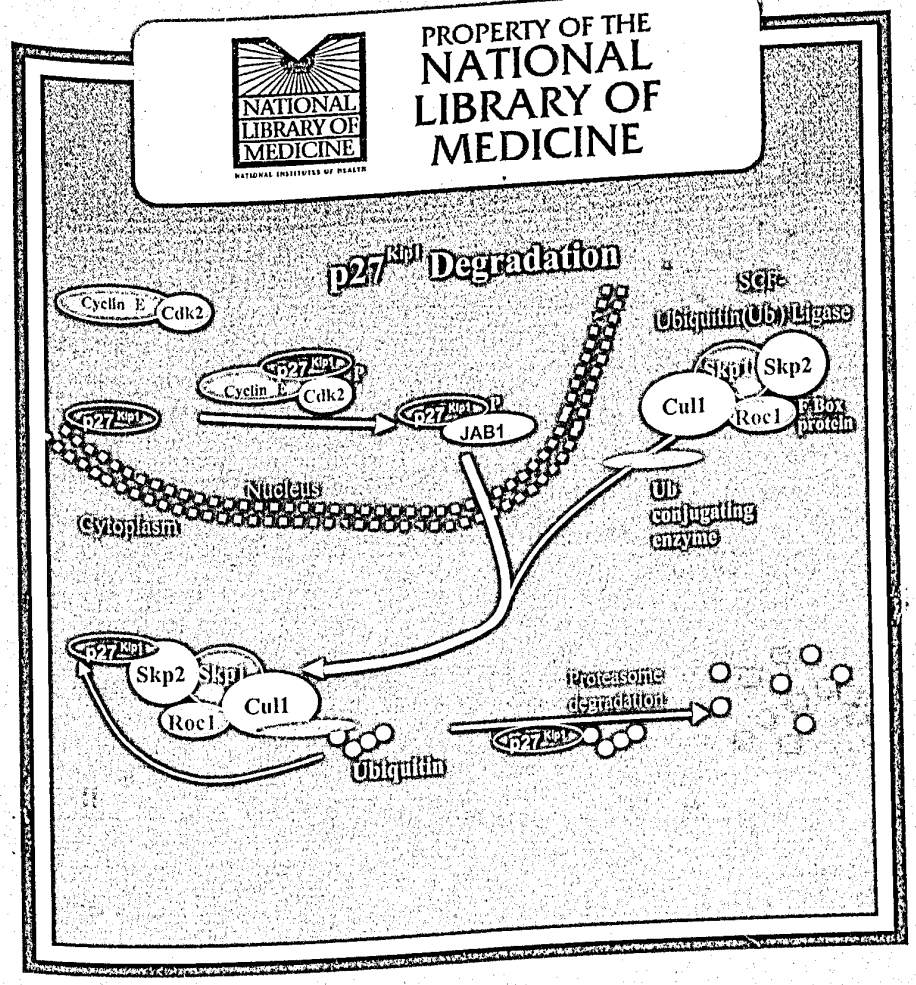
Current Cancer Drug Targets



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Mammalian Target of Rapamycin (mTOR) Inhibitors as Anti-Cancer Agents

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Abstract: Highly specific signal transduction inhibitors are being developed as anti-cancer agents against an array of molecular targets, with the promise of increased selectivity and lower toxicity than classic cytotoxic chemotherapy agents. Rapamycin and its analogues are a promising class of novel therapeutics that specifically inhibit signaling from the serine-threonine kinase, mammalian target of rapamycin (mTOR). mTOR is a key intermediary in multiple mitogenic signaling pathways and plays a central role in modulating proliferation and angiogenesis in normal tissues and neoplastic processes. Rapamycin potently inhibits T-cell proliferation, and is approved for clinical use as an immuno-suppressant following kidney transplantation. Hyperactivation of mTOR signaling has been implicated in tumorigenesis, and promising pre-clinical studies in several tumor types suggest that the anti-proliferative and anti-angiogenic properties of rapamycin may be useful in cancer therapy. These studies have led to several clinical trials evaluating the safety and efficacy of rapamycin analogs in cancer therapy. The goal of this article is to review the mechanism of action of rapamycin as an anti-cancer agent, and to review the clinical experience with rapamycin and rapamycin analogs as immunosuppressive and anti-neoplastic therapeutic agents.

Key words: Rapamycin, CCI-779, temsirolimus, RAD-001, everolimus, AP23573, sirolimus, cancer, cytostatic anti-cancer drugs, mTOR, renal transplant, immunosuppressive agents.

INTRODUCTION

Rapamycin and its analogues are novel, molecularly targeted drugs that are being developed as anti-cancer agents. The parent compound, rapamycin (Sirolimus, Rapamune; Wyeth-Ayerst) is approved by the Food and Drug Administration (FDA) for the prevention of allograft rejection following renal transplantation, and for incorporation into drug-eluting stents to prevent re-stenosis following coronary angioplasty. Experience in the transplant setting suggests that long-term use of this agent is safe and well tolerated. Rapamycin analogues with more favorable pharmacokinetic properties are currently being developed as anti-cancer drugs. Rapamycin and its analogs inhibit the signaling activity of the serine-threonine protein kinase, mammalian target of rapamycin (mTOR). mTOR functions downstream from multiple growth factor receptor tyrosine kinases to promote cell growth and proliferation. Key downstream targets of mTOR include p70S6 kinase and eukaryotic initiation factor 4E-binding protein (4EBP1), which modulate the translation of select mRNA transcripts that ultimately impact on cell growth and cell cycle progression. More recent data have linked mTOR signaling with the cellular response to hypoxia and the expression of vascular endothelial growth factor (VEGF), which suggests that mTOR may be an important mediator of tumor angiogenesis. In tumors that are reliant on mTOR signaling, disruption of these key signaling pathways by rapamycin results in cell cycle arrest and inhibition of angiogenesis, and these effects may account for the anti-neoplastic activities of mTOR inhibitors seen in multiple tumor types. Based on promising pre-clinical studies, rapamycin and its analogs currently are being tested as anti-neoplastic agents, both given alone or in combination with conventional cancer therapies. In this review, the biology of mTOR signaling and the cellular pharmacology of mTOR inhibition will be discussed as will the clinical development of mTOR inhibitors.

History

Rapamycin is a macrocyclic lactone antibiotic that was first isolated from the bacterium *Streptomyces hygroscopicus* found in soil samples taken from Easter Island (called 'Rapa Nui' by its native inhabitants; hence the name 'Rapamycin'). After its isolation and purification, studies revealed that rapamycin was a potent anti-fungal agent and an effective immunosuppressant[1]. Subsequent studies demonstrated that rapamycin inhibited proliferation in several tissues including IL-2 stimulated T-cells, vascular endothelium, smooth muscle, and tumor cells. These observations prompted the development of rapamycin and its analogs as immunosuppressive agents, inhibitors of vascular re-occlusion and as anti-cancer agents.

Rapamycin and three analogs, CCI-779, RAD-001 and AP23573, have been developed for human use (Fig. (**1**)). Among these, only rapamycin (Sirolimus, Wyeth Pharmaceuticals) is currently approved, for preventing kidney allograft rejection following renal transplantation and in drug-eluting stents to reduce the incidence of re-stenosis following coronary artery angioplasty. CCI-779 (Temsirolimus, Wyeth Pharmaceuticals) is an ester of rapamycin, with superior oral bioavailability compared to the parent compound rapamycin. This drug is available in oral and intravenous formulations, and clinical development of this drug is well underway with several phase II and III trials being conducted. RAD-001 (Everolimus, Novartis Pharmaceuticals) is an orally available hydroxyethyl derivative of rapamycin developed by Novartis for applications in the transplant, cardiovascular and oncological

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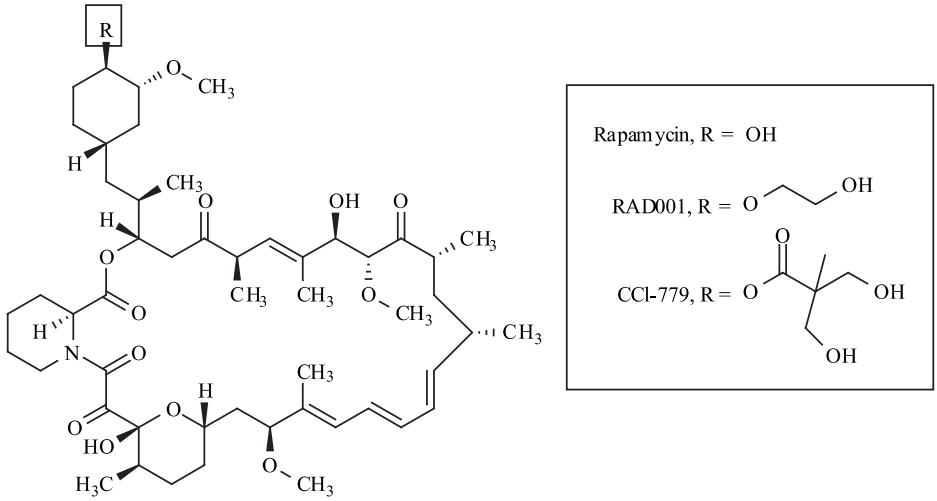

Fig. (1). Structure of rapamycin and rapamycin derivatives.

settings, and clinical testing for all these indications are ongoing. The newest mTOR inhibitory agent to be developed for clinical use is AP23573 (Ariad Pharmaceuticals). Early phase I clinical trials with this agent, which is also an analog of rapamycin, are now underway.

