Will mTOR inhibitors make it as cancer drugs?

Charles L. Sawyers*

Howard Hughes Medical Institute; Departments of Medicine, Molecular and Medical Pharmacology, and Urology and Jonsson Cancer Center, David Geffen School of Medicine at UCLA, University of California, Los Angeles, California 90095
*Correspondence: csawyers@mednet.ucla.edu

Several pharmaceutical and biotechnology companies are actively pursuing the clinical development of inhibitors of the serine/threonine kinase *m*ammalian *t*arget *o*f *r*apamycin (mTOR) for cancer. Rapamycin, the original natural product compound first shown to inhibit mTOR, is already an approved drug for prevention of allograft rejection in recipients of organ transplants due to its potent inhibition of T cell activation. What is the logic behind the use of the same agent for cancer indications? This focus will review the background supporting the potential utility of mTOR inhibitors as anticancer agents, then compare and contrast two different approaches for its clinical development. The first approach is empiric, based on the traditional phase I design of escalation to maximum tolerated dose in a broad patient population, followed by larger trials focused on those tumor types that demonstrate hints of activity in the phase I setting. The second approach is mechanism based, building on knowledge of signaling pathways that activate mTOR, where the dose is selected by measuring target enzyme inhibition in tumor cells and patient eligibility is defined by molecular profiling studies. I will speculate on potential outcomes from both approaches as well as my view of the eventual role that mTOR inhibitors may play in the cancer drug armamentarium.

mTOR: A central regulator of cell growth

Rapamycin, a bacterially derived natural product, induces G1 arrest in various cell types at low nanomolar concentrations. The mechanism was cleverly deciphered through yeast genetic screens that identified a serine/threonine kinase named target of rapamycin (TOR) (Heitman et al., 1991), which is a member of the larger phosphatidylinositol 3-kinase (PI3K) related family that includes PI3K, ATM, and ATR. Rapamycin exerts its action by first binding to the immunophilin FK506 binding protein (FKBP12). The FKBP12/rapamycin complex then binds mTOR, preventing phosphorylation of downstream targets such as S6 kinase (S6K) and 4EBP1 (see Abraham, 2002; Schmelzle and Hall, 2000; Shamji et al., 2003).

mTOR receives a diverse set of signaling inputs. Among the most relevant for a discussion of cancer is mTOR activation by growth factors like IGF-1, which activates the PI3K/Akt signaling pathway. Akt directly phosphorylates a number of proteins that impact cell survival and proliferation (reviewed in Vivanco and Sawyers, 2002), but the details defining the connection to mTOR were unclear until recently. Now a series of biochemical and genetic studies have established a pathway from Akt to mTOR involving the tuberous sclerosis complex proteins tuberin and hamartin, as well as the small Ras-like GTPase Rheb. Tuberous sclerosis complex 2 (TSC2) is a direct substrate of Akt (Inoki et al., 2002; Manning et al., 2002; Potter et al., 2002) (Figure 1). Unphosphorylated TSC2 is bound to TSC1 in a complex that blocks mTOR activation. Akt-mediated phosphorylation of TSC2 disrupts the TSC1/TSC2 complex, allowing unrestrained mTOR kinase activity. Rheb (*R*as *h*omolog *e*nriched in *b*rain) functions in this pathway downstream of TSC2 and upstream of mTOR (Garami et al., 2003; Saucedo et al., 2003; Stocker et al., 2003; Zhang et al., 2003). Interestingly, Rheb is highly expressed in transformed cancer cell lines and functions as an oncogene in fibroblast transformation models, but can also block transformation by Ras or B-Raf (Clark et al., 1997; Im et al., 2002).

A simple linear model of this pathway (Akt-TSC1/2-Rheb-TOR-S6K) cannot account for all experimental findings. For example, PI3K can activate S6K independently of Akt and mTOR through an alternative pathway involving PDK1 (Radimerski et al., 2002). In addition, mTOR functions in a nutrient sensor pathway independent of PI3K and Akt. mTOR is inhibited during conditions of nutrient deprivation, which leads to a slowdown in cell growth (defined as cell size or mass as opposed to cell proliferation). This starvation response makes teleological sense because mTOR plays a key regulatory role in protein translation through modulation of S6K and 4EBP1 action. The recently isolated mTOR binding protein Raptor provides a potential mechanism for how mTOR regulates downstream effectors (Hara et al., 2002; Kim et al., 2002). Because Raptor can also bind S6K and 4EBP1, it may function as a scaffold, keeping this signaling complex primed for rapid response to inputs from various pathways.

