Mammalian Target of Rapamycin (mTOR) Inhibitors

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Current efforts in anticancer drug development are targeting key factors in cell-cycle regulation. Mammalian target of rapamycin (mTOR) is one such protein kinase that facilitates cell growth by stimulating the cell to traverse the G_1 to S phase of the cell cycle. Rapamycin is the first defined inhibitor of mTOR, and the demonstration of its antitumor activity has led to great interest in this pathway as an antitumor mechanism. Analogues with better pharmacologic properties have been developed and have entered clinical trials. Human cell lines of renal cell cancer, among several other tumors, are sensitive to growth inhibition via this pathway. Ongoing clinical trials are evaluating renal cell cancer and other malignancies using therapy with mTOR inhibitors. These agents are more likely to induce growth inhibition rather than tumor regression.

Introduction

Current efforts in anticancer drug development are targeting key regulatory proteins in the cell cycle that may be amenable to control by pharmacologic intervention. An understanding of the pathways and the role of various positive and negative control substances has led to the development of a number of agents that may selectively control growth of tumor cells. Agents that affect certain checkpoints or control points in the cell cycle are of particular interest.

Rapamycin, a natural product derived from *Streptomyces hygroscopicus*, is used as an immunosuppressive agent in renal transplantation, where it was observed to have antitumor as well as immunosuppressive activity [1–3]. A number of pathways of cell regulation were elucidated in subsequent investigations of its mechanism of action and sites of action with respect to the cell cycle. Rapamycin blocks cellular proliferation by inhibition of cell-cycle progression at G_1 to S [4]. The activity of rapamycin as a potential antitumor agent has led to the development of a number of analogues with more favorable pharmacologic properties that are undergoing clinical investigation (Figs. 1 and 2) [5•]. The clinical data from these trials are summarized in this paper.

Rapamycin binds to its immunophilin, FK binding protein (FKBP12), and takes a different pathway than that of the other immunosuppressive agents used in solid organ transplantation (cyclosporine A, FK506). Rapamycin combined with FKBP12 interacts with mTOR and inhibits its activation [6•]. Thus, rapamycin is the first identified mTOR inhibitor.

The inhibition of mTOR appears to be critical to cell-cycle control in malignant cells, and this pathway is of great interest as a possible anticancer target. It is more sensitive to inhibition in malignant cells than in normal cells. As a protein kinase that regulates cell-cycle progression from G_1 to S, mTOR promotes cell growth [5•]. In cells that respond to growth factors through growth factor receptors, the upstream regulator of mTOR, Akt, a serine–threonine kinase, is activated, and then mTOR is activated. In response to activation, mTOR signals two separate downstream pathways, which stimulate translation of specific mRNAs that signal cell growth at the G_1 to S phase of the cell cycle. The first pathway is through phosphorylation of the eukaryotic translation initiation factor, 4E binding protein-1 (4E-BP1), and the second is through the 40S ribosomal protein p70 S6 kinase [5•,7,8]. Cyclin D3 and c-myc are among the other proteins regulated in part by mTOR [8].

An additional component regulating the mTOR pathway is control of the upstream regulator, Akt. Akt is activated by phosphatidylinositol 3-kinase (PI3K), which in turn is controlled by *PTEN* (a tumor suppressor gene). The dysregulation of cell proliferation that occurs in tumors has been attributed in some cases to 1) constitutive activity of Akt, or 2) mutation or deletion of *PTEN*. In cases where either of these two events has occurred, there is uncontrolled activation of mTOR, stimulating increased cell proliferation. Therefore, it is hypothesized that tumors with increased mTOR activity will be highly sensitive to mTOR inhibition.

This process has been investigated in human tumor cell lines. Cell lines with mutations of *PTEN* have increased levels of activated Akt and mTOR [6•,8,9]. Similarly, in tests of sensitivity to rapamycin or its analogue, CCI-779, the pattern of sensitivity matches that of the cell lines with *PTEN* mutations or increased activation of Akt [10].

