

Review

Mammalian target of rapamycin inhibition as a therapeutic strategy in the management of urologic malignancies

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Abstract

The mammalian target of rapamycin (mTOR) is a protein kinase that regulates protein translation, cell growth, and apoptosis. Recently, there has been an enormous increase in our understanding on molecular mechanisms underlying the therapeutics of rapamycin in cancer. Alterations in the pathway regulating mTOR occur in many solid malignancies including prostate, bladder, and kidney cancer; *in vitro* and *in vivo* models of prostate and bladder cancer have established the importance of the mTOR pathway in control of cancer progression and metastasis. Temsirolimus (Torisel) and everolimus (RAD-001), two ester analogues of rapamycin, as well as rapamycin itself have clear antitumor activity in *in vitro* and *in vivo* models and are under clinical trial investigations for prostate and bladder cancer. Phase II and III trials have already established the clinical efficacy of temsirolimus in renal cancer, and current renal trials are evaluating the combined effects of vascular endothelial growth factor and mTOR inhibition. Ongoing studies in prostate and bladder cancer will soon define the activity and safety profiles of everolimus and temsirolimus. Recent molecular advances have uncovered a startling complexity in the macromolecular function of mTOR complexes, with the identification of new mTOR partners (raptor, rictor, FKBP38, PRAS40, and mSIN1), putative cancer therapeutic/prognostic targets for future clinical trials. [Mol Cancer Ther 2008;7(6):1347–54]

Received 12/19/07; revised 3/17/08; accepted 3/19/08.

Grant support: National Cancer Institute R01CA092102 and R01CA102074 (D. Danielpour).

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doi:10.1158/1535-7163.MCT-07-2408

Introduction

Recent advances in cancer research have drawn increased interest in the use of mammalian target of rapamycin (mTOR) inhibitors to treat a variety of cancers, consistent with the key roles of mTOR in cell survival, growth, protein synthesis, cellular metabolism, and angiogenesis. mTOR, which is constitutively activated in many cancers by deregulated activation of oncogenes or loss of tumor suppressor genes, functions in macromolecular complexes that are either rapamycin sensitive or rapamycin insensitive. The rapamycin-sensitive complex of mTOR functions in a negative feedback loop to suppress mitogen activity through insulin receptor substrate-1 (IRS-1). Thus, inhibition of mTOR by rapamycin activates both cytostatic and cell survival responses. Combination therapeutics with agents that counteract this survival effect will likely synergize with mTOR inhibitors for tumor kill. Therefore, further understanding of the molecular mechanisms that regulate the function or mediate the activity of mTOR will undoubtedly affect on the therapeutics of numerous malignancies.

Two mTOR inhibitors [temsirolimus (Torisel) and everolimus (RAD-001)] have already undergone clinical testing in several hematologic and solid malignancies. Recently, in fact, temsirolimus was granted Food and Drug Administration approval for the treatment of metastatic renal cell carcinoma (mRCC) patients. Preliminary phase II data showing the antitumor activity of everolimus in mRCC has led to the design of a large multi-institutional phase III trial evaluating the activity of everolimus plus best supportive care versus best supportive care in patients with previously treated mRCC. Similarly, studies on the molecular pathogenesis of prostate cancer and transitional cell carcinoma of the urothelium (TCC) have also identified that the mTOR signaling pathway promotes tumor growth and proliferation in such cells. Therefore, several phase II trials evaluating these novel compounds in nonrenal genitourinary malignancies are currently underway and will further define the importance of this pathway as a therapeutic target in these solid tumors. In this review, we will discuss the biological rationale behind mTOR inhibition in solid tumors as well as the current clinical data supporting their use in patients with genitourinary tumors.

Discovery of Rapamycin, Rapamycin Analogues, and mTOR

Rapamycin (also known as sirolimus or rapamune), which was first identified as a 914.2-kDa antifungal bacterial

macrolide isolated from *Streptomyces hygroscopicus* (1), later became the preferred immunosuppressant for kidney transplantation because it was shown to be mildly immunosuppressive but did not enhance tumor incidence in contrast to cyclosporin A (2). Rapamycin was rather quickly recognized to have potent and broad antitumor activity, entering the scene for cancer therapy (2). More enthusiasm has been ignited by recent studies showing that rapamycin can sensitize cancers to chemotherapy or radiation therapy (3). Rapamycin and several of its structural derivatives [temsirolimus (Wyeth Pharmaceuticals), everolimus (Novartis Pharmaceuticals), and AP-23573 (Ariad Pharmaceuticals)] are currently under robust and encouraging investigational use in phase I and II clinical trials for a variety of cancers (4).