Because S6K and 4EBP1 play crucial roles in regulating translation, there has been much interest in defining the downstream mRNA targets of mTOR. The 5′ untranslated regions of cyclin D1 and c-Myc mRNAs both have CAP sequences, rendering them subject to regulation by 4EBP1. Cyclin D1 and c-Myc are regulated in part by mTOR since the levels of both proteins can fall in cells exposed to rapamycin in certain contexts (Hosoi et al., 1998; Muise-Helmericks et al., 1998; Takuwa et al., 1999). Global transcriptome analyses using polysome fractions are beginning to define the range of mTOR-regulated mRNAs (Peng et al., 2002; Rajasekhar et al., 2003; Shamji et al., 2000). At a first approximation, these analyses appear to confirm important functional roles of c-Myc and cyclin D1 in rapamycin-induced growth arrest (Gera et al., 2003).

Rapamycin has anticancer activity

The natural products program at the National Cancer Institute identified rapamycin as a potential anticancer agent in the 1970s (Douros and Suffness, 1981). Once the biochemical TOR was identified and more detailed activity profiles against a panel of human tumor cell lines were completed, some very interesting patterns emerged. Specifically, cell lines derived from different cancer types were noted to undergo G1 arrest when exposed to 1 nM rapamycin, a concentration which closely matches that required for biochemical inhibition of mTOR in cells. Notably, several other tumor cell lines that failed to respond to the 1 nM dose did undergo growth arrest at significantly higher concentrations (~1000 nM). While these phenotypic

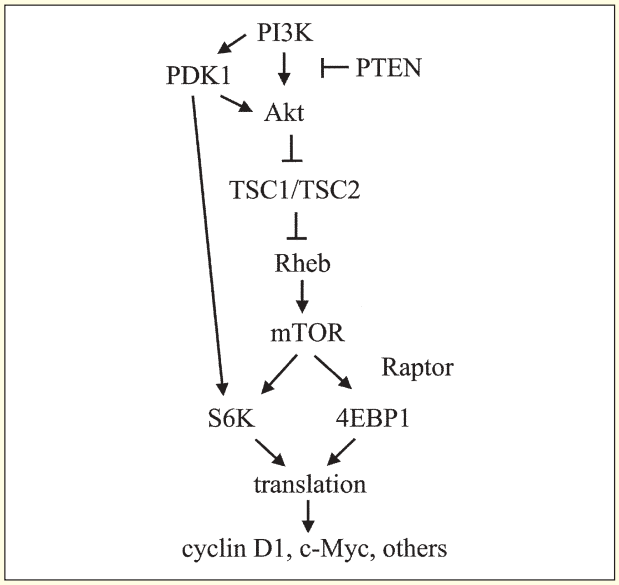

Figure 1. Signaling pathways involving mTOR

The diagram depicts the current view of mTOR regulation through the PI3K/Akt pathway based on biochemical and genetic studies. See text for more details.

screening studies revealed the broad potential for rapamycin as an antiproliferative agent, they did not uncover the mechanism. Nonetheless, the results define two groups of rapamycin-sensitive cell lines—those whose response correlates with inhibition of mTOR (1 nM) (group 1) and those that require significantly higher concentrations (group 2). Of note, the failure of the group 2 cell lines to respond to low "dose" (1 nM) rapamycin cannot be explained by insufficient blockade of mTOR kinase activity because 1 nM rapamycin was equally effective at inhibiting S6K and 4EBP1 phosphorylation in both groups (Neshat et al., 2001). These data support the notion that mTOR is the biologically relevant target of rapamycin for the tumor lines in group 1, whereas other targets are relevant in group 2.

Initial clinical development of mTOR inhibitors—The empiric strategy

In the absence of any histologic subtype or molecular marker that can distinguish between group 1, group 2, and nonresponsive cell lines, the initial clinical development of mTOR inhibitors in cancer has proceeded empirically. Several pharmaceutical companies have compounds in clinical trials for this indication. Among the most advanced is CCI-779 from Wyeth. CCI-779 is an ester of rapamycin with comparable potency and specificity for mTOR but with a longer half-life. Phase I and phase II clinical trials of the intravenous formulation have been completed and show promising enough results to warrant a phase III randomized trial that is underway. A brief review of the rationale and clinical details underlying this empiric approach is warranted to contrast with the molecularly driven approach described subsequently (see Dancey, 2002 for a comprehensive review of the clinical experience with mTOR inhibitors in cancer).