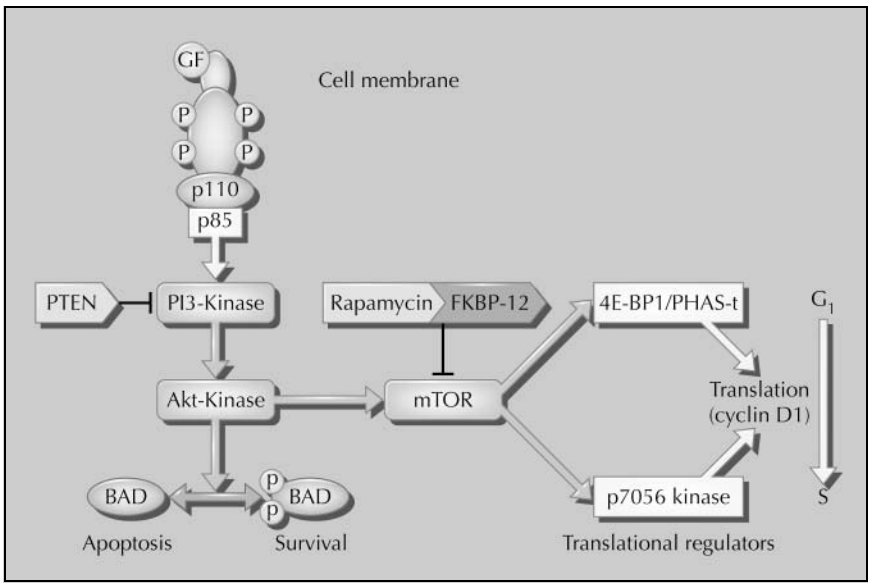

Figure 1. The rapamycin-sensitive signal transduction pathway is shown. Rapamycin and CCI-779 bind to the immunophilin FK506 binding protein 12 (FKBP-12). The rapamycin–FKBP12 complex blocks the kinase activity of the mammalian target of rapamycin (mTOR). The inhibition of mTOR kinase activity inhibits the downstream translational regulators of 4E-BP1/PHAS and p70s6k. The inhibition of 4E-BP1/PHAS and p70s6k decreases the translation of mRNA of specific proteins essential for cell-cycle progression from G_1 to S phase. (*From* Hidalgo and Rowinsky [5•]; with permission.)

With respect to renal cell carcinoma, another pathway influenced by mTOR could also be contributory and may provide another antitumor mechanism. Sporadic renal cell cancer is associated with loss of function of the von Hippel–Lindau (*VHL*) tumor suppressor gene [11•]. The VHL gene product, a protein, is important for causing destruction via the proteosome of the oxygen-sensitive transcription factors, termed hypoxia-inducible factor (HIF)-1α and HIF-2α [12]. Both of these factors are key in stimulating vascular growth in tumors in the setting of hypoxia [13]. When VHL function is lost, these factors are not degraded, and therefore accumulation of HIFs is increased and HIFs continue to function. Both HIFs have been noted to stimulate increased expression of vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and transforming growth factor (TGF)-α, among other growth factors [13]. Thus, loss of function of VHL leads to increases in HIF activity and therefore increases in growth factors key to tumor and vascular growth. This is thought to be a major pathway of growth and persistence of renal cell cancer. Recently, it was determined that mTOR activation increases *HIF-1*α gene expression through mRNA translation and protein stabilization [14,15].

Thus, mTOR inhibition is of general interest as an anticancer approach because it affects multiple pathways in a number of malignancies—through the ribosomal pathway and the factor 4E pathway, and in tumors with mutations of *PTEN*. However, mTOR inhibition may also be of interest in renal cell cancer through the potential effect on HIF-α.