The biological responses of rapamycin appear to mostly depend on mTOR, which is a 290-kDa (2,549–amino acid) serine/threonine kinase with many functional and protein-binding domains. mTOR controls protein synthesis in response to the sufficiency of certain cellular nutrients including amino acids such as leucine (5). The kinase domain of mTOR is located in its COOH-terminal region between a FATC domain and a FKBP-rapamycin binding domain, and mTOR contains HEAT repeats in its NH₂-terminal region (6). Rapamycin binds to and suppresses mTOR only through first associating to the immunophilin FKBP12 (7). One of the downstream targets of mTOR is the eukaryotic initiation factor 4E binding protein (4E-BP1), whose phosphorylation by mTOR stimulates protein translation through releasing eukaryotic initiation factor

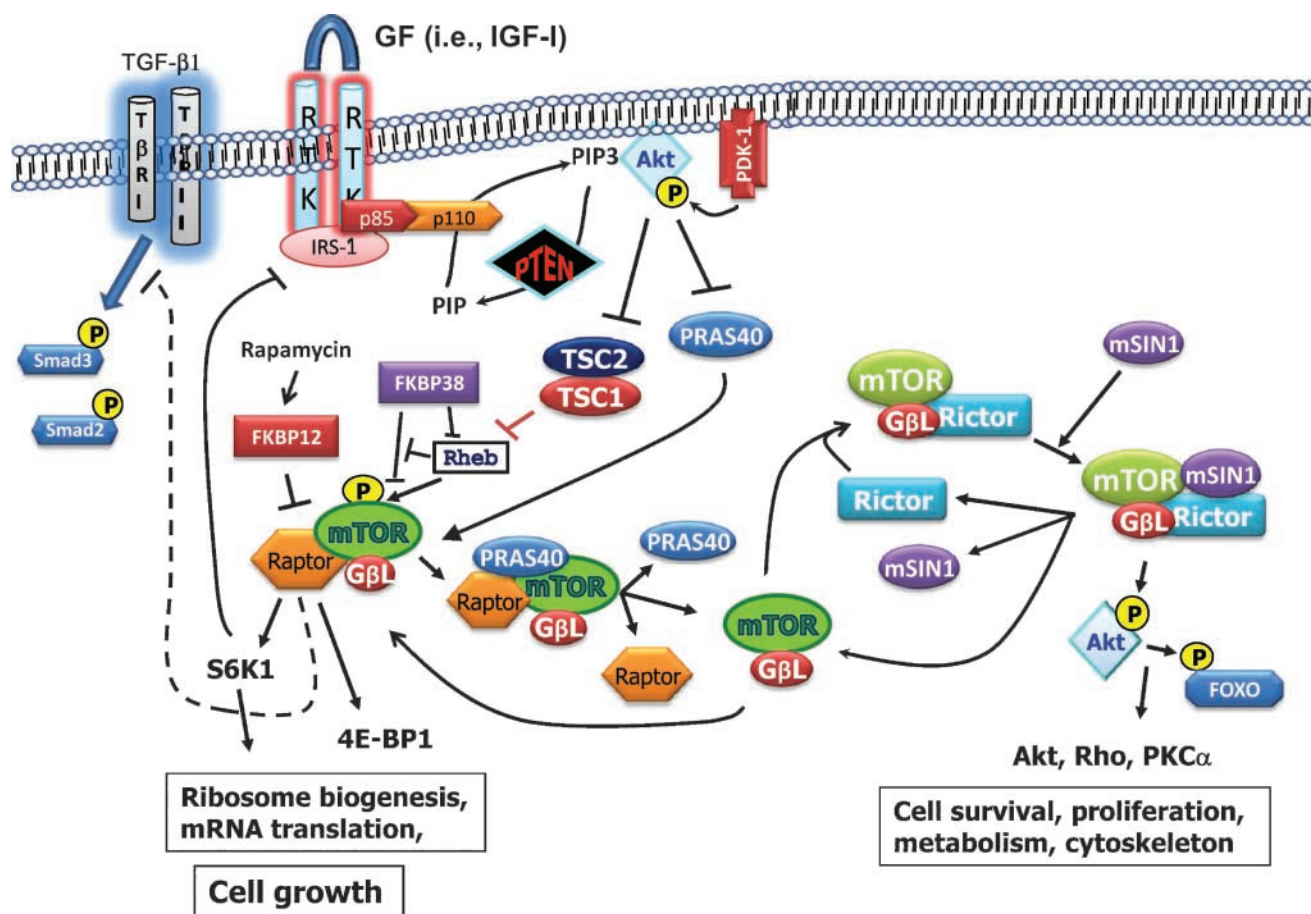


Figure 1. New partners in the mTOR signaling pathway. mTOR signals through two functionally distinct macromolecular complexes, mTORC1 (raptor complex) and mTORC2 (rictor complex), which are rapamycin dependent and rapamycin independent, respectively. The activity of the mTORC1 complex is negatively regulated by the TSC1/TSC2 tumor suppressor complex and by PRAS40. TSC1/TSC2 inactivates Rheb, an enzyme critical to activation of mTORC1. Another protein that inactivates mTORC1 is FKBP38, which interacts with both Rheb and mTOR and interferes with the ability of Rheb to interact with and activate mTORC1. FKBP38 is structurally similar to FKBP12, the protein that mediates the binding of rapamycin to mTORC1. Both TSC2 and PRAS40 are substrates of Akt kinase that are inactivated on phosphorylation by Akt. Through this mechanism, the activation of phosphatidylinositol 3-kinase and Akt (through growth factor signaling or by loss of PTEN) leads to activation of mTORC1, which promotes cell growth. Rapamycin and rapamycin analogues through association with FKBP12 disrupt the mTORC1 complex, leading to growth suppression. However, disruption of mTORC1 may promote the assembly of mTORC2 following the interaction of mTOR with rictor and mSin1, thus counteracting the antitumor activity of current mTOR inhibitors. Moreover, disruption