Following the traditional strategy of defining the maximum tolerated dose (MTD), Wyeth has conducted two phase I dose escalation studies of CCI-779 in patients with solid tumors using two different delivery schedules—weekly versus daily for 5 days every 2 weeks (Hidalgo et al., 2000; Raymond et al., 2000). Toxicities such as low platelet counts and fatigue were observed at high doses, but the drug was generally well tolerated. Importantly, the doses tested in these cancer trials gave peak plasma concentrations well above that required for inhibition of mTOR, but the intermittent nature of the dosing allowed troughs to fall below mTOR inhibition levels. Clinical responses were observed in several patients with advanced stage kidney cancer; therefore, a phase II trial was conducted in this disease. Using three different doses of CCI-779 given weekly, the objective response rate was only 5%, but there was a higher rate of minor responses (29%) and stable disease (40%) (Atkin et al., 2002). Of note, responses were observed equally across all doses (all of which give peak serum levels that block mTOR). On this basis, a phase III randomized trial has been initiated.

Deconvoluting the mechanism of response in kidney cancer

Now that the empiric clinical development plan of CCI-779 has identified renal cell carcinoma as a potential mTOR-dependent cancer, it is interesting to speculate on possible mechanisms. Two scenarios come to mind based on the critical role of angiogenesis in these tumors due to expression of hypoxia inducible factor (HIF) (reviewed in (Kaelin, 2002). The first is based on recent evidence that mTOR inhibitors may be antiangiogenic agents. PI3K, Akt, and mTOR are all critical for vascular endothelial growth factor (VEGF)-mediated endothelial cell proliferation, survival, and migration (Yu and Sato, 1999). In fact, rapamycin-coated coronary artery stents prevent restenosis in patients with coronary artery disease who undergo angioplasty (Morice et al., 2002). Preclinical studies suggest that this effect of mTOR inhibitors on endothelial growth may also apply to tumor angiogenesis since rapamycin blocked the in vivo growth of tumor cells that were resistant to rapamycin in vitro (Guba et al., 2002). A recent antiangiogenesis trial in kidney cancer (not involving an mTOR inhibitor) demonstrated that anti-VEGF antibody was not effective in causing tumors to shrink but caused significant delays in the time to tumor progression (Yang et al., 2003). This clinical outcome is strikingly reminiscent of the phase II results of CCI-779 in kidney cancer and begs the question of whether mTOR inhibitors may work by this mechanism.

A second scenario to explain the activity of CCI-779 in kidney cancer is based on evidence that mTOR can regulate HIF expression through the PI3K/Akt pathway (Hudson et al., 2002; Zhong et al., 2000). Therefore, mTOR inhibitors could have direct effects on tumor cells by reducing HIF levels as well as indirect effects on the endothelial cells recruited for tumor angiogenesis.

Rethinking the empiric clinical development strategy for mTOR inhibitors

The usual justification for pursuing an empiric drug development approach is that the molecular target of the drug is unknown or its disease-specific role is poorly understood. As a result, drug dose is chosen on the basis of what can be tolerated without inordinate toxicity. In this way, it is assumed that any clinical activity will not be missed due to insufficient drug levels. Once dose-limiting side effects are observed, the schedule of drug delivery is typically modified to allow recovery from any

Table 1. Potential mTOR-dependent cancers

Molecular lesion	Clinical disease
Upstream of mTOR	
PTEN loss	Glioblastoma
	Prostate cancer
	Endometrial cancer
	Others[a]
PI3K/Akt activation	Breast cancer (Her2+)
	Chronic myeloid leukemia (Bcr-Abl)
	Ovarian cancer (PI3K or Akt gene amplification)
	Others[a]
TSC1/2 loss	Tuberous sclerosis
Downstream of mTOR	
cyclin D1 overexpression	Mantle cell lymphoma
	Breast cancer
Myc overexpression	Burkitt's lymphoma
	Other Myc-driven cancers?
HIF overexpression	Kidney cancer
	Others?

