

# The rapamycin-sensitive signal transduction pathway as a target for cancer therapy

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The high frequency of mutations in cancer cells which result in altered cell cycle regulation and growth signal transduction, conferring a proliferative advantage, indicates that many of these aberrant mechanisms may be strategic targets for cancer therapy. The macrolide fungicide rapamycin, a natural product with potent antimicrobial, immunosuppressant, and anti-tumor properties, inhibits the translation of key mRNAs of proteins required for cell cycle progression from G<sub>1</sub> to S phase. Rapamycin binds intracellularly to the immunophilin FK506 binding protein 12 (FKBP12), and the resultant complex inhibits the protein kinase activity of a protein kinase termed mammalian target of rapamycin (mTOR). The inhibition of mTOR, in turn, blocks signals to two separate downstream pathways which control the translation of specific mRNAs required for cell cycle traverse from G<sub>1</sub> to S phase. Blocking mTOR affects the activity of the 40S ribosomal protein S6 kinase (p70<sup>s6k</sup>) and the function of the eukaryotic initiation factor 4E-binding protein-1 (4E-BP1), leading to growth arrest in the G<sub>1</sub> phase of the cell cycle. In addition to its actions on p70<sup>s6k</sup> and 4E-BP1, rapamycin prevents cyclin-dependent kinase activation, inhibits retinoblastoma protein (pRb) phosphorylation, and accelerates the turnover of cyclin D1 that leads to a deficiency of active cdk4/cyclin D1 complexes, all of which can inhibit cell cycle traverse at the G<sub>1</sub>/S phase transition. Both rapamycin and CCI-779, an ester analog of rapamycin with improved pharmaceutical properties and aqueous solubility, have demonstrated impressive activity against a broad range of human cancers growing in tissue culture and in human tumor xenograft models, which has supported the development of compounds targeting rapamycin-sensitive signal-transduction pathways. CCI-779 has completed several phase I clinical evaluations and is currently undergoing broad disease-directed efficacy studies. The agent appears to be well tolerated at doses that have resulted in impressive anti-tumor activity in several types of refractory neoplasms. Important challenges during clinical development include the definition of a recommended dose range associated with optimal biological activity and maximal therapeutic indices, as well as the ability to predict which tumors will be sensitive or resistant to CCI-779. *Oncogene* (2000) 19, 6680–6686.

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Cell proliferation is a complex multifaceted process that requires the synthesis of essential regulatory proteins involved in the transduction of extracellular and autocrine proliferative stimuli. Since several of these highly regulated processes are aberrant in many types of cancers, conferring a proliferative advantage, they are potential strategic targets for therapeutic development against cancer (Sherr, 2000). Indeed, several novel classes of therapeutics that interfere with discrete essential elements of aberrant signal transduction and cell cycle regulation, such as inhibitors of various receptor tyrosine kinases, oncogenes, critical proteins involved in signal transduction (e.g. Ras, Raf), and cyclin-dependent kinases, are being developed as anti-cancer agents (Rowinsky *et al.*, 1999, Senderowicz and Sausville 2000). One such agent, rapamycin (sirolimus; Rapamune<sup>®</sup>; Wyeth-Ayerst, PA, USA), a macrolide fungicide isolated from the bacteria *Streptomyces hygroscopicus*, possesses potent antimicrobial, immunosuppressant, and antitumor properties (Baker *et al.*, 1978; Sehgal *et al.*, 1975; Vezina *et al.*, 1975). Because of its profound immunosuppressive actions, rapamycin was initially developed and received regulatory approval for the indication of prevention of allograft rejection following organ transplantation (Sehgal, 1995). The antiproliferative actions of rapamycin have been demonstrated to be due to its ability to modulate critical signal transduction pathways that link mitogenic stimuli to the synthesis of proteins required for cell cycle traverse from G<sub>1</sub> to S (Wiederrecht *et al.*, 1995). Impressive antiproliferative activity has been demonstrated following treatment of a diverse types of experimental tumors with rapamycin (Eng *et al.*, 1984, Muthukkumar *et al.*, 1995; Seufferlein and Rozengurt, 1996). However, the poor aqueous solubility and chemical stability of rapamycin precluded its clinical development as an anti-cancer agent. Recently, a series of rapamycin analogs with improved aqueous solubility and stability have been synthesized and evaluated. CCI-779 (Wyeth Ayerst, PA, USA), a soluble ester analog of rapamycin, was selected for development as an anti-cancer agent based on its prominent anti-tumor profile and favorable pharmaceutical and toxicological characteristics in preclinical studies (Gibbons *et al.*, 2000). Several phase I studies of CCI-779 have been completed and disease-directed efficacy evaluations in a number of tumor types are being performed (Raymond *et al.*, 2000; Hidalgo *et al.*, 2000). This review will summarize the principal mechanisms of anti-tumor action of rapamycin, specifically its effect on rapamycin-sensitive signal transduction pathways, and will discuss the preliminary results of experimental and clinical studies with this novel class of anti-cancer agents.