of mTORC1 by rapamycin may relieve negative feedback suppression on mitogen activation of Akt, leading to elevation of cell survival signals. The ratio of raptor to rictor and the relative levels of PRAS40, TSC2, mSIN1, FKBP38, and IRS-1 in tumor cells are likely to be useful cancer prognostic markers and therapeutic targets.

4E from 4E-BP1 (8). Regulation of protein synthesis via mTOR also occurs through phosphorylation of p70 S6 kinase, a key regulator of cell growth, which phosphorylates the S6 40S ribosomal subunit. The mTOR-associated small G protein ras homologue enriched in brain (Rheb) is an upstream regulator of mTOR-dependent phosphorylation of both 4E-BP1 and p70 S6 kinase (9).

Signaling Pathway

Macromolecular Complexes of mTOR

mTOR functions in macromolecular complexes with at least five other proteins. All complexes appear to have G β L (also called mLST8; ref. 10) and either raptor (11) or rictor (Fig. 1; refs. 12–14). Only the complex containing raptor (mTORC1) is directly suppressed by rapamycin. 4E-BP1 and p70 S6 kinase each associate to mTORC1 through their 5-amino acid TOS signal motif (15, 16) and are phosphorylated by the mTORC1 complex. The FKBP12/rapamycin complex binds to and then disrupts mTORC1 by preventing the interaction of mTOR with raptor. Raptor binds directly to proteins possessing a TOS motif and thus serves as the docking site for both 4E-BP1 and p70 S6 kinase on mTORC1. mTOR complex containing rictor (TORC2) was shown to phosphorylate Akt at Ser⁴⁷³ as shown in an elegant study where raptor, rictor, and mTOR were individually silenced by lentiviral-mediated short hairpin RNA in a variety of tumor cell lines (13). Silencing of either rictor or mTOR reduced the phosphorylation of Akt at Ser⁴⁷³, providing the first evidence of mTORC2 as the long-sought phosphoinositide-dependent kinase 2. Interesting, silencing raptor instead enhanced the phosphorylation of Akt at Ser⁴⁷³ in those cell lines, which may have resulted from increased levels of mTORC2 favored simply by the law of mass action. In that study, silencing of rictor likewise reduced the phosphorylation of Akt at Thr³⁰⁸ but led to the elevation of Akt levels.

