Mammalian Target of Rapamycin Inhibition

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ABSTRACT

The mammalian target of rapamycin (mTOR) is a serine/threonine kinase that has been increasingly recognized as key to the regulation of cell growth and proliferation. mTOR either directly or indirectly regulates translation initiation, actin organization, tRNA synthesis, ribosome biogenesis, and many other key cell maintenance functions, including protein degradation and transcription functions. Inhibition of mTOR blocks traverse of the cell cycle from the G_1 to S phase. Preclinical data show inhibition of tumor growth in a number of cell lines and xenograft models. Clinical trials are ongoing. In metastatic renal cell cancer, both tumor regression and prolonged stabilization have been noted. mTOR inhibition appears to be a key pathway that may be useful in antitumor therapy. Renal cell cancer may be particularly susceptible through both the translation inhibition pathway and pathways that enhance *HIF-1*α gene expression, a factor believed to stimulate growth in metastatic renal cell cancer. Additional clinical trials that use agents that inhibit mTOR are ongoing.

INTRODUCTION

The identification of the mammalian target of rapamycin (mTOR) pathway as a potential target for anticancer therapy emerged from efforts to understand the activity of the immunosuppressive drug rapamycin (sirolimus, Rapamune, Wyeth-Ayerst Laboratories, Collegeville, PA). Rapamycin, a natural product derived from the soil bacteria *Streptomyces hygrosopius*, was approved for use in organ transplantation in 1999 (1–4). During its preclinical evaluation, studies demonstrated potent antitumor activity, although initially the mechanism was unknown (5–7). Subsequently, Dilling *et al.* (8) demonstrated potent inhibition of growth of rhabdomyosarcoma cells by rapamycin at nanogram concentrations. Clues to the pathways involved were derived from this cell line, which required an autocrine loop involving signaling through insulin-like growth factor receptors (9, 10). Subsequent work by many investigators demonstrated that rapamycin produces cell cycle arrest, preventing progression of dividing cells from the G_1 to S phase of the cell cycle (11, 12). Subsequently, the role of mTOR, a serine/threonine kinase, as a key regulator of cell cycle and of many intracellular functions has emerged and presented itself as a potential target for antitumor therapeutics.

mTOR REGULATORY PATHWAYS

Rapamycin does not directly inhibit mTOR but binds to its immunophilin, FK binding protein (FKBP12). Rapamycin plus FKBP12 then interact with mTOR and inhibit its function (12), leading to inhibition of cell growth and proliferation. The downstream effects of this inhibition include inhibition of translational pathways, with loss of phosphorylation of the eukaryotic translation initiation factor, 4E binding protein-1, and inhibition of the 40S ribosomal protein p70 S6 kinase (blocking ribosomal biogenesis; refs. 13–15). This effect results in a 15% to 20% inhibition of overall protein translation and leads to cell cycle arrest (12). Other cellular functions that appear to be regulated by mTOR and, thus, affected by its inhibition include actin organization, membrane traffic, protein degradation, protein kinase C signaling, and tRNA synthesis (16). There are also regulatory effects on synthesis of essential cell cycle proteins, such as cyclin D1 and c-myc (15, 17–19). Recent data suggest that mTOR regulates protein synthesis when cellular ATP levels fluctuate (20).

In addition to the downstream activities of mTOR, which are affected by its inhibition, important upstream regulators of its activity may be altered in malignant cells. This may make this pathway particularly important in antitumor therapeutics. Both phosphatidylinositol 3′-kinase and Akt are upstream to mTOR. Akt activity is regulated by *PTEN*, a tumor suppressor gene, thus regulating mTOR activity. This counteracts Akt activation through phosphatidylinositol 3′-kinase. Aberrations in these upstream regulators, therefore, may lead to alterations in mTOR regulatory activity. The most striking examples of this are the dysregulation of phosphatidylinositol 3′-kinase activity when *PTEN* is mutated, deleted, or methylated (21–23). In these situations, this could lead to uncontrolled activity of mTOR, leading to uncontrolled cell proliferation.

An additional pathway influenced by mTOR that appears to be particularly important in renal cell carcinoma involves the hypoxia-inducible factor (HIF). With loss of *VHL* function commonly seen in clear cell renal cell cancer, there is accumulation of the oxygen-sensitive transcription factors HIF-1α and HIF-2α (24). An increase in accumulation of these factors yields increased stimulation of vascular endothelial growth factor (VEGF), platelet-derived growth factor, and transforming growth factor α (25). This effect is augmented by the activation of mTOR, which stimulates both a protein stabilization function and a protein translational function and, thus, increases HIF-1α activity (26, 27).