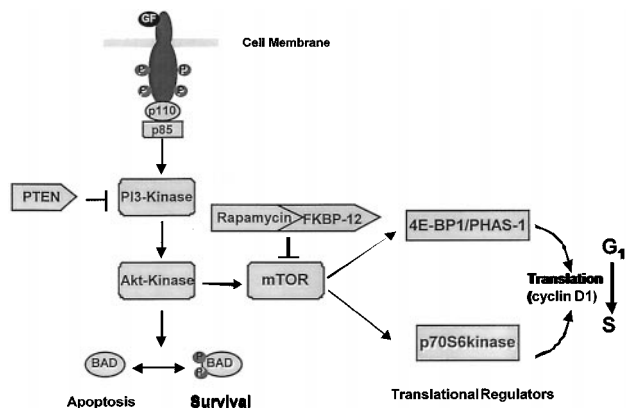
## Mechanism of action of rapamycin and rapamycin analogs

Rapamycin, and its ester analog, CCI-779, uniquely interfere with cell cycle progression from G<sub>1</sub> to S phase in response to proliferative stimuli by blocking the translation of mRNAs of essential cell cycle proteins (Wiederrecht *et al.*, 1995). The principal mechanisms responsible for these actions, which have been elucidated only over the last several years, are graphically depicted in Figures 1 and 2.

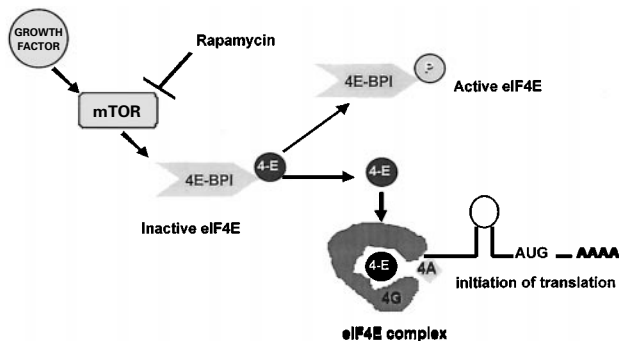
### Upstream actions and the target of rapamycin

Rapamycin binds intracellularly to members of the immunophilin family of FK506 binding proteins (FKBPs), inhibiting their enzymatic activity as prolyl isomerases (Heitman *et al.*, 1991; Koltin *et al.*, 1991; Fruman *et al.*, 1995). Although there are many members of the FKBP family, a large body of biochemical and genetic studies suggest that FKBP12 is the most important binding protein with respect to the rapamycin-sensitive signal transduction pathway (Heitman *et al.*, 1991; Koltin *et al.*, 1991; Fruman *et al.*, 1995). The resultant rapamycin-FKBP12 complex interacts with and inhibits the activity of a 290 kd kinase, termed mammalian target of rapamycin (mTOR) (also known as FRAP, RAFT1, and RAP1) (Figure 1) (Sabatini *et al.*, 1994; Sabers *et al.*, 1995; Brown *et al.*, 1994; Chiu *et al.*, 1994). mTOR is a member of a recently identified family of protein kinases termed phosphoinositide 3-kinase related kinases (PIKKs), which are involved in many critical regulatory cellular functions pertaining to cell cycle progression, cell cycle checkpoints that govern cellular responses to DNA damage, DNA repair, and DNA recombination (Sarkaria *et al.*, 1998).

In response to growth stimuli, quiescent cells increase the translation of a subset of mRNAs whose protein products are required for traverse through the G<sub>1</sub> phase of the cell cycle. mTOR regulates essential signal transduction pathways and is involved in the



**Figure 1** Rapamycin-sensitive signal transduction pathway. 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 4E-BP1/PHAS and p70<sup>S6k</sup>. The inhibition of 4E-BP1/PHAS and p70<sup>S6k</sup> decrease the translation of mRNA of specific proteins essential for cell cycle progression from G<sub>1</sub> to S phase.