Although once thought to be necessary for full activation of Akt, phosphorylation of Akt at Ser⁴⁷³ appears to dictate the substrate specificity of Akt. For example, phosphorylation of this site enables Akt to phosphorylate and thus inactivate the transcriptional factor/apoptosis inducer proteins, FOXO (17). However, studies with rictor knockout mice suggest that Akt Ser⁴⁷³ phosphorylation site does not influence the ability of Akt to phosphorylate and inactivate tuberous sclerosis complex (TSC) 2 (17–19), a key negative regulator of mTORC1.

From Phosphatase and Tensin Homologue Deleted on Chromosome 10 Loss to Akt Activation

Loss of function of the tumor suppressor gene phosphatase and tensin homologue deleted on chromosome 10 (PTEN) is believed to play an important role in the etiology of numerous cancers including ~50% of advanced prostate cancers. PTEN is a lipid phosphatase (20) that suppresses insulin-like growth factor-I receptor signaling by dephosphorylating phosphatidylinositol-3,4,5-triphosphate to phosphatidylinositol-4,5-bisphosphate, leading to inhibition of phosphatidylinositol 3-kinase and downstream Akt responses. Loss or reduced expression of PTEN appears to

promote tumor growth and progression predominantly through elevating the levels of phosphatidylinositol-3,4,5-triphosphate and phosphatidylinositol-3,4-bisphosphate, which associate to the plasma membrane where they recruit Akt and phosphoinositide-dependent kinase 1, respectively, through binding to their PH domains. Phosphoinositide-dependent kinase 1, which a constitutively active kinase, then physically interacts with Akt and activates Akt by phosphorylating Thr³⁰⁸ (21). Akt and phosphoinositide-dependent kinase 1 mediate most if not all the antiapoptotic effects of insulin-like growth factor-I (21), a mitogen significantly implicated in the etiology of prostate cancer (22). Once activated, Akt is transported to cytosolic and nuclear compartments where it phosphorylates numerous proteins having the consensus motif RXXXS/T, many of which are involved in cell growth and apoptosis (23). Although, most studies have focused on the role of a single Akt isoform (Akt1) in cancer, the other two isoforms (Akt2 and Akt3) have unique tissue expression patterns and are also likely to have unique roles in cancer progression (24–27).

mTORC1 Complex

mTORC1 is principally activated through Akt kinase (21). However, the mechanism by which Akt activates mTORC1 appears to be controlled by at least two sets of Akt substrates, one involving the TSC2/Tuberin (see Fig. 1; refs. 28, 29). TSC2 and TSC1/Harmanin form heterodimers that suppress mTORC1 kinase activity through inactivation of the small GTP-binding protein Rheb (30, 31), a protein shown to be critical to the activation of mTORC1. TSC2, which has GTPase activity, inactivates mTORC1 by converting the GTP to GDP on Rheb. The direct phosphorylation of TSC2 by Akt at multiple serines promotes the proteosomal degradation of TSC2, leading to elevation of the GTP-bound Rheb and the subsequent activation of mTORC1 kinase (30). A recent study showed that the FKBP12 homologue FKBP38 inhibits Rheb from activating mTORC1 by two independent mechanisms, one involving a direct association of Rheb to FKBP38 and the other involving the binding of FKBP38 to mTOR at the Rheb binding site (Fig. 1; ref. 32). Similarly, overexpression of Rheb was shown to activate mTORC1 by reversing the inhibitory effect on FKBP38 on mTORC1, suggesting that Rheb activates mTORC1 through reversing the inhibition of mTORC1 by endogenous FKBP38. This study provides fresh insight on the mechanism by which rapamycin may inhibit mTORC1, as the rapamycin/FKBP12 complex may be mimicking the natural ligand FKBP38.