In addition, it has been determined that mutations of tuberous sclerosis complex TSC1 and -2 gene products function together to inhibit mTOR-mediated downstream signaling (28). Mutations of these genes occur in tuberous sclerosis, and their loss of function yields yet another pathway, which leads to

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increased activity of mTOR and disrupts phosphatidylinositol 3′-kinase-Akt signaling through down-regulation of platelet-derived growth factor receptor (28–30). Loss of *TSC1* or *TSC2* gene activity induces VEGF production through mTOR (30). Signaling through this pathway with activation of Akt and mTOR results in increased HIF activity and increased VEGF. However, in TSC2-negative cells, platelet-derived growth factor receptor is markedly reduced (29) TSC2 regulates VEGF through both mTOR-dependent and -independent pathways (31). TSC2 also regulates HIF. Thus, studies evaluating the impact of TSC1 and TSC2 mutations demonstrate the connection of increased VEGF and activated mTOR pathways to angiogenesis. Thus, inhibition of mTOR, leading to an antiangiogenic effect, can be explained by its impact on several proangiogenic pathways.

PRECLINICAL EVALUATION OF mTOR INHIBITION

As stated previously, early work evaluating the immunosuppressive activity of rapamycin also demonstrated antitumor activity (5, 6), but this was not initially further evaluated. Subsequently, studies in rhabdomyosarcoma cells suggested an antitumor effect, particularly in cells that required stimulation by insulin-like growth factor (8). Subsequent studies in multiple other cell lines have shown both cytostatic and cytotoxic effects of rapamycin (8, 15, 22, 32–41). Of interest, during these evaluations, it became apparent that rapamycin not only produced cell cycle arrest in G_1 but also produced effects that led to cell death. Experimental findings have demonstrated induction of programmed cell death (apoptosis) in B-cells (42, 43) and rhabdomyosarcoma cells (35, 36).

In tests performed by the National Cancer Institute (NIH, Bethesda, MD) human tumor cell line panel, rapamycin and its derivatives showed significant growth inhibition in breast cancer, prostate cancer, leukemia, melanoma, renal cell cancer, glioblastoma, and pancreatic cancer (22). It is known that half of glioblastomas have *PTEN* mutations, which could make them increasingly sensitive to mTOR inhibition, and this is being investigated clinically (21, 22, 44).

Studies in human tumor xenografts in mice have also demonstrated prolonged time to tumor growth (37). In studies in a pediatric brain tumor model that used CCI-779, an ester of rapamycin, there was significant growth inhibition and an additive effect when CCI-779 was administered with cisplatin (37). This interesting evaluation demonstrated new clinical characteristics: (1) there was not a linear-dose response effect but more of a threshold level effect, (2) intermittent dosing was effective, and (3) 2 weeks of daily dosing was superior to 1 week, one large single dose, and dosing for >2 weeks.

Additionally, renal transplantation investigators have compared the tumor-promoting effect of the immunosuppressive agents used in renal transplantation in a murine model of metastatic human renal cell cancer (45). Of interest, and consistent with these preclinical data, the number of pulmonary metastases was reduced when the animals were exposed to rapamycin but increased when exposed to cyclosporine, another immunosuppressive agent used in transplantation (45). In these studies, rapamycin also reduced circulating levels of VEGF-A and transforming growth factor β1 (45).

Because rapamycin has poor water solubility and stability in solution, it is a poor candidate for parenteral administration. Therefore, two ester analogues of rapamycin have been developed with improved pharmaceutical properties and cellular effects similar to rapamycin in the cell line screening evaluations: CCI-779 (Wyeth-Ayerst Research, Cambridge, MA) and RAD-001 (everolimus, Novartis AG, Basel, Switzerland). Both of these agents have entered clinical trials (46–48). A third mTOR inhibitor, ap23573 (ARIAD Pharmaceuticals, Cambridge, MA; ref. 49), is completing preclinical trials and is scheduled for clinical trials in late 2004.