Preclinical Evaluation of mTOR Inhibition

Considerable preclinical work has been done in human tumor cell lines and animal models of human xenografts to evaluate the effect of mTOR inhibition on cell growth and tumor growth. A striking effect was initially evaluated using rapamycin, the prototype agent to induce mTOR inhibition [16,17]. The rapamycin analogues, CCI-779, RAD001, and ap23573, are now being evaluated. CCI-779 is an ester of rapamycin, and its active metabolite is sirolimus. RAD001 is also a derivative of rapamycin, and all are inhibitors of mTOR with cell-line sensitivity patterns of inhibition similar to that of rapamycin.

In tests of the National Cancer Institute human tumor cell-line panel, antitumor activity for rapamycin and its derivatives showed significant growth inhibition at concentrations of less than 0.01 μM for breast cancer, prostate cancer, leukemia, melanoma, renal cell cancer, glioblastoma, and pancreatic cancer [10]. In addition, some cell lines of virtually every tissue tested showed sensitivity. Half of glioblastomas have *PTEN* mutations, which could make them increasingly sensitive to mTOR inhibition. Additionally, tumors with lesions of other proteins that regulate progression through G_1 may also be more susceptible to mTOR inhibition, such as deletions of p16 to glioma and pancreatic cancer (Wyeth, Personal communication). In studies of human tumors as xenografts in mice, evaluation of growth inhibition demonstrated prolonged time to tumor growth in a similar spectrum of diseases. Growth inhibition rather than tumor regression appeared to be the most consistent outcome, and compared with untreated control subjects, there was significant increase in time. Geoerger *et al.* [18] investigated the antitumor activity of CCI-779 in human primitive neuroectodermal tumor/medulloblastoma (PNET/MB) models, the most common form of malignant pediatric brain tumor. In cell lines and in xenografts, significant growth inhibition was exhibited. An additive effect was also seen in the xenograft when CCI-779 was given in combination with cisplatin, another active agent in PNET [18]. This carefully done study exemplifies the unusual features of mTOR inhibitors: 1) A linear dose-response effect is not apparent; there appears to be a dose threshold that is effective with minimal benefit from incremental increases in dose; 2) intermittent dosing is

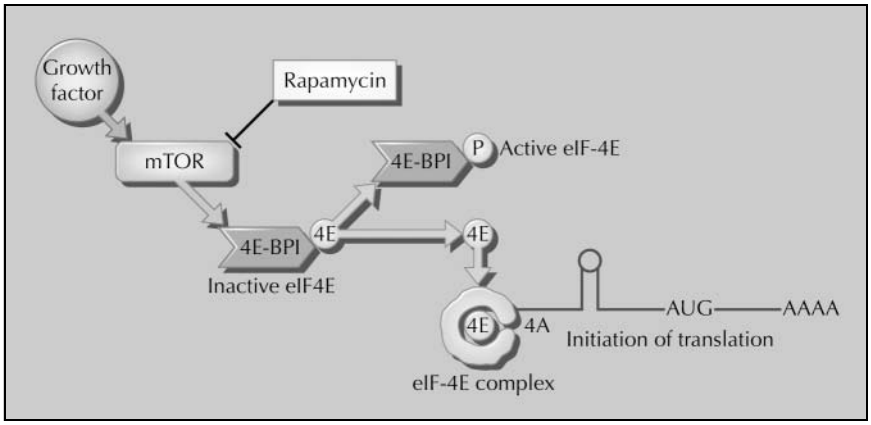

Figure 2. Rapamycin and CCI-779 inhibit the phosphorylation of 4E-BP1/PHAS, preventing the release of the eIF-4E and the activation of the eIF4F complex. (*From* Hidalgo and Rowinsky [5•]; with permission.)

effective; and 3) a 2-week period of daily dosing was superior to 1 week, superior to one large single dose, and superior to longer than 2 weeks of treatment. Thus, sufficient preclinical data demonstrating antitumor activity of rapamycin, and subsequently of its analogues, have led to the initiation of clinical trials to investigate the effectiveness of mTOR inhibition as an anticancer modality.