[a]For more complete listings, see Vivanco and Sawyers, 2002.

toxicities. Hence, an intermittent regimen using doses just below the toxic level is the typical outcome of phase I evaluations of novel agents. Based on this approach, the early phase CCI-779 studies converged on a weekly dosing regimen. However, mTOR inhibitors represent an unusual example because of the broad clinical experience with rapamycin as an immunosuppressive agent for patients receiving organ transplants. For this indication, the dose is based on that required to inhibit T cell activation, which correlates with mTOR inhibition in blood cells. These patients take rapamycin daily at relatively low doses (in comparison to the weekly "cancer" dose of CCI-779) with minimal side effects. A recent phase I clinical report of a second mTOR inhibitor, RAD-001 (Novartis), measured S6K in blood cells to guide dose selection in cancer patients (O'Donnell et al., 2003).

If mTOR is the relevant target in cancer cells, why wasn't this low dose, daily schedule evaluated initially? And why not select patients whose tumors are more likely to be mTOR dependent? Obviously, the details underlying the decision-making processes at pharmaceutical companies are not public knowledge, but I will speculate on a few potential reasons. First, a daily dosing schedule would lead to constitutive T cell suppression, a side effect that one would prefer not to have in cancer patients. My personal view is that immune suppression is of secondary concern in the initial stages of developing an anticancer agent and should be factored into decision making only after the primary goal of tumor response has been achieved. Second, in vitro studies have defined a second population of rapamycin-sensitive tumor cell lines (group 2, see above) where the relevant target of drug action is not mTOR (perhaps another PIKK family kinase?). In hopes of capturing this second group of tumors, one would not want to restrict clinical evaluation to daily, low dose mTOR inhibitor because this would only be effective against those tumors presumed to be mTOR dependent (group 1). Third, it would be difficult to convince upper management to support a clinical development program focused exclusively on mTOR-dependent cancers without any knowledge of the size of the target population or the tools to identify the patients. Finally, marketing departments would presumably be concerned about the confusion generated by parallel use of the same drug for immune suppression and cancer. Reformulation into a new compound with a different schedule of administration could address this issue (albeit, cosmetically). I reiterate that my comments in this paragraph are highly speculative, but I hope that they help illustrate the complex set of scientific, economic, and regulatory considerations that influence decisions underlying a clinical development path.

An alternative clinical development strategy using molecularly selected patients

Studies of kinase inhibitors such as imatinib (also called Gleevec or STI571) indicate that these drugs can have tremendous clinical activity in appropriately selected patients. This experience has led to the notion that certain cancers are kinase dependent, typically due to fusions, point mutations, or amplification affecting the kinase gene that is targeted by the inhibitor (reviewed in Sawyers, 2003). In general, these genetic events enhance enzymatic activity of the kinase and serve as oncogenic events driving the growth of the cancer. Since mTOR is a kinase, can a similar paradigm of clinical development be applied here?

Strict application of the imatinib paradigm is unlikely since there is currently no evidence that the mTOR gene is mutated or amplified in human cancer. However, preclinical observations suggest that tumors with primary genetic abnormalities affecting pathways that regulate mTOR are, in fact, dependent on mTOR. These abnormalities include upregulation of the PI3K/Akt pathway, directly or by loss of the tumor suppressor phosphatase PTEN, as well as mTOR upregulation by TSC2 loss. PTEN null tumors are sensitive to mTOR inhibitors in several different human and murine preclinical models (Grunwald et al., 2002; Neshat et al., 2001; Podsypanina et al., 2001; Shi et al., 2002). Transformation induced by oncogenic alleles of Akt, but not Myc or Ras, is also reversed by mTOR inhibition (Aoki et al., 2001). Recent studies in conditional PTEN knockout or transgenic Akt mouse models confirm a role for mTOR in either aberrant cell growth (Kwon et al., 2003) or transformation through the PI3K/Akt pathway (E. Holland, personal communication; W. Sellers, personal communication). Similarly, tumors caused by loss of TSC2 also show enhanced sensitivity to rapamycin in growth assays (Kenerson et al., 2002).

The mTOR dependency of these tumors, whether induced by loss of PTEN or TSC2 or by activation of PI3K or Akt, shares conceptual similarity to synthetic lethal relationships originally described in yeast. Loss of PTEN or TSC2 seems to render mTOR essential in tumor cells but not in surrounding normal cells. However, genetic studies in worms and flies make it clear that TOR is essential for normal development (Long et al., 2002; Oldham et al., 2000; Zhang et al., 2000). Because TOR is highly conserved, it is likely that certain normal mammalian functions, including T cell function, will be mTOR dependent. Nonetheless, the clinical experience with rapamycin as an immunosuppressive agent indicates that mTOR inhibitors are well tolerated.

