinase activity as assayed in peripheral blood mononuclear cells (2) and inhibition of S6 phosphorylation as measured by immunohistochemistry of posttreatment neuroendocrine tumors (15). Molecular determinants of response have also been investigated. Baseline expression of phosphorylated S6 (15, 29) or phosphorylated p70 S6 kinase (12) was identified as associated with objective response to temsirolimus in small populations of patients. Further, a decrease in p70 S6 kinase activity in paired pre- and post-temsirolimus peripheral blood mononuclear cells was associated with a longer time to treatment failure (2). These translational efforts require expansion and validation in large prospective sample sets but support target inhibition by temsirolimus and generate hypotheses about prediction of response.

In summary, the mTOR pathway is likely critical across a broad spectrum of tumor types. Temsirolimus has shown anti-tumor activity, most notably in poor-risk advanced RCC where a demonstration of overall survival benefit has been observed. A randomized trial of RAD001 versus placebo in treatment-refractory RCC has completed accrual and results are pending. The lack of significant antitumor effect of temsirolimus-mediated mTOR inhibition in some tumors, especially those with predicted sensitivity based on alterations such as PTEN mutation, underscores the complex interplay of multiple signaling pathways within a single tumor. Other rapamycin analogue inhibitors of mTOR have also begun clinical testing. Everolimus (RAD001, Novartis) and AP23573 (Ariad Pharmaceuticals) have shown tolerability and preliminary antitumor activity in small trials (30, 31). Potential differences in antitumor effect or tolerability among these agents due to route/schedule of administration, potency against mTOR, or other drug effects are not well characterized at present. More potent or complete mTOR inhibition (e.g., through agents that inhibit both mTORC1 and mTORC2), inhibition of multiple signaling pathways simultaneously, and/or more precise molecular phenotyping of tumors to define mTOR pathway reliance are needed to build on the clinical benefits of temsirolimus observed to date.

References

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