PHASE I CLINICAL INVESTIGATION IN CANCER PATIENTS

Most of the current clinical data with agents that inhibit mTOR come from clinical trials of CCI-779 (Wyeth-Ayerst). This agent has been evaluated extensively in two Phase II trials, one using a weekly schedule and one using a daily for 5 days schedule. Raymond *et al.* (46) studied the weekly dosing as a 30-minute infusion at doses ranging from 7.5 to 220 mg/m^2 per week. There were no dose-limiting toxic effects, although grade 3 mucositis was observed. There was a partial response in one patient each with renal cell cancer, breast cancer, and a neuroendocrine tumor. The second Phase I trial, by Hidalgo *et al.* (47) evaluated daily 30-minute infusions given for 5 days every 2 weeks. Dose-limiting toxic effects were grade 3 thrombocytopenia, grade 3 elevations of liver function tests, and grade 3 hypocalcemia. The maximally tolerated dose was 19 mg/m^2 daily for minimally pretreated patients and 15 mg/m^2 daily for heavily pretreated patients. A partial response was noted in one patient with non–small cell lung cancer.

Other relatively frequent toxic effects that occurred with some frequency included dermatologic effects such as eczematoid reactions, maculopapular rash, nail bed changes, and folliculitis. The hematologic effect was thrombocytopenia. Elevations in liver function tests and hypertriglyceridemia and hypercholesterolemia were noted. There were reversible decrements in testosterone. There was occasional mucositis. All of these effects were described as being mild.

PHASE II CLINICAL TRIAL OF CCI-779 IN PATIENTS WITH METASTATIC RENAL CELL CANCER

A Phase II trial has been completed and reported involving patients treated previously with metastatic renal cell cancer (50, 51). This study used weekly administration of one of three dose levels of CCI-779: 25 mg/m^2, 75 mg/m^2, and 250 mg/m^2. The dose used was blinded to the treating physician and patient. Dose reductions were prescribed for grade 3 toxic effects. Treatment was continued until evidence of progression or unacceptable toxic effects. Patients were premedicated with diphenhydramine to preclude allergic reactions that were observed early in the trial. There were 111 patients enrolled and 110 received treatment, 36 at 25 mg/m^2, 38 at 75 mg/m^2, and 36 at 250 mg/m^2. Ninety percent of patients had received prior therapy for metastatic disease (usually a cytokine), and more than half had

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received more than one prior regimen. Sixty-five percent of patients were Eastern Cooperative Oncology Group performance status 1, and 35% were Eastern Cooperative Oncology Group performance status 0.

As in the Phase I trials, the most common toxic effects observed were maculopapular rash (76%) and mucositis (70%). Additionally, with prolonged therapy, patients developed asthenia (50%) and nausea (43%). Grade 3 or 4 laboratory adverse events were hyperglycemia (17%), hypophosphatemia (13%), anemia (9%), and hypertriglyceridemia (6%). There did not appear to be a dose-toxicity relationship, although there were more dose reductions at the higher dose levels. Responses were observed with all of the dose levels.

The objective response rate was 7%, with one complete and seven partial responses. Median time to tumor progression was 5.8 months, and median survival was 15 months. The cumulative response rate, including complete responses, partial responses, minor response, and stable disease for >24 weeks, was 51% (51).

In this study, patients were evaluated for prognosis, using the prognostic factor criteria developed by Motzer *et al.,* (52) in an analysis of >300 patients treated with interferon at Memorial Hospital, New York, NY. The five factors that were determined to have significant prognostic impact were performance status, lactate dehydrogenase, serum calcium, hemoglobin, and time from initial diagnosis to treatment (52). Although initially developed for untreated patients, the factors appear to segregate patients in the second-line setting as well (51). In this study of CCI-779, survival was decidedly different based on prognostic group among all of the dose-level patient groups (51).

Eighty-seven percent of CCI-779 patients fell into the intermediate or poor prognosis categories for metastatic renal cell cancer, with <10% in the good prognosis group. Median survival for these CCI-779–treated patients in the intermediate or poor groups appeared to be 1.6- to 1.7-fold longer than those in the original study by Motzer *et al.* (52) of interferon-treated patients when compared with prognostic group by prognostic group (of first-line patients). Thus, mTOR inhibition may be of particular value in the poorer prognosis patients. This will be additionally tested in a randomized Phase III trial.