An alternative group of tumors that might also be mTOR dependent are those that express high levels of mTOR-regulated mRNAs, such as cyclin D1 or Myc. Because both genes are

also regulated by mTOR-independent mechanisms, it is difficult to anticipate how effective an mTOR inhibitor might be in reducing the expression of either protein. This, as well as the mTOR dependency of PTEN or TSC2-deficient cancers, can be tested in clinical trials where patient selection is based on documenting the relevant molecular pathway abnormality in tumor tissue.

The greatest challenge in designing these clinical trials is identification of molecularly defined patient cohorts (Table 1). Traditional inclusion criteria using tumor histology and site of origin will fail miserably because the molecular phenotype cannot be discerned from the clinical phenotype (tuberous sclerosis may be an exception). The most appropriate tools for discerning these phenotypes in the context of a clinical trial have not been defined. Among the potential approaches are proteomic or gene expression profiling to recognize signatures of kinase dependency. A first iteration of this approach, through the use of phospho-specific antibodies against specific kinase targets or substrates, shows promise in initial immunohistochemical applications (Choe et al., 2003). Limitations include the tricky performance characteristics of certain antibodies and the potential need for large numbers of optimized antibodies for comprehensive evaluation. It may also be possible to recognize kinase activation through gene expression profiling (Allander et al., 2001; Shai et al., 2003). Tissue availability and tumor heterogeneity present additional obstacles. Nonetheless, several of these approaches are under evaluation in small clinical studies of mTOR inhibitors. One possibility is that pilot trials will identify biomarkers that can be incorporated more easily into large scale studies.

Expectations

mTOR inhibitors are now far along on the clinical development path as anticancer agents, but it remains unclear how the story will unfold. The empiric approach has uncovered a low but reproducible objective response rate in kidney cancer patients. There is the impression of a much larger rate of disease stabilization, but this must be confirmed by a randomized trial. mTOR inhibitors will also be combined empirically with other agents (like interferon in kidney cancer) in an effort to increase the response rate, but these trials will be conducted without molecular insight into the mechanism of response. Although we all hope for success, this strategy is strikingly similar to recent combination trials of EGFR inhibitors with chemotherapy in advanced stage lung cancer (reviewed in Dancey and Freidlin, 2003). Single agent response rates with EGFR inhibitors in these patients are low but reproducible; the molecular basis of response is unknown; and four large randomized trials of EGFR inhibitors plus chemotherapy were all negative.

The alternative approach of using molecular insights from preclinical work to select patients is just now being evaluated. Efforts to identify appropriate patients based on immunohistochemical staining of tumor biopsies have been convincing enough to launch exploratory trials, but the robustness of these assays in realtime clinical settings remains to be defined.

If we assume that these assays are accurate and that continuous daily dosing of mTOR inhibitors effectively blocks mTOR in tumor cells, what clinical outcomes might we expect? There are several issues to consider. First, mTOR inhibition in sensitive tumor cell lines typically causes G1 arrest rather than apoptosis. Therefore, objective response rates may be low but disease stabilization could be high. Second, even though we have the tools to recognize tumors with loss of PTEN or activation of Akt, we do not know if this molecular abnormality represents an early or late event in the history of that tumor. This issue could be critical because an inhibitor that blocks an initiating oncogenic event is, presumably, more likely to induce a clinical response than one that blocks a later event involved in disease progression.

No matter the outcome, it is likely that combination therapy will be required to fully evaluate the potential of mTOR inhibitors in order to maximize response rates and prevent drug resistance. To avoid mistakes of the past, we need to select combinations based on mechanistic insights into why certain patients respond and others do not. One possibility is an mTOR plus EGFR inhibitor combination, particularly in a disease like glioblastoma where PTEN loss and genome-based EGFR activation can occur in the same tumor (Choe et al., 2003). Furthermore, perturbations in one signaling pathway may alter the cellular response to inhibition of another, as has been observed with PTEN and EGFR in laboratory models (Bianco et al., 2003; She et al., 2003). The good news is that we finally have a very nice selection of signaling pathway inhibitors, and we have the tools to select the patients. We just have to get to work and do the right clinical experiments.

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