BIOLOGY OF mTOR

mTOR is a serine-threonine-directed kinase that belongs to the family of phosphatidyl-inositol 3-kinase-related kinases (PIKK). All members of this PIKK family contain a C-terminal kinase domain that shares significant homology with that of the phosphatidyl-inositol 3-kinase (PI3K); other members of this family include ataxia telangiectasia mutated (ATM), ATM and Rad3 related (ATR) and DNA-dependent protein kinase (DNA-PK) [2,3]. These latter 3 kinases play key roles in orchestrating DNA damage checkpoint responses and DNA repair [4]. In contrast, mTOR monitors intracellular nutrient and energy availability and promotes cell growth and proliferation following mitogenic stimuli, dependent upon the availability of requisite nutrients [5].

Rapamycin is a highly specific inhibitor of mTOR function. Rapamycin is unable to bind directly to mTOR, but forms a complex with the immunophilin, 12 kDa FK506-binding protein (FKBP12; FK-506 is an unrelated immunosuppressant); it is this drug-protein complex that binds to mTOR through an FKBP12-rapamycin binding (FRB) domain[6]. The FRB domain is adjacent to the kinase domain in mTOR and formation of this tri-molecular complex markedly attenuates downstream signaling from mTOR. Interestingly, rapamycin treatment does not inhibit mTOR catalytic kinase activity directly, since autophosphorylation of mTOR is unaffected by rapamycin treatment. Instead, binding of the FKBP12/rapamycin complex is thought to prevent interaction of mTOR with its kinase substrates and thus prevent downstream signaling [7]. The interaction of the rapamycin/FKBP12 complex with mTOR is highly specific and is so stable that inhibition of mTOR by rapamycin is essentially irreversible. The cellular and biochemical effects of rapamycin are generally believed to result exclusively from inhibition of mTOR signaling [8,9].

The mTOR signaling network (Fig. (**2**)) is important for driving cell growth and proliferation in multiple tumor types. Several receptor tyrosine kinases (RTKs), including the epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR) and insulin-like growth factor (IGFR) can activate PI-3 kinase activity, which, in turn, phosphorylates phosphatidylinositol (PI) on the D-3 position [10]. The resulting accumulation of phosphatidylinositol-3, 4, 5-triphosphate on the cytoplasmic surface of the plasma membrane leads to activation of a number of kinase signaling pathways including that regulated by protein kinase B (PKB, Akt). Akt stimulates mTOR function both through direct phosphorylation of a negative regulatory domain within mTOR [11] as well as through its effects on the tuberous sclerosis complex-2 (TSC2) protein [12-15]. TSC2, in a complex with tuberous sclerosis complex-1 (TSC1) protein, functions as a GTPase-activating protein towards the Rheb1 GTPase. Akt-mediated phosphorylation of TSC2 disrupts the TSC1/TSC2 complex and relieves inhibition of Rheb1 activity; activated Rheb1 then can stimulate mTOR phosphorylation and signaling (see Fig. (**2**)) [16-19]. The inhibitory effects of TSC2 on mTOR activity are stimulated in nutrient deprived conditions by activation of LKB-1, which signals through AMP-activated protein kinase (AMPK) to enhance TSC2 activity [20,21]. Signaling from mTOR also is regulated by association with Raptor, which probably functions as a scaffolding protein to promote transient association and phosphorylation of downstream targets [22,23]. Collectively, these data highlight the idea that mTOR functions within a molecular complex of multiple proteins that regulate its activity [24].

PI3K-mediated activation of Akt is normally opposed by the lipid phosphatase PTEN (phosphatase and tensin analogue), which dephosphorylates phosphatidylinositol at the D-3 position. Deletion or mutation of the gene encoding this tumor suppressor protein commonly occurs in multiple tumor types and results in constitutive activation of PI3K-dependent signaling pathways that include Akt and activated Ras as signaling mediators. Consistent with the potential role of the Akt/mTOR signaling pathway in tumorigenesis, overexpression of activated Akt and activated Ras in glial progenitor cells leads to formation of GBM-like tumors in a

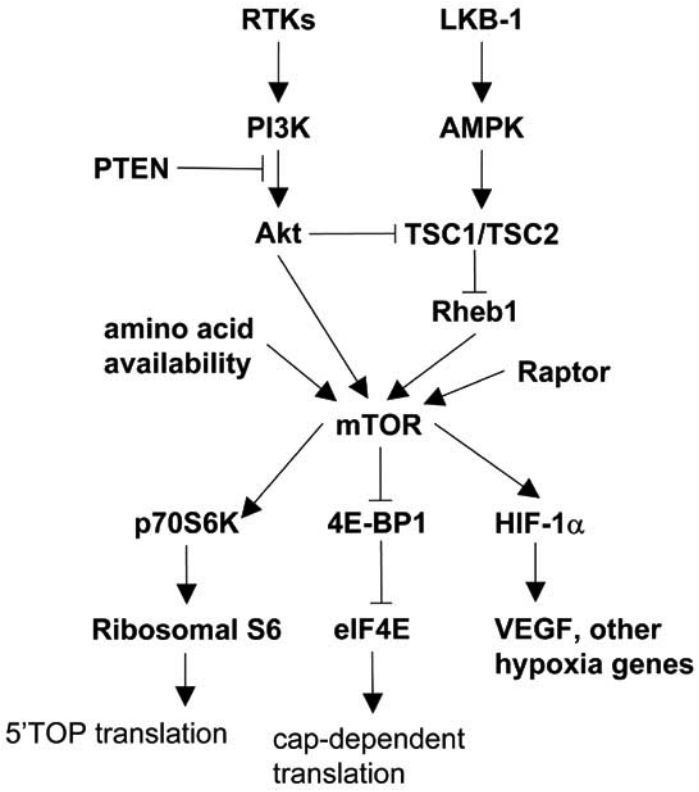

Fig. (2). The mTOR signaling network.

transgenic mouse model [25], and these tumors are exquisitely sensitive to treatment with rapamycin. Germ-line inherited deficiencies in PTEN, TSC or LKB-1 result in Cowden's disease, Tuberous Sclerosis and Peutz-Jeghers syndrome, respectively, which are all characterized by the development of multiple benign hamartomas. Loss-of-function in any one of these three proteins results in hyperactivation of mTOR signaling, and this presumably accounts for the development of the characteristic hamartomatous lesions. Thus, constitutive activation of mTOR signaling can be an important contributor to tumorigenesis in both benign and malignant tumors.

mTOR-Dependent Signaling Pathways

mTOR signals downstream to multiple protein targets: p70 S6 kinase (p70S6K), eukaryotic translation initiation factor 4E binding protein (4E-BP1), and the hypoxia-inducible transcription factor, HIF-1α. These 3 well-characterized downstream signaling targets have been implicated in control of hypoxia- and mitogen-induced tumor proliferation and disruption of these pathways may play an important role in the anti-tumor effects of rapamycin. In the sections below, we will describe the potential links between the anti-tumor effects of mTOR inhibitors and disruption of downstream signaling to p70S6K, 4EBP1 and HIF-1α in more detail.

p70S6K

mTOR regulates translation of select mRNA transcripts containing 5'-terminal oligopyrimidine (5'TOP) tracts through phosphorylation of p70S6K. Following PI3K-dependent phosphorylation of residues within an auto-inhibitory domain, mTOR regulates the phosphorylation of Thr-389 [26]. Modification of this residue is essential for subsequent phosphorylation of other residues within the activation loop of the kinase domain, which allows for full catalytic activity. After mitogen stimulation, activated p70S6K phosphorylates the S6 component of the 40S ribosomal subunit, and this promotes translation of mRNA containing 5'TOP [27]. Because transcripts for many ribosomal proteins and translation elongation factors contain this 5'TOP motif, rapamycin-mediated suppression of p70S6K activity may inhibit cell growth and proliferation by limiting ribosomal biogenesis and restricting protein synthesis capacity.