Recent evidence support that a proline-rich Akt substrate of 40 kDa (PRAS40) is also involved in Akt-dependent activation of mTOR (33, 34). Before getting phosphorylated by Akt, PRAS40 binds to raptor and sequesters raptor from mTORC1 (33, 35); through this mechanism, PRAS40 disrupts the mTORC1 complex similar to the effect of rapamycin (Fig. 1). The interaction of PRAS40 with raptor competes with the interaction of raptor with S6K1 and 4E-BP1 (35, 36). Moreover, this interaction of PRAS40 is very specific for the mTORC1 complex, as PRAS40 does not

associate with or disrupt TORC2. Ironically, rapamycin has been shown to decrease the coimmunoprecipitation of PRAS40 with mTOR, suggesting that rapamycin/FKBP12 competes with PRAS40 for binding to mTOR (35). However, the inhibitory activity of PRAS40 on mTORC1 is lost following the activation of Akt, which promotes proteosomal degradation of PRAS40 following phosphorylation of Thr²⁴⁶ (33). The mechanism for such proteosomal-dependent degradation is not clear but may involve 14-3-3, as once phosphorylated by Akt, PRAS40 binds to 14-3-3. Thus, the constitutive activation of Akt occurring in many cancers favors the formation of mTORC1 over that of mTORC2 and hence therapeutic responses to rapamycin. Taken together, these studies indicate that PRAS40 may play a pivotal role in the mechanism by which Akt activates mTORC1. Interestingly, PRAS40 has been recently reported to also be a substrate or downstream target of mTORC1, which phosphorylates PRAS40 at Ser¹⁸³, and occurs through a mechanism that is blocked by rapamycin (36). Mutation of Ser¹⁸³ to Asp, to mimic this phosphorylated form of PRAS40, was shown to disrupt its interaction with raptor, whereas mutation to Ala retains association to raptor, suggesting that phosphorylation of PRAS40 at Ser¹⁸³ by mTORC1 disables PRAS40 from binding to raptor and disrupting mTORC1 (36). Although several groups have shown that PRAS40 disrupts mTORC1, one group recently reported that PRAS40 may function as a downstream mediator of TOR signaling, because knockdown of PRAS40 by small interfering RNA impaired insulin-induced phosphorylation S6 and 4E-BP1 (15). However, the *in vivo* role of PRAS40 in controlling or mediating mTOR responses awaits the development of knockout mice. The role of PRAS40 in controlling oncogenesis and tumor progression remains a relatively unexplored and promising area of future investigation (37, 38).

In line with a finely regulated homeostatic control mechanism, mTORC1 is under negative feedback control (39). One such negative feedback reported by several investigators occurs through S6K and IRS-1. Once activated by mTORC1, S6K phosphorylates and inactivates IRS-1 (refs. 40, 41; Fig. 1). Inactivation of IRS-1 quenches growth factors, particularly insulin-like growth factor-I, from activating the phosphatidylinositol 3-kinase/Akt/mTORC1 pathway. Although adaptive in normal tissues, this negative feedback may account for resistance of tumors to killing by rapamycin, as inhibition of mTORC1 activates Akt, which in turn inactivates apoptotic proteins. Thus, strategies that prevent the activation of IRS-1 or Akt by rapamycin are likely to have synergistic activity on tumor kill (40, 42–44).

mTORC2 Complex

Another recently identified mTOR-binding protein is mSIN1, which selectively associates with mTORC2 but does not interact with mTORC1 (Fig. 1; refs. 45, 46). These studies revealed that mSIN1 is critical to the function of mTORC2, particularly for the ability of the mTORC2 complex to phosphorylate Akt at Ser⁴⁷³. *In vivo* and *in vitro* studies conducted with mSIN1 knockout mice

showed that loss of mSIN1 rendered mTORC2 unable to activate Akt at Ser⁴⁷³ (17, 19), indicating that mSIN1 is a key regulator of the substrate specificity of Akt. This effect may at least partly occur through the activity of mSIN1 in stabilizing the mTORC2 complex (46). Moreover, those studies support mSIN1 is a potentially important therapeutic target of mTOR, as interfering with the interaction of mSIN1 with mTORC2 is likely to prevent the potentially deleterious activation of Akt that occurs with rapamycin and that may also occur with rapamycin analogues. Five splicing isoforms of mSIN1 have been identified, three of which bind to mTORC2 to form three distinct mTORC2s; however, only two of them mediate the activation of Akt by insulin (46). Those studies suggest the generation of three unique forms of mTORC2, each with different cellular functions. Although mTORC2 is considered to be the rapamycin-insensitive complex, long-term treatment with rapamycin has been shown to suppress the mTORC2 complex in certain cells through a mechanism that is not well understood (47). It is thus likely that the levels of mSIN1 or the composition of its various isoforms may influence the sensitivity of mTORC2 to rapamycin.