**Figure 2** Rapamycin and CCI-779 inhibits the phosphorylation of 4E-BP1/PHAS, preventing the release of the eIF-4E and the activation of the eIF4F complex

coupling of growth stimuli with cell cycle progression. Phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) (PI3K/Akt) appears to be the key modulatory factor in the upstream pathway by which growth factor-growth factor receptor interactions affect the phosphorylation state of mTOR (Figure 1) (Downward 1998; Scott *et al.*, 1998; Nave *et al.*, 1999). PI3K plays a central role in cellular proliferation, motility, neovascularization, viability, and senescence and is upregulated in cancer cells (Shayesteh *et al.*, 1999; Cantley *et al.*, 1991). Its main physiological function is the phosphorylation of the D3 portion of membrane phosphoinositols (Cantley *et al.*, 1991; Carpenter *et al.*, 1990). Although the role of PI3K and its lipid products in signal transduction processes is not clear, the activity of this enzyme on tyrosine kinases induces mitogenesis, cellular growth, and cellular transformation (Carpenter *et al.*, 1990; Varticovski *et al.*, 1994; Hu *et al.*, 1995). Recently, several studies have investigated the role of small molecule-inhibitors of PI3K as potential tumor suppressor agents. For example, the flavonoid derivative, LY294002 (Eli Lilly, Indianapolis, IN, USA), a potent PI3K inhibitor, is a competitive, reversible inhibitor of the ATP binding site of the enzyme (Vlahos *et al.*, 1994; Hu *et al.*, 2000). The agent induces G<sub>1</sub> arrest in proliferating cells, leading to almost complete inhibition of melanoma cell proliferation, partial inhibition of MG-63 osteosarcoma cell growth, and inhibitor of OVCAR-3 ovarian carcinoma inducing prominent apoptotic effects (Hu *et al.*, 2000; Casagrande *et al.*, 1998; Thomas *et al.*, 1997). The inhibitor also completely inhibits the retinoblastoma protein (pRb) hyperphosphorylation that normally occurs during G<sub>1</sub> progression and induces up-regulation of the cyclin-dependent kinase inhibitor p27 (Casagrande *et al.*, 1998).

There are ample experimental data indicating that mTOR functions downstream of the PI3K/Akt pathway and is phosphorylated in responses to stimuli that activate the PI3K/Akt pathway (Scott *et al.*, 1998; Nave *et al.*, 1999; Hu *et al.*, 1995; Sekulic *et al.*, 2000). PI3K and Akt are considered proto-oncogenes, and the pathway is inhibited by the tumor suppressor gene *PTEN* (Wu *et al.*, 1998). There are other signaling pathways that are activated downstream of PI3K, but the Akt pathway is of particular interest because of its role in inhibiting apoptosis and promoting cell proliferation by affecting the phosphorylation status

of cell-survival and apoptosis-inducing proteins like BAD (Downward, 1998).

### Downstream effects

Following activation via phosphorylation, mTOR modulates two separate downstream pathways that control translation of specific subsets of mRNAs including the eukaryotic initiation factor 4E binding protein-1 (4E-BP1), also known as PHAS-1 (phosphorylated heat- and acid-stable protein), and the 40S ribosomal protein S6 kinase (p70<sup>s6k</sup>) (Figure 1) (Brunn *et al.*, 1997; Gingras *et al.*, 1998; Hara *et al.*, 1997). There is ample evidence indicating that the activation of either PI3K or Akt is sufficient to induce the phosphorylation of both 4E-BP1/PHAS-1 and p70<sup>s6k</sup> through mTOR (Chung *et al.*, 1994; Petrisch *et al.*, 1995). Furthermore, treatment of activated PI3K- or Akt-expressing cells with rapamycin blocks the phosphorylation of p70<sup>s6k</sup> and 4E-BP1/PHAS-1, suggesting that mTOR is required for these activities (Gingras *et al.*, 1998; Burgering and Coffer, 1995).