Phase I Clinical Investigations in Cancer Patients

The greatest amount of clinical data amassed for the analogues concerns CCI-779, for which phase I and II trials have been completed. Although animal data provide some guidance, with any new agent, phase I clinical trials often demonstrate new information. Phase I trials with CCI-779 were conducted with intermittent schedules to avoid prolonged immunosuppression, as is seen and desired with rapamycin.

Raymond *et al.* [19] conducted a trial using a 30-minute weekly intravenous infusion. The maximally tolerated dose was not reached with this study. The doses ranged from 7.5 to 220 mg/m^2/wk. Grade 3 mucositis was observed. A partial response (PR) was seen in one patient each with renal cell cancer, breast cancer, and neuroendocrine tumor. Hidalgo *et al.* [20] conducted a second phase I study, with a schedule of daily 30-minute infusion for 5 days, repeated every 2 weeks. For heavily pretreated patients, the maximally tolerated dose was 15 mg/m^2/d, and for minimally pretreated patients the maximally tolerated dose was 19 mg/m^2/d. The dosage ranged from 0.75 to 19.1 mg/m^2/d. Dose-limiting toxicities included grade 3 hypocalcemia, grade 3 elevation in liver function tests, and grade 3 thrombocytopenia. One patient with non–small-cell lung cancer experienced a PR.

Toxicities observed with some frequency included folliculitis, nail bed changes, maculopapular rash, and eczematoid reactions as dermatologic changes. Hematologic changes were primarily thrombocytopenia. Biochemical alterations included abnormalities of liver function tests, hypertriglyceridemia, hypercholesterolemia, and reversible decrements in testosterone. Some patients also developed mucositis. All of these toxicities were reported as relatively mild in the phase I studies.

The responses in these phase I studies, at doses that were tolerable, have led to development of disease-directed phase II trials and trials evaluating this drug in combination with chemotherapy. Two phase I studies have been initiated with combinations of chemotherapy, one of CCI-779 plus gemcitabine, and one with 5-fluorouracil and leucovorin. The results of these two studies are yet to be presented. (Wyeth, Personal communication).

Phase II Clinical Trial in Renal Cell Carcinoma

In single-agent phase II studies initiated with a weekly schedule, CCI-779 appeared to have activity and was tolerated well over a large range of doses. Because selection of a dose for the weekly schedule was problematic, three doses were used in a study of patients with metastatic renal cell cancer: 25 mg/m^2, 75 mg/m^2, and 250 mg/m^2, each administered weekly (Submitted manuscript) [21•]. Both the investigator and the patient were blinded to the dose level. Dose reductions were required for grade 3 or greater toxicity. Treatment was continued until evidence of progression was shown or unacceptable toxicity was reached. Patients were premedicated with diphenhydramine to preclude allergic reactions that had been seen earlier in the trial. One hundred eleven patients with renal cell cancer who had previously received some type of systemic therapy (usually a cytokine) were enrolled, and 110 were treated. The patients were randomly assigned to dose levels and evenly distributed, with 36 treated at 25 mg/m^2/wk, 38 at 75 mg/m^2/wk, and 36 at 250 mg/m^2/wk. Sixty-two percent were classified as Eastern Cooperative Oncology Group (ECOG) performance status 1, and 38% were classified as ECOG performance status 0. The most common clinical adverse events were rash (72%), mucositis (65%), asthenia (39%), nausea (36%), and acne (30%). The most common laboratory adverse events were thrombocytopenia (24%), hypertriglyceridemia (24%), and anemia (23%). Most adverse events were grade 1 or 2. the most common grade 3 or 4 adverse events were hyperglycemia and anemia. Some occurrences of nonspecific pneumonitis, the majority asymptomatic, were observed. Median time to progression and overall survival were 5.8 months and 15 months

respectively, for the entire group. Although the response rate (complete response [CR] + PR) was 7% for the entire group, the cumulative responses (CR + PR) added to minor responses and stable disease greater than 24 weeks comprised 51% of the entire group.