Suppressing a Tumor Suppressor Pathway

Transforming growth factor- β (TGF- β) is an important regulator of numerous cellular functions, including cell growth, differentiation, apoptosis, cell motility, and cell adhesion. Deregulation of TGF- β expression and function is implicated in the pathogenesis of cancer (22) and is regarded as an important tumor suppressor in many early-stage cancers. There is substantial cross-talk between insulin-like growth factor-I and TGF- β receptor signaling pathways (21). Loss of the tumor suppressor function of TGF- β in prostatic carcinoma may occur through activation of the insulin-like growth factor-I signaling pathway (21, 22, 48, 49). Akt can block TGF- β responses through multiple mechanisms, not limited to the suppression of TGF- β -induced apoptosis. In the NRP-152 rat prostate epithelial cell line, Akt has been shown to block TGF- β responses through suppressing phosphorylation of Smad3 (21). This occurs through an mTOR-dependent mechanism, as it is reversed by rapamycin and mTOR small interfering RNA (48). These results support that mTOR interferes with the ability of TGF- β receptors to phosphorylate Smad3 (Fig. 1), molecular details of which are currently under investigation. We hypothesize that rapamycin can activate Smad3 by reversing the inhibitory activity of mTORC1 on TGF- β receptors. Through a different mechanism, involving the inhibitory association of FKBP12 to TGF- β type I receptor (T β RI), this macrolide was proposed previously to enhance TGF- β signaling (50). In that model, FKBP12 was reported to interact with T β RI and prevent ligand-independent activation of T β RI by T β RII (51–53). Here, TGF- β first associates with T β RII, causing dimerization of T β RII with T β RI and release of FKBP12 from T β RI, allowing the T β RII kinase to activate T β RI. Activated T β RI would then phosphorylate and activate the transcription

factor/transcription coregulators, Smad2 and Smad3. However, in the absence of ligand, T β RII has low affinity for T β RI, and FKBP12 would prevent the ligand-independent activation of T β RI by T β RII in that complex.

mTOR Inhibition in Prostate and Bladder Cancer

Genetic alterations, including loss of *PTEN*, mutation of the phosphatidylinositol 3-kinase, and amplification of *AKT1* and *AKT2* leading to activation of Akt kinase activity have been linked with the development of castrate progressive prostate cancer (54). Transgenic mice with *PTEN* knockout confirmed a functional role of this pathway in acquisition of castrate progressive prostate cancer (55). It is also known that alterations of the Akt signaling cascade (mTOR dependent) can lead to the development of prostate intraepithelial neoplasia (54). Similarly, preliminary *in vitro* findings in prostate cancer cell lines show a correlation between *PTEN* loss or Akt activation and sensitivity to growth inhibition by temsirolimus (56, 57). Although the *in vitro* data in TCC are less prominent, it is well established that *PTEN* mutations are present in ~30% of TCC patients and that the phosphatidylinositol 3-kinase pathway regulates TCC cell invasion (58). Thus, mTOR inhibition has been an attractive therapeutic strategy for this disease. Unfortunately, the development of clinical trials in this setting has been quite slow. To date, there are no active clinical trials evaluating mTOR inhibitors in TCC; however, with the availability of everolimus and temsirolimus and the current published clinical and safety data, it is expected that these agents will be tested in patients with cisplatin-refractory TCC.

Preliminary data in prostate cancer patients suggest that mTOR inhibition may have antitumor activity in these patients. A single phase II study led by Lerut et al. (59) evaluated everolimus in patients with newly diagnosed, localized prostate cancer. In this study, patients received 4 weeks of everolimus administered as either weekly (30, 50, or 70 mg) or daily (5 or 10 mg) doses before undergoing radical prostatectomy. Changes in immunohistochemical scores for total and phosphorylated forms of S6, Akt, and 4E-BP1 between pretreatment and posttreatment tissue were evaluated. Fifteen patients completed treatment: 4 patients in each of the 30 and 50 mg/wk, 5 mg/d cohorts, and 3 patients in the 10 mg/d cohort. No dose-limiting toxicities were observed. Most frequently observed adverse events are common toxicity criteria grade 1 to 2 stomatitis and rash. Initial immunohistochemical observations indicate highly heterogeneous phospho-S6 staining in hyperplasia, intraepithelial neoplasia, and cancer cells and no correlation between immunohistochemical score for phospho-S6 and Gleason score or proliferation index.