The first downstream regulator modulated by the phosphorylation status of mTOR, 4E-BP1/PHAS-1, is a low molecular-weight protein that inhibits the initiation of translation through its association with eIF-4E, the mRNA cap-binding subunit of the eukaryotic initiation factor-4 (eIF-4F) complex (Figure 2). The binding of 4E-BPs to eIF-4E is dependent on the phosphorylation status of 4E-BP. In the unphosphorylated state that predominates in quiescent cells, 4E-BP1/PHAS-1 binds to eIF-4E which inhibits its activity (Sonenberg *et al.*, 1998). In response to proliferative stimuli, 4E-BP1/PHAS-1 becomes phosphorylated through the action of mTOR and other kinases which decrease its binding affinity for eIF-4E. These actions promote the dissociation of the 4E-BP1/PHAS-1 complex, increasing the availability of eIF-4E, which can then bind to eIF-4G, -4B, and -4A, forming the multisubunit eIF-4F complex. These interactions lead to an increase in the translation of mRNAs with regulatory elements in the 5'-untranslated region such as cyclin D1 and ornithine decarboxylase (Sonenberg and Gingras, 1998; Rosenwald *et al.*, 1995; Shantz and Pegg 1995). In contrast, growth factor deprivation or treatment with rapamycin results in the dephosphorylation of 4E-BP1/PHAS-1, an increase in eIF-4E binding, and a concomitant decrease in the translation of mRNAs for cell cycle progression from G<sub>1</sub> to S phase, as shown in Figure 2 (Brunn *et al.*, 1997; Gingras *et al.*, 1998).

The second downstream target modulated by mTOR is the kinase p70<sup>s6k</sup>. Upon activation by proliferative stimuli mediated by the PI3K/Akt signal transduction pathway, mTOR phosphorylates/activates p70<sup>s6k</sup>, which, in turn, phosphorylates the 40S ribosomal protein S6 (Hu *et al.*, 2000). The phosphorylation of S6 leads to the recruitment of the 40S ribosomal subunit into actively translating polysomes, thereby enhancing the translation of mRNAs with a 5' terminal oligopolypyrimidine including those that encode for ribosomal proteins, elongation factors, and insulin growth factor-II. Rapamycin treatment results in a rapid and profound dephosphorylation of p70<sup>s6k</sup>, suppressing its activity (Seufferlein and Rozengurt 1996; Grewe *et al.*, 1999).

In addition to its well characterized inhibitory actions on p70<sup>s6k</sup> and 4E-BP1/PHAS-1, rapamycin interferes with other intracellular processes involved in cell cycle progression which undoubtedly contribute to its antiproliferative actions. These other actions are particularly important in exponentially growing cells where inhibition of p70<sup>s6k</sup> phosphorylation by rapamycin does not result in G<sub>1</sub> cell cycle arrest (Kawamata *et al.*, 1998). Rapamycin increases the turnover of cyclin D1 at the mRNA and protein level (Hashemolhosseini *et al.*, 1998). This effect, in addition to the decrease in translation of cyclin D1 mRNA subsequently to 4E-BP1/PHAS inhibition, results in a relative deficiency of cyclin D1 in the cdk4/cyclin D1 complexes required for retinoblastoma protein phosphorylation (Morice *et al.*, 1993; Nourse *et al.*, 1994). Rapamycin also blocks the elimination of the cyclin dependent kinase (cdks) inhibitor p27 and facilitates the formation of the cyclin/cdks-p27 complexes (Nourse *et al.*, 1994; Luo *et al.*, 1996). Furthermore, in exponentially growing cells, rapamycin upregulates p27 at both the mRNA and protein level and inhibits cyclin-A-dependent kinase activity (Kawamata *et al.*, 1998). It appears that, although rapamycin treatment results in cell cycle arrest at the G<sub>1</sub>/S transition, the precise mechanism responsible for this effect is both cell cycle- and cell type-specific.

By inhibiting the translation of critical mRNAs involved in the G<sub>1</sub> to S phase transition in response to mitogenic stimuli, and by interfering with the balance of cyclin/cyclin-dependent-kinase/cyclin-dependent kinase inhibitors in the early phases of the cell cycle, rapamycin inhibits the growth of cancer cells. Rapamycin exerts concentration-dependent inhibition of cell proliferation and tumor growth in a variety of murine and human cancers growing in both cell culture and xenograft models including B16 melanoma, P388 leukemia, MiaPaCa-2 and Panc-1 human pancreatic carcinoma and tumors derived from B-cell lymphoma, small cell lung cancer carcinoma, and childhood rhabdomyosarcoma (Muthukkumar *et al.*, 1995; Seufferlein and Rozengurt 1996; Hosoi *et al.*, 1999). Rapamycin also induces p53-independent apoptosis in childhood rhabdomyosarcoma cell lines and enhances the apoptotic-inducing effects of cisplatin in murine T-cell, human HL-60 promyelocytic leukemia, and human ovarian SKOV3 cancer cell lines (Hosoi *et al.*, 1999; Shi *et al.*, 1995).