4EBP1

mTOR modulates protein translation initiation by regulating the assembly of the eukaryotic initiation factor 4F (eIF4F) complex on the 5'-methyl-GTP cap of mRNA transcripts. The eIF4F complex is a heterotrimer composed of the mRNA cap-binding protein eIF4E, a scaffolding protein eIF4G, and a helicase eIF4A (reviewed in [5]). This tripartite complex regulates the rate of cap-dependent protein translation by mediating the rate-limiting step of mRNA loading onto the small 40S ribosomal subunit. Formation of a functional eIF4F complex is controlled by the phosphorylation status of an eIF4E binding protein (4E-BP1). In nutrient- or growth factor-deprived cells, the association of hypophosphorylated 4E-BP1 with eIF4E blocks binding of eIF4G to the cap structure and inhibits

cap-dependent translation. In contrast, nutrient- or mitogen-induced phosphorylation of 4E-BP1 disrupts association with eIF4E and allows formation of a functional eIF4F complex [28,29]. 4E-BP1 is phosphorylated on at least 5 serine or threonine sites, and these mitogen-induced modifications are regulated, in part, by mTOR. Several studies have suggested that mTOR directly phosphorylates all 5 sites on 4E-BP1 [30,31], while others have argued that direct mTOR phosphorylation of 2 of the sites (Thr-37 and Thr-46) serves as a priming event that allows subsequent phosphorylation of the other sites (Ser-65, Thr-70, and Ser-83) by other signaling pathways [32]. Rapamycin diminishes 4E-BP1 phosphorylation, prevents dissociation of 4E-BP1 from eIF4E, and results in inhibition of cap-dependent translation. Because translation is less efficient for transcripts with complex secondary structures, rapamycin preferentially inhibits translation of mRNA transcripts containing complex 5' untranslated regions (UTR) [33]. Transcripts with complex 5'UTRs whose translation is inhibited by rapamycin include key proteins involved in cell proliferation and angiogenesis, such as cyclin D1, ornithine decarboxylase and vascular endothelial growth factor (VEGF).

HIF-1α

Hypoxia induces the expression of multiple genes containing hypoxia response elements (HREs), and transcription from this response element is regulated primarily by the HIF-1 transcription factor. HIF-1 is a heterodimer composed of HIF-1α and HIF-1β. Under normoxic conditions, HIF-1 heterodimer levels are undetectable due to rapid degradation of HIF-1α subunit. The stability of HIF-1α is regulated through post-translational modification of an oxygen-dependent degradation (ODD) domain. In the presence of oxygen, prolyl hydroxylases modify two conserved proline residues (Pro-402 and Pro-564) within the ODD [34,35]. Hydroxylation of these residues promotes HIF-1α association with the von Hippel Lindau-containing ubiquitin ligase complex and subsequent ubiquitin-mediated proteosomal degradation. Because molecular oxygen is required for catalysis of this reaction, hypoxic conditions prevent hydroxylation of the ODD, which results in stabilization of HIF-1α. Other transactivating post-translational modifications and dimerization with the HIF-1β subunit result in formation of an active HIF-1 transcriptional complex. HIF-1 drives expression of genes, such as *VEGF*, which contain HREs within their promoter region. The repertoire of hypoxia-inducible genes enables tumor or normal tissues to adapt to low oxygen environments and include genes involved in oxygen and glucose transport, glycolysis, growth-factor signaling, immortalization, genetic instability, invasion and metastasis, apoptosis and pH regulation [36].

Signaling through the PI3K/mTOR pathway regulates HIF-1α expression and activity [37]. The link between PI3K signaling and HIF-1α activity was first established in Ras-transformed cells, where hypoxia-induced signaling to HIF-1 was blocked by genetic or pharmacological inhibition of PI3K activity [38]. Subsequent studies have demonstrated that restoration of wild-type PTEN function, expression of a dominant-negative Akt construct, or treatment with rapamycin blocks hypoxia and mitogen-induced HIF-1 signaling [39-41]. Moreover, a recent study demonstrated that rapamycin blocks both hypoxia-induced HIF-1α accumulation and transactivation, and that this effect of rapamycin is specifically due to pharmacological inhibition of mTOR [42]. Collectively, these data suggest the existence of a PI3K/Akt/mTOR signaling pathway that regulates HIF-1α expression, stability or activation.

ANTI-TUMOR EFFECTS OF RAPAMYCIN

The central role for mTOR in modulating cell proliferation in both tumor and normal cells and the importance of mTOR signaling for the hypoxic response suggests that rapamycin-based therapies may exert anti-tumor effects primarily through either inhibition of tumor cell proliferation or suppression of angiogenesis. Pre-clinical and early clinical results demonstrate that only a subset of tumors will respond to rapamycin-based therapies. In the following sections, we will review the pre-clinical efficacy data, the evidence supporting the anti-angiogenic and cytostatic properties of rapamycin, and discuss potential mechanisms of resistance to mTOR inhibition.

Pre-clinical studies have demonstrated efficacy of rapamycin analogs and the parent compound in multiple tumor types. In the National Cancer Institute 60 tumor cell line panel, both rapamycin (NSC 226080) and CCI-779 (NSC 683864) demonstrated growth inhibitory activity against a broad spectrum of tumors with a subset of leukemia, lung, brain, prostate, breast, renal and melanoma tumor cell lines being inhibited at low nanomolar concentrations (http://dtp.nci.nih.gov/). Early animal studies at the NCI and at Ayerst Research Laboratories demonstrated modest growth inhibitory properties of rapamycin in murine tumor models of B16 melanoma, P388 lymphocytic leukemia, EM ependymoblastoma, CD8F1 breast carcinoma, Colon 38, CX-1 and 11/A colon cancer models [43]. Subsequent published studies have documented significant tumor growth inhibition with rapamycin or CCI-779 treatment of DAOY medulloblastoma, U251 or SF295 glioma, PC-3, DU-145, LAPC4, or LAPC9 prostate carcinoma, and Rh-18 rhabdomyosarcoma human tumor xenografts [44-47]. These data have provided the impetus for development of mTOR inhibitor therapy in a variety of tumor types.

Treatment of tumor cells *in vitro* with rapamycin results in an accumulation of cells in the G_1 phase of the cell cycle. Similarly, rapamycin treatment in tumor-bearing animals results in decreased tumor cell proliferation as indicated by BrdU labeling index [48]. At the molecular level, this drug-induced cell cycle arrest is associated with an accumulation of the cyclin-dependent kinase inhibitor, $p27^{kip1}$, decreased expression of cyclinD1, and a corresponding decrease in phosphorylation of the retinoblastoma protein. CyclinD1 mRNA contains a complex 5'UTR, and reduction in cyclinD1 levels presumably results from rapamycin-mediated inhibition of 4EBP1 phosphorylation and the resulting inhibition of eIF4F function. Likewise, the mRNA encoding for multiple oncogenes or proteins involved in DNA metabolism and S-phase progression contain complex

5'UTRs and are regulated by mTOR [49]. Although rapamycin can induce apoptosis in select tumor models, rapamycin treatment typically slows tumor growth but does not induce tumor regression, suggesting that increased tumor cell loss through apoptosis or other mechanisms of cell death likely are not major contributors to drug effect in most tumors. Collectively, these data support the idea that rapamycin exerts a cytostatic effect on tumor cell growth.

Rapamycin also has cytostatic effects on normal and tumor vasculature. As discussed above, mTOR plays an important role in modulating the cellular response to hypoxia through stabilization and activation of HIF-1α. HIF-1 drives expression of hypoxia-responsive genes, and these genes include those such as vascular endothelial growth factor (VEGF) that are important for tumor angiogenesis. Translation of VEGF mRNA also is regulated in an mTOR-dependent manner *via* its complex 5'UTR. Consistent with these mechanisms of regulation, treatment of tumor-bearing animals with rapamycin results in decreased expression of VEGF mRNA in tumors [50] and decreased circulating levels of VEGF protein [51]. VEGF drives endothelial proliferation through interaction with its cognate receptor tyrosine kinases, VEGF receptor 1 and 2, and these receptors can signal downstream through the PI3K/Akt pathway to mTOR. Thus, proliferation of smooth muscle and endothelial cells is inhibited by mTOR inhibition [52,53], and this effect is likely related to the efficacy of rapamycin-eluting stents in preventing vascular re-occlusion. In animal models, rapamycin inhibits tumor growth and neo-vascularization of CT-26 colon tumors grown in a dorsal skin-fold model and suppresses serum levels of VEGF in tumor bearing animals[51]. Thus the anti-angiogenic effects may contribute to the efficacy of mTOR inhibitors in cancer therapy.