Although these results are encouraging, the evaluation and measurement of everolimus induced antiprostate cancer activity and its correlation with biological effects remains unclear. Clinical trials evaluating the activity of AP-23573 in refractory solid malignancies including metastatic castrate progressive prostate cancer have been

initiated. Similarly, trials evaluating the combination of bevacizumab and temsirolimus in chemotherapy-refractory castrate progressive prostate cancer have been initiated at our institution. Trials evaluating mTOR inhibitors early on in localized prostate cancer are also under way. We hope the tissue and correlative endpoints of these trials will help to clarify the antitumor activity of mTOR inhibitors in prostate cancer patients.

Activity of mTOR Inhibitors in mRCC

Although the importance of this signaling cascade in RCC has not been well characterized, several *in vitro* studies have shown that the activation of the mTOR pathway leads to gene overexpression of hypoxia-inducible factor-1 α a crucial molecule in the pathogenesis of clear cell RCC (60). Other authors (61) have also reported that temsirolimus can reduce the expression of both hypoxia-inducible factor-1 α and hypoxia-inducible factor-2 α regardless of oxygen saturation. Another potential rationale for mTOR inhibition in RCC is through *PTEN*. In RCC, the gene expression of *PTEN* can be heterogeneous as *PTEN* mutations in RCC are not commonly seen. Nevertheless, *PTEN*-deficient cells are more sensitive to the activity of mTOR inhibitors through an increased phosphorylation state in the Akt pathway. Further studies will help determine whether such enhanced sensitivity may be due to elevated ratio of mTORC1 to mTORC2, from loss of PRAS40 by Akt, from differences in the S6K/IRS-1-negative feedback control, or from differences in levels of mSIN1. Next, we will review the existing clinical data with the two mTOR inhibitors most commonly studied in mRCC, everolimus and temsirolimus.

Everolimus

Everolimus is an orally active derivative of rapamycin. In preclinical models, the administration of everolimus is associated with reduction of mTOR downstream phospho-S6 and p-4E-BP1 and occasionally with increase in upstream phospho-Akt. Several phase I and II studies evaluating dose, schedule, and pharmacodynamics of everolimus have been reported and are discussed elsewhere (62–65). Abid et al. (66) conducted a phase II trial where 41 previously treated mRCC patients received oral everolimus at a dose of 10 mg/d without interruption (28-day cycle), but with dose modifications for toxicity. The overall response rate observed was 33% (12 of 37), 19 patients had stable disease ≥ 3 months with a median duration of therapy of 8 months (range, ≥ 1 –20). Treatment-related adverse events included mucositis, skin rash, pneumonitis, hypophosphatemia, hyperglycemia, thrombocytopenia, anemia, and elevated liver function tests.

Currently, a large phase III trial evaluating everolimus plus best supportive care versus placebo plus best supportive care in patients with previously treated mRCC is under way in Europe. This is an attractive trial evaluating the concept of sequential therapy in RCC that plans to accrue >300 patients with tyrosine kinase inhibitor-refractory mRCC.