### Clinical development

The unfavorable pharmaceutical properties of rapamycin, particularly its poor aqueous solubility and instability, preclude its clinical development as an anti-cancer agent, and, therefore, soluble ester analogs were synthesized and evaluated as a collaborative effort between investigators at Wyeth-Ayerst and the National Cancer Institute. CCI-779, a water soluble ester analog of rapamycin, was selected for further development based on favorable pharmaceutical, toxicologic, and antitumor profiles in preclinical evaluations. In the National Cancer Institute human tumor cell line screen, CCI-779 and rapamycin demonstrated similar anti-tumor profiles and potencies (Pearson correlation coefficient, 0.86), with IC<sub>50</sub> values frequently less than



$10^{-8}$  M (Gibbons *et al.*, 2000). Platelet-derived growth factor stimulation of the human glioblastoma line T98G was markedly inhibited ( $IC_{50}$ , 1 pM), consistent with its proposed mechanism of action as an inhibitor of signal transduction, and growth-inhibited cells were arrested in  $G_1$  (Gibbons *et al.*, 2000). In studies involving cancer cell lines growing in tissue culture, human prostate, breast, and small cell lung carcinomas, glioblastoma, melanoma, and T-cell leukemia were among the most sensitive cancers to CCI-779, with  $IC_{50}$  values in the nanomolar range (Gibbons *et al.*, 2000). Significant growth inhibition was also observed following treatment of a variety of human tumor xenografts with CCI-779, but the preponderance of tumor growth inhibition, in contrast to overt tumor regression, suggests that subsequent disease-directed trials should be designed to assess this potential outcome. In addition, several intermittent CCI-779 dosing regimens were effective in human tumor xenograft studies, which is important in view of the possibility that prolonged immunosuppression can result from both rapamycin and CCI-779 administered on continuous dose-schedules and since the immunosuppressive effects of rapamycin analogs have been demonstrated to resolve in approximately 24 h following treatment (Gibbons *et al.*, 2000).

Thus far, CCI-779 has been evaluated in two phase I studies, in which the agent has been administered as a 30-min IV infusion weekly and as a 30-min IV infusion daily for 5 days every 2 weeks (Raymond *et al.*, 2000; Hidalgo *et al.*, 2000). These schedules were selected for initial clinical evaluation to avoid prolonged drug-induced immunosuppression which resolves shortly after drug treatment. However, similar to the traditional paradigm used to develop nonspecific cytotoxic agents, these phase I studies were designed to determine the maximum tolerated dose based on dose-limiting toxicities as classically defined. To date, 16 patients have been treated with CCI-779 at doses ranging from 7.5 to 220 mg/m<sup>2</sup>/week on the weekly schedule and 35 patients have received doses ranging from 0.75 to 24 mg/m<sup>2</sup>/day on the daily for-5-days every-2-week schedule. The principal toxicities of CCI-779 have included dermatologic toxicity, myelosuppression, reversible elevations in liver function tests, and asymptomatic hypocalcemia. The cutaneous effects, which have been relatively more common with CCI-779 on the weekly schedule, have been multifaceted. Dermatologic manifestations have included aseptic folliculitis, erythematous maculopapular rashes, eczematoid reactions, dry skin, vesicular lesions, and nail disorders. The principal hematologic toxicity has been thrombocytopenia, whereas anemia, leukopenia, and neutropenia have generally been less common and severe. Other toxicities and biochemical abnormalities, which have generally been mild to moderate in severity, reversible, and noted over wide dosing ranges include mucositis, hypertriglyceridemia, hypercholesterolemia, and reversible decrements in serum testosterone. The maximum tolerated doses of CCI-779 on the daily-for-5-day-every-2-week schedule are projected to be 15 and 24 mg/m<sup>2</sup>/day in patients with minimal or extensive prior myelotoxic therapy, respectively, whereas the maximum tolerated dose has not been determined for CCI-779 administered on a weekly schedule. The preliminary results of pharmacokinetic studies indicate