The anti-tumor effects of rapamycin therapies are likely secondary to both inhibition of tumor cell proliferation and inhibition of tumor angiogenesis. The relative contribution of these two effects in any given tumor may be difficult to delineate. However, pre-clinical and clinical data suggest that that only a subset of tumors will respond to rapamycin; one of the challenges will be to identify patients most likely to respond to this class of agents. Future studies delineating the relative contributions of the anti-proliferative and anti-angiogenic effects to the overall anti-tumor efficacy of rapamycin will be invaluable for identifying the relevant cellular targets that are responsible for the anti-tumor effects of rapamycin.

COMBINATIONS OF MTOR ANTAGONISTS WITH OTHER ANTI-CANCER AGENTS

Angiogenesis and tumor cell proliferation have been implicated as important mediators that can influence the efficacy of traditional cytotoxic cancer therapies; therefore, much research effort has focused on the evaluation of

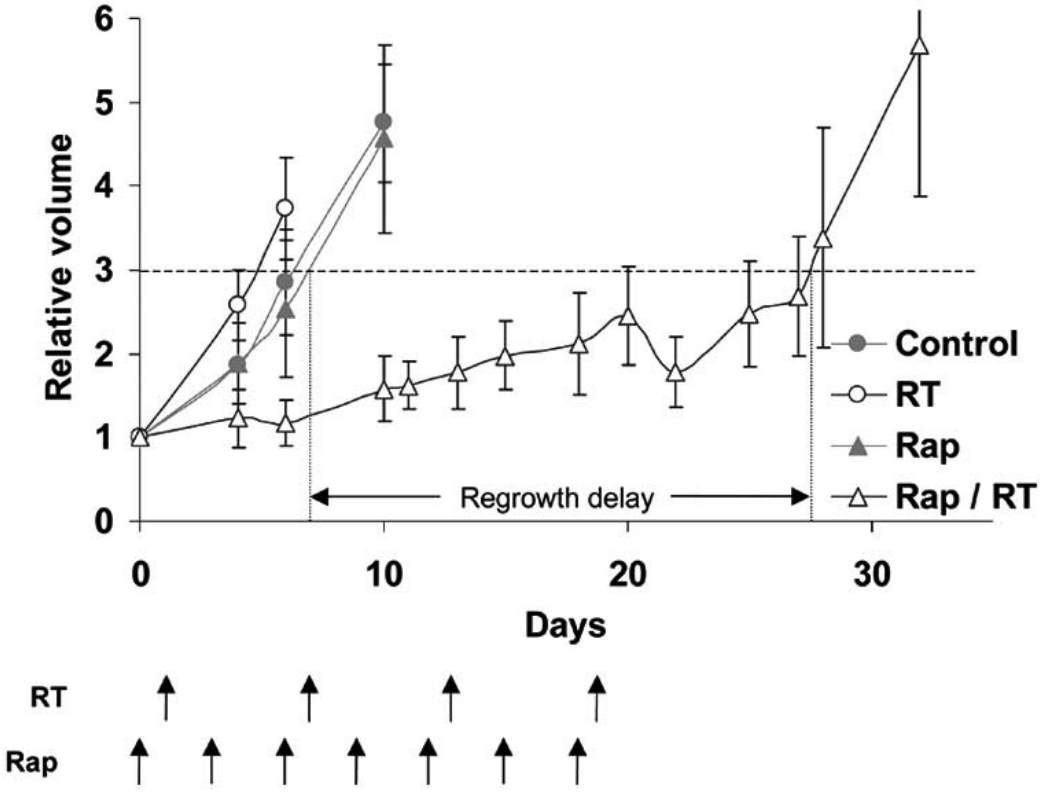

Fig. (3). Rapamycin enhances the efficacy of radiation in U87 xenografts. Nude mice with established U87 flank xenografts were randomized into four treatment groups: 1) placebo, 2) radiation only (4 Gy x 4), 3) rapamycin only (1 mg/kg), or 4) radiation and rapamycin. The tumor re-growth for each treatment group is shown. Data points represent the mean relative tumor volume ± SE. Treatment was initiated on Day 0 with the first injection of rapamycin (Rap). The schedule for Rap and radiation (RT) treatments is depicted below the x-axis. Highly similar results were obtained in two independent experiments. (Reproduced with permission, *Cancer Res.*, **2002**, *62*, 7291-7297).

combinations of mTOR inhibitors with standard cancer therapies. Rapamycin potentiates cisplatin induced apoptosis in multiple cell lines including HL-60 leukemia cells and SKOV3 ovarian cancer cells [54]. Likewise, insulin-like growth factor-induced resistance to cisplatin in Rh30 rhabdomyosarcoma cells can be reversed by rapamycin treatment [55]. Geoerger *et al.* demonstrated that combinations of rapamycin with either cisplatin or camptothecin provides additive growth inhibition in the rapamycin-sensitive DAOY medulloblastoma cell line but not in the rapamycin-resistant D283 cell line [46]. Consistent with this *in vitro* data, CCI-779 therapy provides for additive tumor growth inhibition in animals when combined with doxorubicin or cisplatin in PC3 or DAOY xenografts, respectively [46]. Several other investigators have confirmed the *in vitro* effect of rapamycin on sensitizing cancer cells to chemotherapeutic agents such as adriamycin, VP-16, and cisplatin[1] and hormonal therapies such as tamoxifen[2] dexamethasone[3], and the anti-estrogen ERA-923[4]. Rapamycin also has been combined with other molecularly targeted therapeutics. In our laboratory, we found that the combination of rapamycin with the EGFR inhibitor EKI-785 resulted in synergistic growth inhibition (unpublished data), and similar results were reported with the combination of RAD-001 and the VEGFR/EGFR inhibitor AEE788[5]. Likewise, rapamycin combined with the bcr/abl inhibitor Imatinib mesylate (Gleevec) provided synergistic growth inhibition of chronic myeloid leukemia (CML) cell lines[6]. Taken together, these data suggest that mTOR-dependent signaling may be important for resistance to chemotherapy-induced apoptosis and provides a rationale for the combination of rapamycin with specific chemotherapy agents.

Rapamycin also can enhance the efficacy of radiation therapy. Based on significant pre-clinical and clinical data demonstrating that tumor proliferation during fractionated radiotherapy contributes to clinical radiation resistance [56,57], we hypothesized that rapamycin-mediated inhibition of tumor proliferation during radiotherapy would enhance the efficacy of radiation [48]. Consistent with the idea that mTOR is not involved in DNA damage responses, rapamycin had no effect on the *in vitro* radiation sensitivity of several glioma cell lines including U87 cells. In contrast, intermittent dosing with rapamycin throughout a fractionated course of radiation significantly enhanced the efficacy of treatment in radio-resistant U87 xenografts (Fig. (**3**)). On the basis of these data and the previously described effects of cisplatin, we have initiated a phase I clinical trial evaluating the combination of definitive radiation therapy, cisplatin and rapamycin in patients with unresectable lung cancer.

SENSITIVITY AND RESISTANCE TO MTOR INHIBITION

Because rapamycin is a highly specific inhibitor of mTOR signaling, response to rapamycin therapies only would be expected in tumors where mTOR activity is specifically required for tumor proliferation or angiogenesis. Thus, sensitivity or resistance to inhibitor therapy will, at least in part, be defined by the molecular signaling characteristics of individual tumors. Although there is no clinical data regarding rapamycin resistance, many laboratory studies have defined multiple molecular characteristics that predispose to rapamycin resistance, including normal PTEN function, low 4EBP1 levels, c-Myc overexpression, and a collection of other less-well characterized molecular features.