Subsequently, in renal cell cancer, a study of CCI-779 plus interferon (IFN) alfa was initiated, based on data showing significant synergy in murine systems [22]. This phase I study attempted to escalate doses of CCI-779, given as a weekly intravenous infusion, and IFN, given as a subcutaneous injection three times weekly [22]. The initial dose of IFN was 6 MU three times weekly. IFN alone was given during the first week. The initial dose of CCI-779 was 5 mg/m^2/wk. CCI-779 dose escalation steps were 10 mg/m^2, 15 mg/m^2, and 25 mg/m^2. Once the maximum dose of CCI-779 was determined, IFN was to be escalated if possible to 9 MU three times weekly. As of November 2002, 20 patients were entered at four different dose levels of CCI-779. Fifteen had remained on study for greater than 7 months. Partial responses were reported in two patients and stable disease in six, and the study was continuing to accrue. At this early evaluation, the combination of CCI-779 and IFN was considered tolerable, and antitumor activity was observed. It is too early to determine if the level of activity is additive or synergistic, or if it is consistent with expectations with either drug alone.

Phase II Trial in Metastatic Breast Cancer

A study in patients with locally advanced or metastatic breast cancer was initiated, using two weekly dose levels of CCI-779, 75 mg/m^2 and 250 mg/m^2 [23•]. Patients had been treated previously with anthracyclines (45%), taxanes (5%), or both (50%). One hundred nine patients were enrolled, and 106 were treated. Liver, lung, and bone disease were reported in 59%, 36%, and 42% of patients, respectively. Dose reductions were required in 31% of patients treated at 75 mg/m^2 and in 51% of those treated at 250 mg/m^2. Fewer grade 3 or 4 toxicities occurred among the patients treated at 75 mg/m^2, and it is clear that prior chemotherapy had an impact on the level and frequency of toxicities from CCI-779. Leukopenia and thrombocytopenia were seen in both groups. Clinical benefit was observed in 49% of the patients, based on the report of one CR, eight PRs, and 43 patients who remained stable for more than 8 weeks, including eight unconfirmed PRs. Activity was observed at both dose levels and was seen in patients with liver metastases. Further evaluation of the data is ongoing, and plans for combination studies are being evaluated.

Phase I Trial in Primary Brain Tumors

An additional phase I study is being conducted in patients with primary brain tumors, again with 50% or more expressing loss or mutation of *PTEN* [24]. Patients with primary brain tumors were treated for multiple cycles of eight weekly doses, and some received as many as 17 cycles. Higher doses were administered due to the prometabolic effect of corticosteroids in patients with primary brain tumors.

Oral Formulations of mTOR Inhibitors

An oral formulation of CCI-779 is under development and has been used in treatment of rheumatologic patients and in normal control subjects (Wyeth, Personal communication). Its activity in cancer patients will be evaluated if the level of intravenous activity is deemed sufficient to continue studies. RAD001 is an oral formulation of another mTOR inhibitor. A phase I study with weekly administration of this agent has been reported, and combination studies are in progress (Novartis, Personal communication) [25]. Similarly, ap23573, a third mTOR inhibitor, is undergoing animal studies and is expected to enter clinical trials in humans (ARIAD Pharmaceuticals, Personal communication) [26].

Conclusions

Inhibition of mTOR appears to be a viable approach to anticancer therapy. Ongoing clinical trials suggest that agents that inhibit this pathway mediate changes in cell proliferation but may not cause regression in the majority of patients. Combination studies are still at early stages and may be difficult to execute. This pathway should be evaluated very carefully and thoroughly because it may be difficult to elucidate precisely what determines a beneficial effect. The gestalt from treating physicians and nurses is that inhibition of this pathway is beneficial to patients with renal cell cancer in that the duration of stable disease is prolonged. Somehow, this observation needs to be quantified, and that is the difficulty. Nevertheless, mTOR inhibition is a promising area for clinical development.

Acknowledgments

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