dose-dependent pharmacokinetics, elimination half-life values of approximately 15–17 h, and preferential partitioning of CCI-779 in red blood cells (Raymond *et al.*, 2000; Hidalgo *et al.*, 2000). Major tumor responses (partial responses; >50% reduction in the sum of the bidimensional product of all measurable lesions) have been noted in previously-treated patients with renal cell carcinoma and non-small cell lung carcinoma, and minor tumor responses (<50% reduction in the sum of the bidimensional product of all measurable lesions) have been observed in previously-treated patients with soft tissue sarcoma, serous papillary carcinoma of the endometrium, breast carcinoma, squamous cell carcinoma of the skin, and non-Hodgkins lymphoma. The fact that CCI-779 consistently induced tumor regressions at relatively nontoxic doses in the phase I studies is particularly noteworthy, since this observation suggests that the optimal therapeutic dose of CCI-779 may be lower than the maximum tolerated dose.

Disease-directed efficacy studies of CCI-779 in a broad range of tumor types will be initiated following the completion of phase I studies. Based on the results of experimental studies involving malignant gliomas that rely on paracrine or autocrine stimulation of receptors that trigger the PI3/Akt pathway, studies directed at assessing the anti-tumor activity of CCI-779 in patients with glioma are planned. However, since CCI-779 is principally metabolized by cytochrome P450 mixed function oxidases that are induced by many types of anticonvulsant agents commonly co-administered to patients with malignant gliomas, the toxicities, pharmacokinetics, and optimal dose-schedule of CCI-779 are being evaluated in patients concurrently receiving treatment with P450 mixed function oxidase-inducing anticonvulsant agents. In addition, phase I studies evaluating the feasibility of administering CCI-779 in combination with various cytotoxic chemotherapeutics such as 5-fluorouracil and gemcitabine are underway. An oral formulation of CCI-779, which would increase the feasibility of protracted drug administration, is also in early clinical development.

For CCI-779 and other rationally developed agents, in which tumor growth inhibition and delay are predominant therapeutic effects in preclinical studies, traditional phase II tumor screening paradigms that focus solely on tumor regression may be suboptimal since clinically significant inhibitory effects on tumor growth may not be detected (Gelmon *et al.*, 1999). Although regressions of well established tumors have been noted in preclinical studies of CCI-779, tumor growth delay is the principal therapeutic effect of the agent. In addition to the traditional screening endpoint, tumor regression, disease-specific screening studies or phase II clinical trials, at which time 'go or no go' decisions are made about the subsequent development of CCI-779 and other antiproliferative agents, must be designed to evaluate inhibition of tumor growth. Possible clinical endpoints that have been proposed for the evaluation of antiproliferative agents include time to progression, the proportion of patients without progressive disease as their best response, symptomatic benefit, and reduction in tumor markers. Potential surrogate endpoints include inhibition at the target, particularly if this effect can be validated to reflect tumor growth delay and/or

regression in preclinical models, and assessments of cell proliferation by functional imaging (e.g. positron emission tomography, nuclear magnetic resonance scanning). However, randomized clinical trials are the optimal means to evaluate any agent's effect on tumor growth and/or related surrogate endpoints. Nevertheless, tumor regression has been observed with CCI-779 and other signal transduction inhibitors in both preclinical studies and phase I clinical trials, which may occur when the inhibition of tumor growth is of sufficient magnitude so that tumor cell death, possibly through apoptosis, exceeds the rate of tumor cell proliferation (Adjei *et al.*, 2000; Ferry *et al.*, 2000). Therefore, the traditional endpoint of tumor regression, which is used to screen for the activity of nonspecific cytotoxic agents, may be an appropriate endpoint in nonrandomized phase II studies of antiproliferative agents that have the potential for inducing tumor regression as discerned from preclinical studies.