PTEN Function

Several studies have demonstrated that lack of PTEN function is associated with an increased sensitivity to mTOR [45,58-60]. Heterozygous PTEN knock-out mice spontaneously develop tumors associated with inactivation of the remaining PTEN allele, and these tumors show a high level of Akt activity [61]. Treatment with rapamycin prevents tumor formation in these PTEN +/- mice, and mouse embryonic fibroblasts obtained from PTEN-null (-/-) embryos are significantly more sensitive to rapamycin than the corresponding wild-type MEFs. PTEN-deficient prostate cancer cell lines are more sensitive to rapamycin than cell lines with wild type PTEN [62], and a similar relationship between PTEN loss and rapamycin sensitivity has been noted in glioblastoma, breast cancer, and myeloma cell lines [63]. These data imply that cells without normal PTEN function are more likely to depend on hyperactivation of Akt/mTOR signaling to drive tumor cell proliferation. Conversely, tumors with wild-type PTEN are less likely to utilize mTOR-dependent signaling pathways as a major driver of proliferation.

4EBP1, eiF4E, and c-Myc Levels

The data presented above suggest that tumor cells which depend on mTOR signaling for survival or proliferation are most likely to be sensitive to rapamycin therapies. This concept can be extended to the influence of downstream mTOR signaling pathways on sensitivity and response to drug therapy. Of the three downstream pathways described

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previously, the 4EBP1/eIF4E signaling pathway is emerging as an important effector of mTOR-mediated cell proliferation. In a recent study, selection of rhabdomyosarcoma cells for rapamycin resistance resulted in the isolation of resistant clones with decreased 4EBP1 protein expression relative to eIF4E [64]. Subsequent over-expression of exogenous 4EBP1 in these resistant clones restored normal sensitivity to rapamycin. Likewise, within a panel of colorectal cell lines, a low ratio of 4EBP1 to eIF4E was associated with resistance to rapamycin that could be reversed by 4EBP1 overexpression [64]. As discussed previously, rapamycin blocks mTOR-mediated phosphorylation of 4EBP1, and hypophosphorylated 4EBP1 binds to eIF4E and prevents translation of transcripts with complex 5'UTRs. With a decreased ratio of 4EBP1 to eIF4E, levels of 4EBP1 will be inadequate to suppress eIF4E function, even in the presence of rapamycin, and this unrestrained activity of eIF4E presumably results in rapamycin resistance.

These previous data suggest that signaling from mTOR through 4EBP1 to control eIF4E function is a key pathway and that other pathways modulating eIF4E or 4EBP1 function also could contribute to resistance to rapamycin therapy. In fact, several studies have associated c-Myc amplification and overexpression with resistance to rapamycin [65,66]. The *eIF4E* gene is a direct target of the c-Myc transcription factor, and overexpression of c-Myc can drive eIF4E transcript expression [33,67,68]. Also, in a positive-feedback loop, c-Myc mRNA contains a complex 5'UTR, so Myc translation is facilitated by high-level eIF4E expression [69,70]. Ultimately, increased expression of eIF4E could result in a decreased 4EBP1/eIF4E ratio and this might contribute to rapamycin resistance as described above. In a similar manner, overexpression of eIF4E can transform cells and is an oncogenic event in several tumor types including colon, breast, prostate and head and neck cancer [33,69,71-73], and it might be expected that tumors exhibiting eIF4E overexpression may be resistant to rapamycin therapy.

Other Factors Associated with Resistance or Sensitivity

Rapamycin treatment induces expression of the cyclin dependent kinase (cdk) inhibitor, $p27^{kip1}$. $p27^{kip1}$ interacts with and inhibits the activity of cyclin/cdk complexes responsible for driving cell cycle progression from G1 to S-phase. However, it is unclear whether changes in $p27^{kip1}$ levels are a cause of, or an effect of, rapamycin-induced cell cycle arrest since conflicting studies have either shown rapamycin resistance or no change in rapamycin sensitivity in murine cells derived from $p27^{kip1}$ null mice [74-76]. In rhabdomyosarcoma cell lines, rapamycin treatment of cells expressing mutant p53 was associated with failure to arrest in G1 and subsequent apoptosis, while enforcement of a G1 arrest in these cells by ectopic expression of wild-type p53 prevented apoptosis [77]. A recent study has correlated higher phospholipase D (PLD) activity in MDA-MB-231 cells with rapamycin resistance compared to MCF-7 cells, and expression of a dominant-negative PLD construct in the MDA-MB-231 cells was associated with a significant increase in rapamycin sensitivity [78]. Cells transformed by overexpression of the GLI transcription factor are highly sensitive to inhibition by rapamycin [79]. Lack of wild-type function of the ataxia-telangiectasia mutated (ATM) protein also is associated with rapamycin resistance. The ATM protein, like mTOR, is a member of the PI3K-related family of protein kinases and is involved in DNA damage recognition and repair. The mechanism of rapamycin resistance in these cells is unknown [80]. Engineered mutations in the FRB domain of mTOR or mutations in FKBP12 are associated with failure of rapamycin to bind and inhibit mTOR signaling. Other studies in yeast models have suggested that mutations of 14-3-3 scaffolding proteins or PP2A-related phosphatases also result in rapamycin resistance.

Collectively, the data presented suggest that multiple molecular characteristics contribute to rapamycin resistance, and the challenge will be understand how these various features interact to influence tumor response to rapamycin-based therapies.

CLINICAL USE OF RAPAMYCIN

In the following sections, we will review the clinical data supporting the FDA approval for the transplant and cardiovascular indications and then review the early clinical data available regarding therapy with rapamycin analogs in cancer treatment.

Immunosuppressive Agent

Rapamycin (Sirolimus) is used commonly in the renal transplant setting in combination with or as an alternative to standard therapy with calcineurin inhibitors (such as cyclosporine and tacrolimus). Initial FDA approval for rapamycin was based on 2 large multi-center randomized trials that evaluated the benefit of rapamycin added to cyclosporine A-based regimens. In the U.S. trial, 38 centers randomized 719 patients to rapamycin doses of 0, 2, or 5 mg/day in combination with cyclosporine A, azathioprine, and steroids [81]. In the Global trial, 34 centers from Europe, North America and Australia randomized 576 patients to the same dosing options for rapamycin combined with cyclosporine A and steroids [82]. In both trials, a significant reduction in acute graft rejection was observed with rapamycin therapy. In the U.S. trial, the cumulative acute rejection rates at 24 months post transplant were 33%, 25% and 18% for treatment with 0, 2 or 5 mg rapamycin, respectively. Likewise, in the Global study, the cumulative failure rates were 44%, 30% and 26%, respectively. Rapamycin-based therapies also have been developed that allow avoidance of calcineurin inhibitors in those patients intolerant of these agents. The typical dosing regimen used at the Mayo Clinic for patients on a rapamycin-based regimen is a loading dose of 10 mg for 2 days and then 5 mg daily. In the early post-transplant setting, rapamycin drug levels are checked daily and doses are adjusted to provide trough serum levels of 15-20 ng/mL rapamycin (personal communication, T. Larson, MD). Rapamycin is rapidly absorbed after oral dosing with peak drug concentrations achieved approximately 1.5 hours after administration; however, the terminal elimination half-life of rapamycin is quite long (approximately 60 hours). Thus,

stable drug levels may not be achieved for several days following a dose adjustment.

Rapamycin therapy is generally well tolerated with the most common toxicities being hyperlipidemia, thrombocytopenia and diarrhea. Hyperlipidemia (both hypercholesterolemia and hypertryglyceridemia) occurred in approximately half of patients on rapamycin therapy enrolled on the pivotal Global trial, as opposed to only a quarter of those on cyclosporine alone. Rapamycin-induced-hyperlipidemia responds to standard lipid lowering drugs. However, patients with pre-existing hyperlipidemia that is poorly controlled with a single lipid-lowering agent generally are considered poor candidates for rapamycin therapy. Thrombocytopenia is typically mild and was observed in 6, 13, and 28% of patients treated on the Global trial with 0, 2, and 5 mg of rapamycin, respectively. Due to concerns about delayed wound healing, rapamycin typically is not used in the immediate post-transplant setting. The rare occurrence of non-infectious pneumonitis has been reported. Specifically of interest to oncology applications, rapamycin therapy has not been associated with an increased rate of systemic infections. The incidence of aphthous ulceration in the mouth, commonly observed in the pivotal clinical trials in transplant patients, has now decreased with the routine use of a capsule formulation of rapamycin. Thus, experience in the transplant setting suggests that rapamycin is a relatively non-toxic drug and that chronic oral dosing is generally well tolerated.