Temsirolimus

Preliminary phase I data led to the design of a phase II trial in patients with treatment-refractory mRCC. In this study, 111 patients were randomized to one of multiple dose levels (25, 75, or 250 mg i.v. weekly; ref. 67). The overall response rate was 7%, with additional patients showing minor responses. Given the high number of dose reductions and treatment discontinuations at the higher dose levels, the investigators advocated the 25 mg i.v. weekly dose for future temsirolimus studies. Retrospective assignment of risk criteria to patients in this study identified a poor prognosis group ($n = 49$). Temsirolimus-treated patients in this poor prognosis group had a median overall survival of 8.2 months compared with 4.9 months for first-line IFN- α -treated patients (historical controls, $n = 437$). Loss of PTEN may be more common in poor-risk patients and may account for this finding, because mutation of this tumor suppressor gene would activate mTOR and potentially increase the relevance of mTOR targeted therapy in this subgroup (68). A subsequent randomized phase III trial was conducted in patients with poor-risk metastatic RCC as defined by existing prognostic schema (69). Patients with mRCC and no prior systemic therapy were enrolled in this open-label study if they had three or more of six adverse risk factors [Karnofsky performance status <80%, time to metastatic disease <1 year, hemoglobin less than the lower limit of normal, serum lactate dehydrogenase >1.5 times upper limit of normal, corrected serum calcium >10 mg/dL, and >1 metastatic disease site; ref. 70]. Patients were equally randomized to receive IFN- α up to 18 million units s.c. three times weekly, temsirolimus 25 mg i.v. once weekly, or temsirolimus 15 mg i.v. once weekly + IFN- α 6 million units s.c. three times weekly. The primary study endpoint was overall survival and the study was powered to compare each of the temsirolimus-containing arms with the IFN- α arm. The study showed that patients treated with temsirolimus had a statistically longer survival than those treated with IFN- α alone (10.9 versus 7.3 months; $P = 0.0069$). Overall survival of patients treated with IFN- α or temsirolimus + IFN- α were not statistically different (7.3 versus 8.4 months; $P = 0.6912$). Similarly, the overall response rate for each treatment arm was 7% IFN- α , 9% temsirolimus, and 11% for the combination arm. These results led to the recent Food and Drug Administration approval of temsirolimus in mRCC. Of importance, the lack of survival benefit in the combination arm is thought to be the result of a lower dose of temsirolimus coupled with an increased number of patients unable to receive temsirolimus secondary to IFN- α -related toxicities. Similar to the everolimus toxicity profile, some of the most common toxicities observed in patients receiving weekly temsirolimus include hyperglycemia, hypertriglyceridemia, hypercholesterolemia, and asthenia, edema, and dyspnea.

More recently, Dutcher et al. (71) did a subgroup analysis of the IFN- α - and temsirolimus-treated patients on the phase III study. In their retrospective analysis, overall survival and progression-free survival correlated with type of histology, age, and Memorial Sloan-Kettering Cancer

Center prognostic risk group (70, 72). Caution should be used when interpreting subgroup analyses as their sample size and lack of statistical power to detect meaningful differences between groups may not reflect the entire population studied. Despite its limitations, it is provocative that patients with histologies other than clear cell carcinoma ($n = 37$; 18%) receiving temsirolimus appeared to have a superior median progression-free survival and overall survival (7 versus 1.8 months and 11.6 versus 4.3 months, respectively). Similarly, when poor-risk and intermediate-risk patients were stratified using the standard prognostic criteria, intermediate-risk patients did not have any benefit by receiving temsirolimus when compared with IFN- α -treated patients. Other variables that appeared to predict a better outcome in temsirolimus-treated patients include age <65 years and prior nephrectomy, although the patients with primary tumors in place also received benefit from this agent.

Based on the current available data, temsirolimus is a standard of care for patients with poor-risk features as well as for patients with non-clear cell RCC. Further investigation of this agent is planned in patients with fewer adverse risk features and in combination with other vascular endothelial growth factor targeting strategies.

Future of mTOR Inhibitors in Genitourinary Malignancies

Current *in vitro* and *in vivo* data clearly show the importance of the mTOR signaling pathway in the pathogenesis of genitourinary malignancies. Although current existing data in prostate and bladder are not as robust as in renal cancer, ongoing studies will further define the clinical efficacy and safety profile of these agents in this subset of patients. Once their activity as single agents has been defined, the endpoint of future studies should be aimed to identify biological markers of response and potential combination strategies that could lead to improvement in antitumor activity. Clinical studies evaluating dual inhibition (mTOR and vascular endothelial growth factor inhibitor) have initiated. Preliminary data using bevacizumab and temsirolimus in advanced RCC have shown clinical efficacy and no new safety signal of concerns. This type of combinations will soon be tested in patients with castrate progressive prostate cancer.

The mTOR field has witnessed an explosion of information in the past 5 years, with major advances in the basic biology of mTOR signaling, particularly the composition and function of macromolecular components of mTOR that confer responses to mTOR inhibitors. These studies have provided new tissue markers (raptor, rictor, PRAS40, mSin1, FKBP38, and IRS-1) that are likely to not only predict therapeutic response but also serve as new therapeutic targets for intervention of cancer.

Disclosure of Potential Conflicts of Interest

J.A. Garcia: Novartis and Wyeth research funds, Wyeth advisory board/consultation. D. Danielpour reports no potential conflicts of interest.

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