Another important issue pertaining to the development of CCI-779 and other rationally designed inhibitors of signal transduction is the need to assess relevant target effects in order to guide dose selection and facilitate the assessment of benefit in clinical trials. The selection of an appropriate dose for broad disease-directed trials of CCI-779 is especially challenging since objective anti-tumor activity has been observed in patients treated with a wide range of doses. Unlike the situation with nonspecific cytotoxic agents, in which the relationship between dose and response is roughly linear and the maximum tolerated dose is sought, preclinical data suggest that therapeutic activity generally plateaus above a 'threshold' dose. In contrast, since the relationship between dose and toxicity is still likely to be linear, the selection of an optimal biological dose for clinical trials is desirable. However, efforts to precisely assess target effects during clinical investigations are extremely complex, requiring an understanding of the agent's precise mechanism of activity, and the availability of assays that measure relevant drug effects at the tumor target or in surrogate tissues that reflect inhibition at the target. Still, the availability of feasible biological assays capable of assessing inhibition at the target does not ensure that such effects will relate to clinical benefit. Since the intracellular target of CCI-779 and its downstream signaling pathways have been well characterized, the development of assays that measure the phosphorylation status of either 4E-BP1/PHAS and/or p70<sup>s6k</sup>, enabling evaluation of whether patients are receiving pharmacodynamically-active doses, is feasible (Yatscoff and Aspeslet, 1998). Nevertheless, a critical issue is whether these downstream effects correlate with the anti-tumor activity of CCI-779, particularly since malignant cells can traverse the cell cycle and proliferate despite the dephosphorylation of 4E-BP1/PHAS and the inactivation of p70<sup>s6k</sup> by rapamycin (Kawamata *et al.*, 1998; Yatscoff and Aspeslet, 1998). These observations suggest that either these pathways are not the only mechanisms by which cell cycle progression is regulated, or that mTOR-4E-BP1/PHAS-1 and mTOR-p70<sup>s6k</sup> pathways are redundant. Although assessing drug effects with such assays may facilitate the determination of whether a delivered dose is pharmacologically-active, the assay may not neces-

sarily predict drug efficacy since the assays may be assessing targets that are not related to the effects of the agent on proliferation. Alternatively, downstream factors may render the tumor cells resistant. Therefore, optimal assessments of target inhibition in tumor or surrogate tissues may require additional information about the relevance of target inhibition in terms of cell proliferation, cell cycle arrest, apoptosis, and/or angiogenesis. Such assays will ultimately be validated following the acquisition of late-stage clinical outcome data, particularly information pertaining to tumor regression, time to progression, clinical benefit, and survival, in large numbers of patients. At this juncture, the utility and feasibility of assays measuring the inactivation of p70<sup>s6k</sup> in peripheral blood mononuclear cells are being assessed in the course of early disease-directed efficacy studies of rapamycin.

Another important issue regarding the optimal development of CCI-779 is whether tumors with specific molecular abnormalities are particularly sensitive or resistant to the antiproliferative effects of CCI-779. Tumors that rely on paracrine or autocrine stimulation of receptors that constitutively stimulate the PI3/Akt/mTOR pathway or tumors with mutations that activate the PI3K/Akt signal transduction pathway may depend on rapamycin-sensitive pathways for growth and thus may be especially sensitive to rapamycin analogs. For example, mutations of the *PTEN* tumor suppressor gene which encode a lipid phosphatase that inhibits PI3K-dependent activation of PKB/Akt, occur commonly in a wide variety of tumor types (Hosoi *et al.*, 1998; Besson *et al.*, 1999; Teng *et al.*, 1997). The PI3K/Akt pathway is constitutively activated in tumors with *PTEN* mutations and these neoplasms are generally resistant to apoptosis. As predicted, the preliminary results of experimental studies in our laboratory indicate that breast cancer cell lines with *PTEN* mutations are extraordinarily sensitive to rapamycin (Hidalgo M, personal communication). In addition to increased sensitivity to rapamycin conferred by mutations of the *PTEN* suppressor oncogene, abnormalities of regulators of the G<sub>1</sub> checkpoint such as *pRB*, *p16*, *p27*, and cyclin D1 may also increase the sensitivity of tumors to rapamycin and may predict for drug efficacy (Sausville *et al.*, 1999). It is clear that defining the molecular characteristics of tumors of patients enrolled in clinical trials of CCI-779 may help to identify which patients may benefit from treatment.

## Conclusion

The natural product rapamycin, and its water-soluble ester analog, CCI-779, have potent immunosuppressant and antiproliferative properties stemming from their ability to modulate signal transduction pathways linking growth stimuli to the synthesis of specific proteins required for cell cycle progression from G<sub>1</sub> to S phase. Both rapamycin and CCI-779 profoundly inhibit the proliferation of a broad range of human tumors both *in vitro* and *in vivo*, which have supported the clinical development of this class of compounds. At this juncture, CCI-779, which possesses more favorable pharmaceutical properties than rapamycin, is completing several phase I evaluations and is broad disease-

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