Rapamycin-Eluting Cardiac Stents

Rapamycin-eluting cardiovascular stents were recently approved by the FDA for prevention of re-stenosis following coronary artery angioplasty. The mechanical injury to the endothelial lining of the vessel resulting from angioplasty leads to an injury response that is characterized by proliferation of neo-intimal endothelial cells and vascular smooth muscle cells. This injury response can result in re-occlusion following angioplasty. The observation that rapamycin inhibits endothelial proliferation led to the development of rapamycin-eluting stents. In these stents, a rapamycin-containing polymer coats the metal stent, and the drug slowly elutes from the stent to maintain high local concentrations of rapamycin for several weeks. Several large randomized clinical trials have demonstrated that the risk of requiring target vessel re-vascularization at 9 to 12 months following stent implantation is reduced from 11-21% in patients treated with standard bare metal stents to approximately 4% in those patients treated with rapamycin-eluting stents [83-85]. Oral rapamycin therapy following deployment of bare metal stents also has some efficacy in this setting, with a correlation between serum levels and efficiency in reducing re-stenosis rates[86]. However, oral dosing appears to be less effective than using drug-coated stents, presumably due to the lower drug concentrations achieved in the target tissues with the former [86,87]. Given the significant reduction in coronary re-occlusion rates with rapamycin-eluting stents, their use has been rapidly integrated into routine clinical practice.

Rapamycins as Anti-Cancer Agents

The rapamycin analogs CCI-779, RAD-001 and AP23573 are being developed for use in oncology. While AP23573 is now completing phase I clinical trials, CCI-779 and RAD-001 are being studied in phase II and phase III trials. Experience with these agents suggests that the toxicities are quite similar to those experienced by transplant patients treated with rapamycin. Summaries of the clinical trials that have been reported at least in abstract form are presented in Table 1. Within the context of phase I and II clinical trials, there are reports of partial responses and stable disease with rapamycin analogs in non-small cell lung cancer, renal cell cancer, breast cancer, sarcoma, mesothelioma, mantle cell lymphoma and glioblas-toma. Data in renal cell cancer suggest that mTOR inhibitors may prolong survival in patients with an intermediate to poor prognosis[7].

Table 1. Results of Clinical Trials of mTOR Inhibitors

Tersirolimus, CCI-779 (Wyeth Pharmaceuticals)				
Reference (see footnotes)	Institutional setting	Trial details	Disease group	Conclusion
Raymond [8]	Multicenter European, n=16	Phase I study, weekly IV.	Advanced solid tumors	Toxicity minor, DLTs not seen. Anti-tumor activity noted at doses >15 mg/m^2/w in patients with RCC, neuroendocrine tumor, soft tissue sarcomas, melanoma, rectal and adrenal cortical carcinoma.
Hidalgo 2000 [9]	Multicenter US trial n=35	Phase I study CCI-779 administered as a 30-minute IV infusion daily for 5 days, delivered every 2 weeks.	Advanced solid tumors	Main toxicities noted were mucositis, thrombocytopenia and hypersensitivity. MTD determined to be 15mg/m^2 in heavily pre-treated groups and 19.1 mg/m^2 in minimally pre-treated patients. Efficacy noted in patients with soft-tissue sarcoma, cervical, uterine, NSCL ca and RCC.

[7]·Hidalgo, M.; Atkins, M. B.; Stadler, W. M.; Logan, T.; Dutcher, J. P.; Hudes, G.; Marshall, B.; Liou, S. H.; Dukart, G. A randomized double-blind phase 2 study of intravenous (IV) CCI-779 administered weekly to patients with advanced renal cell carcinoma (RCC): Prognostic factor analysis. *Proc. Am. Soc. Clin. Oncol*. **2003**, *22,* Abstract # 804.

[8]·Raymond, E.; Alexandre, J.; Depenbrock, H.; Mekhaldi, S.; Angevin, E.; Hanauske, A.; Baudin, E.; Escudier, B.; Frisch, J.; Boni, J.; Pierre Armand, J.P. CCI-779, a Rapamycin Analog with Antitumor Activity: A Phase I Study Utilizing a Weekly Schedule. *Proc. Am. Soc. Clin. Oncol.* **2000**, Abstract # 728.

[9]·Hidalgo, M.; Rowinsky, E.; Erlichman, C.; Drengler, R.; Marshall, B.; Adjei, A.; Hammond, L.; Speicher, L.; Galanis, E.; Edwards, T.; Boni, J.; Dukart, G.; Buckner, J.; Tolcher, A. CCI-779, a Rapamycin Analog and Multifaceted Inhibitor of Signal Transduction: a Phase I Study. *Proc. Am. Soc. Clin. Oncol.* **2000,** Abstract # 726.

(Table 1). contd.....

Forouzesh[10]	Multicenter US trial n=24	Phase I, daily oral therapy for 5 days every 2 weeks.	Advanced solid tumors	Toxicity minor. Preliminary evidence of anti-tumor activity of oral CCI-779 noted. Several patients with RCC, NSCLC, myxoid chondrosarcoma, mesothelioma, and leiomyosarcoma had prolonged stabilization of disease. Recommended oral dosage of CCI-779 determined to be 75 mg/day at this schedule.
Buckner[11]	Multicenter US study n=25	Phase I, daily IV therapy for 5 days every 2 weeks.	Most patients with advanced CNS tumors (e.g. 15 had astrocytomas).	Toxicity minimal. Stable disease (>4 m) seen in 6 patients. MTD higher in patients on CYP3A4 inducing anti-convulsant drugs (>37mg/m^2) suggesting a pharmacokinetic interaction.
Smith[12]	Multicenter, US trial. n=71	Phase 1/2 study testing the combination of IV weekly CCI-779 and IFN-α (subcutaneous, three times a week).	Advanced Renal Cell cancer	Minimal toxicity. Weekly IV CCI-779 15 mg, and 6 million Units IFN-α determined as the MTD. Partial response rate was 13% and stable disease in 71% of patients at these doses. A phase III study was initiated based on these results (table 2).
Chan [13]	Multicenter, European trial (CESAR /European CCI-779 Breast Group). n=106	Phase II study of two dose levels of IV CCI-779 (75 or 250 mg) delivered once a week.	Metastatic breast cancer	Toxicity minimal. Clinical benefit noted in half of enrolled patients. No difference in activity between the 2 dose levels.
Witzig [14]	NCCTG Multicenter, study n=18	Phase II study, weekly IV CCI-779 (250mg).	Heavily pre-treated patients with mantle cell lymphoma	Hematological toxicity common at dose selected and required dose reductions. Objective responses noted in 44% of patients.
Galanis[15]	NCCTG multi-center study N=41	Phase II study, weekly IV CCI-779 (150 mg weekly IV).	Recurrent glioblastoma multiforme	Minor radiological responses seen in 10 patients (of 44), but none met response criteria.
Margolin[16]	Multicenter, US trial n= 33	Phase II study of weekly IV CCI-779 (250 mg).	Metastatic melanoma	Primary end point (prolongation of time to progression) not reached. 1 partial response seen. Toxicity was mild and managable.
Atkins[17]	Multicenter, US trial n=110	Randomized double blind phase II study of weekly IV CCI-779. 3 dose levels (25, 75 or 250 mg) were tested.	Metastatic RCC	Clinical benefit seen in 74%. Prolonged survival in treated patients (compared to historic controls). All 3 doses had equal efficacy.

[10]·Forouzesh, B.; Buckner, J.; Adjei, A.; Marks, R.; Hammond, L.; Molpus, K.; Boni, J.; Dukart, G.; Friedman, R. R. Phase 1, bioavailability, and pharmacokinetic study of oral dosage of CCI-779 administered to patients with advanced solid malignancies, 2002 EORTC-NCI-AACR Symposium on Molecular Targets and Cancer Therapeutics. *Eur. J. Cancer* **2002**, *38*(Suppl. 7), pp. 54. Abstract # 168.

[11]·Buckner, J.; Prados, M.; Rowinsky, E.; Moynihan, T.; Burton, J.; Marshall, B.; Boni, J.; Dukart, G. A phase I study of the safety, tolerability, and pharmacokinetics of intravenous CCI-779 given once daily for 5 days every 2 weeks to patients with CNS tumors. *Neuro-Oncol.* **2002**, *4*, Abstract # 208.

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[13]·Chan, S.; Johnston, S.; Scheulen, M. E. First report: a phase 2 study of the safety and activity of CCI-779 for patients with locally advanced or metastatic breast cancer failing prior chemotherapy. *Proc. Am. Soc. Clin. Oncol*. **2002**, Abstract # 175.

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[15]·Galanis, E.; Buckner, J.; Maurer, M.; Ballman, K.; Hidalgo, M.; James, C.; Jenkins, B.; Walsh, D. NCCTG Phase II trial of CCI-779 in recurrent glioblastoma multiforme (GBM). *Proc. Am. Soc. Clin. Oncol.* **2004**, *23*, Abstract # 107.