Preclinical data have suggested synergy of CCI-779 administered in combination with interferon α, which has led to the conduct of a Phase I/II clinical trial of this combination in renal cell cancer (53, 54). The study design began with an initial dose of interferon at 6 million units three times per week and of CCI-779 at 5 mg/m^2 weekly. In the initial Phase I component of the study, the dose of CCI-779 was escalated, and interferon αwas kept at 6 million units three times per week. Doses of CCI-779 ranged from 5 to 25 mg/m^2. A small cohort was also treated at 15 mg/m^2 and 9 million units of interferon α. The Phase II component of the trial has extended the number of patients treated at 15 mg/m^2 of CCI-779 and 6 million units of interferon α. As of December 2003, 71 patients have been entered, and several continue with treatment. The median time undergoing treatment for all of the cohorts is 7 months, and more than half have continued treatment for >6 months. Responses have been confirmed, and evaluation is continuing. The combination appears to show favorable activity and safety. A Phase III study has been initiated in which the combination of CCI-779 and interferon α is compared with either agent alone as first-line therapy in patients with a poor prognosis for metastatic renal cell cancer. An additional randomized study is planned for patients who have had prior therapy.

OTHER AGENTS

The other agents that inhibit mTOR are undergoing early phases of evaluation. RAD001 is an oral formulation and is currently in Phase I clinical trials (48). The agent ap23573 is currently in preclinical evaluation, with human clinical trials planned (49).

DISCUSSION

Preclinical data demonstrate the importance of mTOR as a regulator of cell growth and proliferation. Additional evaluation of malignant cells demonstrates that the constitutive activity of mTOR leads to unregulated growth. Human malignancies have been demonstrated to have a constitutive activation of mTOR or its upstream regulators, leading to enhanced mTOR activity. Now clinical data are beginning to demonstrate that this pathway is a viable target for antitumor therapeutics, and the outcome demonstrated in the Phase II trial of CCI-779 in metastatic renal cell cancer is promising. The ability to evaluate and interpret prolonged stable disease in metastatic renal cell cancer trials continued to be problematic but appears to be a real outcome and will need to be carefully quantitated in future randomized trials with many of the agents currently undergoing evaluation, including the inhibitors of mTOR.

As noted in the early *in vitro* work by Dilling *et al.* (8), the rhabdomyosarcoma cell line that they studied is regulated by an autocrine loop involving secretion of type 2 insulin-like growth factor, and signaling is through the insulin-like growth factor receptor. In previous studies (8, 12), the cells most sensitive to rapamycin were dependent on this autocrine pathway. Others have reported the interaction of rapamycin with control of glucose and lipids and the role of mTOR in the insulin-signaling pathway (55, 56). In view of the incidence of hyperglycemia and hypertriglyceridemia observed during treatment with CCI-779, it is possible that these biochemical parameters may correlate with the degree of mTOR inhibition (51). This will be evaluated by additionally investigating the clinical outcome in the current trials with CCI-779 and the levels of glucose and lipids. This will need additional confirmation by observations during trials of the other mTOR inhibitors, as to whether these clinical effects indeed do reflect a pharmacodynamic surrogate for mTOR inhibition.

The inhibition of mTOR appears to be a promising targeted approach to antitumor therapy. Additional evaluation of these agents for schedule, route of administration, and combination therapy approaches is clearly warranted. Renal cell cancer appears to be sensitive to this therapeutic approach, and it will be of great interest to determine whether combinations of mTOR inhibitors with other agents will enhance the therapeutic efficacy in this difficult disease. It will also be important to evaluate the biochemical parameters that might predict favorable outcome.

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OPEN DISCUSSION

Dr. Robert J. Motzer: Can you comment on the lung toxicity or the pulmonary infiltrates that are associated with the drug?

Dr. Janice P. Dutcher: There have been some asymptomatic patients, but we did not see them at all in our group.

Dr. Michael B. Atkins: In the paper that was published in JCO, there were 6 patients out of approximately 100 who had pulmonary infiltrates (J Clin Oncol 2004;22:909–18). Some of these individuals had associated symptoms. All had their treatment held, 2 were not restarted probably because their disease progressed, 4 were restarted when their symptoms got better. Two did not have a recurrence of the problem, and 2 had a recurrence of the pulmonary infiltrates. Five of 6 happened at the 75-mg dose level, 1 at the 250-mg level, and none at the 25-mg dose level. It is hard to say whether any of these side effects were dose dependent.