[16]·Margolin, K. A.; Longmate, J.; Baratta, T.; Synold, T.; Weber, J.; Gajewski, T.; Quirt, I.; Christensen, S.; Doroshow, J. H. CCI-779 in metastatic melanoma: A phase II trial of the California Cancer Consortium. *Proc. Am. Soc. Clin. Oncol.* **2004**, *23*, Abstract # 7523.

[17]·Atkins, M. B.; Hidalgo, M.; Stadler, W. A randomized double-blind phase 2 study of intravenous CCI-779 administered weekly to patients with advanced renal cell carcinoma. *Proc. Am. Soc. Clin. Oncol.* **2002**, Abstract # 36.

(Table 1). contd.....

Everolimus, RAD-001 (Novartis Pharmaceuticals)				
Pacey [18]	European, multicenter. n=8	Phase I study of the combination of RAD001 (IV, 20 mg/week) and Gemcitabine 600 mg, IV (delivered weekly for 3 of 4 weeks).	Advanced solid tumors	Hematological toxicity was excessive at dose/schedule selected.
Van Oosterom[19]	US, single institusion. N=12	Phase I/II study of the combination of Imatinib mesylate (Gleevec) and RAD-001.	Patients with GIST who became refractory to mono-therapy with Imatinib.	Drug-drug interaction noted (RAD-001 levels increased with concurrently therapy with Imatinib). 1 patient had prolonged stable disease. A radiological measure of activity noted (reduction in FDG-avidity with initiation of therapy).
O'Donnell[20]	Multicenter European study. n= 16	Phase I dose escalation study of oral weekly RAD-001. 4 dose levels tested: 5, 10, 20 and 30 mg.	Advanced solid tumors	Toxicity minimal. 20 mg/week appeared to inhibit target. Stabilization of disease in patients with HCC, NSCLC and fibrosarcoma.
AP23573 (Ariad Pharmaceuticals)				
Mita[21]	US trial, n=22	Phase I, dose escalation study. MTD determined as 18.75 mg/d IV delivered 5 days every 2 weeks.	Advanced solid tumors	DLTs were mucositis, rash, fatigue. Of the 16 patients who were evaluable, 2 had minor responses, and 6 had stable disease for varying periods. Pharmacodynamic evaluation (inhibition of 4EBP1 phosphorylation) revealed biological activity that lasted for up to 10 days after last infusion.

Abbreviations: DLT, Dose limiting toxicity; IFN, Interferon; IV, intravenous; MCL, Mantle cell lymphoma; MTD, maximal tolerated dose; NSCL ca, Non Small cell lung cancer; RCC, Renal cell cancer.

[18]·Pacey, S.; Rea, D.; Steven, N.; Brock, C.; Knowlton, N.; Shand, N.; Hazell, K.; Zoellner, U.; O'Donnell, A.; Judson, I. Results of a phase 1 clinical trial investigating a combination of the oral mTOR-inhibitor Everolimus (E, RAD001) and Gemcitabine (GEM) in patients (pts) with advanced cancers. *Proc. Am. Soc. Clin. Oncol*. **2004**, *23*. Abstract # 3120.

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[20]·O'Donnell, A.; Faivre, S.; Judson, I.; Delbado, C.; Brock, C.; Lane, H.; Shand, N.; Hazell, K.; Armand, J. P.; Raymond, E. A phase I study of the oral mTOR inhibitor RAD001 as monotherapy to identify the optimal biologically effective dose using toxicity, pharmacokinetic (PK) and pharmacodynamic (PD) endpoints in patients with solid tumours. *Proc. Am. Soc. Clin. Onc.* **2003**, *22*, pp. 200, Abstract # 803.

[21]·Mita, M. M.; Rowinsky, E. K.; Goldston, M. L.; Mita, A. C.; Chu, Q.; Syed, S.; Knowles, H. L.; Rivera, V. M.; Bedrosian, C. L.; Tolcher, A. W. Phase I, pharmacokinetic (PK), and pharmacodynamic (PD) study of AP23573, an mTOR Inhibitor, administered IV daily X 5 every other week in patients (pts) with refractory or advanced malignancies. *Proc. Am. Soc. Clin. Oncol*. **2004**, *23*, Abstract # 3076.

Temsirolimus, CCI-779 (Wyeth Pharmaceuticals)

The clinical development of CCI-779 is the most advanced of the 3 analogs of rapamycin. Both oral and intravenous formulations are available. CCI-779 is rapidly metabolized to rapamycin in the liver, and both the parent drug and the rapamycin metabolite are active in patients. Two administration schedules have been tested in Phase I studies for the IV formulation: daily injection for 5 days every other week or a single injection each week. The maximum tolerated dose of CCI-779 when delivered daily for 5 days using a bi-weekly schedule is 15 mg/m^2 in heavily pretreated patients and 19.1 mg/m^2 in minimally pretreated patients. When delivered intravenously using a weekly schedule, the maximally tolerated dose is >220 mg/week. However, correlative laboratory studies suggest that mTOR is effectively inhibited in surrogate normal tissues at doses as low as 10-15 mg/week on this weekly schedule. Inhibition of mTOR by rapamycin can persist for days following removal of drug in cell culture experiments, and given the long half-life of rapamycin in patients, it is likely that weekly bolus treatment with CCI-779 will provide for continuous inhibition of mTOR signaling in tumors throughout any given week of treatment. Data from a phase I trial conducted in patients with recurrent glioma suggests that the concurrent use of drugs that induce cytochrome P450 (such as anti-seizure medications), can lead to modest decreases in the levels of the active metabolite (rapamycin)[15]. Phase I trials evaluating the oral formulation of CCI-779 are ongoing and results should be reported soon.

Evidence of anti-tumor activity has been noted in patients with a variety of tumor types. Published results are available from a large phase II trial of CCI-779 in renal cell cancer, and preliminary results are available from phase II studies in patients with breast cancer and mantle cell lymphoma. In the renal cell cancer study, 110 patients were randomized to receive weekly IV infusions of 25, 75, or 250 mg [88]. One patient achieved a complete response, and 7 patients had partial responses with greater than 50% reduction in tumor size. Approximately half of patients had at least stabilization of disease for greater than 24 weeks. Survival for patients who were classified, on the basis of known prognostic factors, as being in an intermediate or

Table 2. Ongoing Phase II Clinical Trials with mTOR Inhibitors Registered Through the NCI

Investigator	Study title
Tersirolimus, CCI-779 (Wyeth Pharmaceuticals)	
North American Brain Tumor Consortium. (Protocol chair: Susan Chang)	Phase I/II Study of CCI-779 in Patients With Malignant Glioma
Eastern Cooperative Oncology Group (Protocol chair: Kishan J. Pandya)	Phase II Randomized Study of CCI-779 in Patients With Extensive stage small cell lung cancer
Ireland Cancer Center. Cleveland, OH. (Protocol Chair: Beth Overmoyer)	Phase II Randomized Study of Letrozole With or Without CCI-779 in Postmenopausal Women With Locally Advanced or Metastatic Breast Cancer
Jonsson Comprehensive Cancer Center, UCLA. (Protocol Chair: Charles Sawyers)	Phase II Randomized Study of Neoadjuvant CCI-779 Followed By Radical Prostatectomy in Patients With Newly Diagnosed Prostate Cancer Who Have a High Risk of Relapse
NCI-Canada. (Protocol Chair: Amit Oza)	Phase II Study of CCI-779 in Patients With Metastatic or Locally Advanced Recurrent Endometrial Cancer
Jonsson Comprehensive Cancer Center, UCLA. (Protocol Chair: Robert Figlin)	Phase III Randomized Study of Interferon alfa Versus CCI-779 Versus Interferon alfa and CCI-779 in Patients With Poor Prognosis Stage IV or Recurrent Renal Cell Carcinoma
North Central Cancer Treatment Group (Protocol chair: Thomas E. Witzig)	Phase II Study of CCI-779 in Patients With Previously Treated Mantle Cell Non-Hodgkin's Lymphoma
University of Texas - MD Anderson Cancer Center (Protocol chairs: Henry Qinghua Xiong, and James L. Abbruzzese)	Phase II Study of CCI-779 in Patients With Locally Advanced or Metastatic Pancreatic Cancer
Arthur G. James Cancer Hospital-Ohio State University (Protocol chair: Sherif Farag)	Phase II Study of CCI-779 in Patients With Relapsed or Refractory Multiple Myeloma
University of Texas - MD Anderson Cancer Center (Protocol chair: Francis Giles)	Phase II Study of CCI-779 in Patients With Relapsed or Refractory Acute Myeloid Leukemia, Acute Lymphoblastic Leukemia, Myelodysplastic Syndromes, or Chronic Myelogenous Leukemia in Blastic Phase
University of Chicago Cancer Research Center (Protocol chair: Koen Van Besien)	Phase II Study of CCI-779 in Patients With Recurrent or Refractory B-Cell Non-Hodgkin's Lymphoma or Chronic Lymphocytic Leukemia
North Central Cancer Treatment Group (Protocol chair: Alex Adjei)	Phase II Study of CCI-779 in Patients With Stage IIIB (With Pleural Effusion) or IV Non-Small Cell Lung Cancer
North Central Cancer Treatment Group (Protocol Chair: Evanthia Galanis)	Phase II Study of CCI-779 in Patients With Recurrent Glioblastoma Multiforme
Everolimus, RAD-001 (Novartis Pharmaceuticals)	
Memorial Sloan-Kettering Cancer Center (Protocol chairs: Howard Scher, Neal Rosen and Lauren Abrey)	Phase I/II Study of Everolimus and Gefitinib in Patients With Progressive Glioblastoma Multiforme or Progressive Castrate Metastatic Prostate Cancer