Dr. Robert A. Figlin: My understanding of signal transduction inhibition with CCI-779 is that administered once a week it will not inhibit the target for an extended period. Do we have any readout from laboratory biology that can help us understand whether once a week, which is very convenient for the patient but might not be good at impacting the tumor, is a better strategy? Is that born out by laboratory models? How do we need to be targeting these pathways via pharmacological interventions?

Dr. William G. Kaelin, Jr.: I think these are the right questions.

Dr. Atkins: There were a lot of patients in this study who had minor responses. I think it was 26% of the patients who had more than 25% tumor regression without satisfying the criteria for partial response. A lot of the patients who were benefiting were patients who we thought would never have benefited from immunotherapy. There were people with hypercalcemia, fatigue, a performance status of 2, and multiple prior treatments. We were seeing the disease course change in those patients, so it suggested to us that maybe there was a different patient population that was responding to CCI-779 than would typically respond to immunotherapy. What would be the appropriate targets to measure in an mTOR inhibition trial and when would be the appropriate time to measure them?

Dr. Figlin: That's in fact what our trial will be looking at in patients who are undergoing cytoreductive nephrectomy with metastatic disease. We will be looking at inhibition of all of the targets in the mTOR pathway.

Dr. Walter M. Stadler: There is a lot of publicity about these drugs being more active in the PTEN-deficient tumors. Has any of that been born out clinically?

Dr. Figlin: The mTOR pathway appears to have importance not just inside the tumor cell but also in terms of the angiogenesis. There may be some synergy in terms of that pathway being important in multiple places.

Dr. Atkins: What about the connection between mTOR inhibition and HIF? Is that a direct effect or is that a more general effect on protein synthesis or on molecules that are turning over quicker?

Dr. Kaelin: That is still being worked out, and I certainly don't want to oversell the HIF connection. There clearly is a HIF connection, but mTOR is a fairly important enzyme that does other things. An emerging theme that is coming out of at least preclinical studies with various targets and cancer is this notion that cancer cells, after mutating a particular pathway, become addicted to that pathway and hypersensitive to inhibitors of that pathway. This happens to be one of those scenarios that have been described for some other targets as well.

Dr. Michael S. Gordon: Those have been the settings where the greatest results, positive results, have been shown.

Dr. Kaelin: Preclinically, in retrospect, there was evidence that leukemic cells become addicted to BCR-ABL signaling and become hypersensitive to an inhibitor. In some of these models, for example, after you introduce BCR-ABL into cells, they become hypersensitive and they will undergo apoptosis after acute interruption of that signal, which might favor intermittent dosing to allow normal cells to recover. If I am not mistaken, rapamycin is primarily antiproliferative, meaning cytostatic. There is an influence of PTEN status on responsiveness, but still what you see is a cytostatic effect. In that scenario, you could make the case for a continuous exposure.

Dr. Daniel J. George: It sounds like there is still a huge dose range that people are struggling with. Obviously, there is toxicity, but does that toxicity vary? Should we be using a dose lower than 25 mg?

Dr. Dutcher: The sense was that the toxicity was clearly manageable at all dose levels, and the dose that is in the Phase III trial is the 25-mg dose. There are some that would argue that you should stay at the 250 mg, because it would allow dose reductions and there was a slight difference in survival.

Dr. Atkins: When we looked at all of the efficacy parameters of response, time to progression, and survival, there was no clearcut difference between doses, indicating that even the 25-mg dose was probably high enough to hit the target. Although the survival was longer in the 250-mg patients, the time to progression was less, so there may have been patient selection that accounted for their prolonged survival. It is interesting that in the more recent studies with CCI-779 and interferon, we have seen several patients who have had significant hypertension as well. It was not reported in the single-agent Phase II study, because it probably did not get to exceed the 10% to 15% range. Now we are seeing patients who have had hypertension because they have been on treatment for a while, which may be an indication that CCI-779 is hitting VEGF or HIF.

Dr. Gordon: Is the rash an EGFR-type rash? Is it the same as we would see with gefitinib?

Dr. Dutcher: It is not as severe, but it is similar. It usually did not last through the whole treatment.

Dr. Atkins: A lot of patients got steroids to control the rash, which could be a potential problem. It is sort of strange to think about combining this with interferon even though there was synergy, but it is clearly an immunosuppressive agent if you give it frequently enough.

Dr. Dutcher: Some of the people got antibiotics for the nail bed problems.

Dr. Gordon: But that, again, is something that we have been seeing with the targeted therapies.

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Mammalian Target of Rapamycin Inhibition

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