poor risk group, is approximately 1.7-fold higher than historical controls treated with standard interferon therapy. Additional enrollment at the MTD also demonstrated a high rate of clinical benefit (stable disease and partial response)[12]. Based on these data, a phase III study in renal cell cancer is now underway (see Table 2). CCI-779 also demonstrated preliminary clinical activity for patients with advanced stage breast cancer who were refractory to prior chemotherapy[13]. In this study, PR rates were 12% among patients receiving either 75 or 250 mg CCI-779 IV each week. Promising results for the use of CCI-779 have also come from its use in patients with mantle cell lymphoma. In a phase II clinical trial run through the North Central Cancer Treatment Group, Witzig *et al.* noted a response rate of 44% with weekly doses of up to 250 mg CCI-779 in the 18 heavily pretreated patients with mantle cell lymphoma. This trial has now accrued a total of 35 patients, and a subsequent cohort of patients will be enrolled onto this trial and treated at a lower dose of 25 mg/week to evaluate whether equal efficacy can be achieved at this low dose[14]. In an NCCTG phase 2 clinical trial in recurrent glioblastoma multiforme, radiographic evidence of anti-tumor effects with CCI-779 alone was seen in 10 of 31 patients[15]. In contrast, monotherapy with CCI-779 in patients with metastatic melanoma did not significantly affect disease progression[16]. In addition to the trials discussed here, several phase II clinical trials using CCI-779 are currently ongoing (Table 2) in a variety of tumor types.

CCI-779 is relatively well tolerated. Acute hypersensitivity reactions have been noted soon after the start of infusions; these resolve if the infusion is stopped. To prevent this reaction, patients are routinely pre-medicated with antihistamines prior to infusion. Similar to effects observed with rapamycin in other clinical settings, thrombocytopenia, hyperlipidemia, leukopenia, and diarrhea are commonly observed in the treatment of cancer patients. Other toxicities noted include acne like rash, stomatitis, liver enzyme abnormalities, hyperglycemia, asthenia, and nausea. Pneumonitis is a rare side effect of CCI-779 therapy, and is characterized by dyspnea as well as cough and fever,

and these typically resolve following discontinuation of drug.

Several clinical trials using the combination of CCI-779 with other anti-cancer agents also are underway. At Mayo Clinic, a phase I study of CCI-779 and EKB-569 (an oral EGFR tyrosine kinase inhibitor; Wyeth Pharmaceuticals) in solid tumors, and a phase I study of rapamycin, cisplatin and radiation in lung cancer are currently accruing patients. Such combination regimes require careful evaluation of potential toxicities in Phase I studies. As an example, the combination of CCI-779 with weekly 5-fluorouracil and leukovorin, as piloted by others, was abandoned due to overlapping gastrointestinal toxicity with the combination [89]. The results of other such combination studies using CCI-779 are eagerly awaited.

Everolimus; RAD-001 (Novartis)

RAD-001 (40-*O*-(2-hydroxyethyl)-rapamycin) is an analogue of rapamycin that is currently being tested both as an immunosuppressive and anti-cancer agent. The hydroxyethyl group increases the polarity and solubility of the compound, which allows for improved oral absorption. This drug is currently undergoing phase III testing in renal transplant patients, while phase II clinical trials are also underway in several tumor types.

RAD-001 is rapidly absorbed after oral administration. The elimination half-life is 30 hours. Absorption is retarded by fatty foods. The drug is mainly (80%) bound to erythrocytes in the blood. Similar to CCI-779 and rapamycin, RAD-001 is metabolized by CYP3A4 in the liver. Therefore, there is a significant potential for interactions with other agents that are substrates for CYP3A4 (such as anti-convulsant drugs, anti-fungals, and grapefruit juice). The oral absorption of RAD-001, CCI-779 and rapamycin is at least partially dependent on the activity of P-glycoprotein (Pgp) within the gut mucosa, and intrinsic differences in Pgp activity may account for a significant proportion of inter-patient variability in drug absorption. In phase I trials, weekly doses of up to 30 mg were well tolerated. The main toxic events noted in early clinical studies include headaches, fatigue, mucositis, elevations of serum lipids and oral aphthous ulcers[20]. The phase II dose has been determined at 20 to 30 mg per week.

Two trials testing RAD-001 in combination with other agents have recently been reported. In one of these, RAD-001 was combined with Imatinib mesylate (Gleevec) to treat patients with gastrointestinal stromal tumors (GIST) who had become refractory to single agent therapy with Imatinib. With combination therapy, there appeared to be a restoration of sensitivity to Imatinib in some patients[19]. While these results are preliminary, they are exciting in that they suggest that combinations of signal transduction inhibitors may be useful to overcome drug resistance. In contrast, combination of RAD-001 (weekly) and gemcitabine (weekly, 3 weeks out of every 4) is associated with excessive hematological toxicity[18]. Thus, as with CCI-779, combination studies require careful evaluation of toxicities, and the results of multiple other trials currently testing combination therapies with mTOR inhibitors will provide important directions for future clinical testing.

AP23573 (Ariad Pharmaceuticals)

AP23573 is a rapamycin analogue that, unlike CCI-779 and RAD-001, is not a pro-drug of rapamycin. Preliminary results of a phase I study have been reported at the time of this manuscript. AP23573 is administered intravenously; the MTD has been determined to be 18.75 mg/d, delivered 5 days out of every 2 weeks. The toxicity profile of this agent appears to be similar to that of other mTOR inhibitors (mucositis, rash, fatigue, and myelosuppression). Patients with a variety of tumor types were enrolled in this study, and preliminary evidence of efficacy was noted in a few patients[21]. Further data on this agent is awaited.

RAPAMYCIN METABOLISM

Drug-drug interactions can be a significant factor that may influence serum and consequently tumor drug levels. Rapamycin is primarily metabolized by cytochrome P450 3A4 (CYP3A4) iso-enzyme to inactive metabolites, and increased enzymatic activity by concomitant administration of rapamycin with other CYP3A4-inducing drugs can significantly decrease the achievable rapamycin levels in serum and tumor. Common CYP3A4-inducing drugs used in oncology patients include anti-fungal agents such as ketoconazole and anti-seizure medications such as phenytoin and phenobarbital. In fact, in a Phase I clinical trial run at Mayo Clinic, patients with high-grade gliomas receiving enzyme-inducing anti-convulsants required a 30% higher dose of the rapamycin analog CCI-779 compared with patients not on enzyme-inducing anti-convulsants[11]. This issue was further studied in a phase II trial of CCI-779 in patients with recurrent GBM, several of whom were concurrently on CYP3A4 inducing anti-convulsants. The pharmacokinetic data of patients who were on CYP3A4 inducers was compared to data from a different study of CCI-779 in renal cell cancer patients (who were not taking CYP3A4 inducers). The area under the curve (AUC) of CCI-779 was comparable between the 2 studies, however the AUC of rapamycin, which is the primary active metabolite of CCI-779, was significantly reduced (by more than 50%) in patients on CYP3A4 inducers. Thus, the interaction between CYP3A4-inducing agents and CCI-779 may be clinically relevant, especially when low doses of CCI-779 are used[15]. Another potential drug interaction is with grapefruit juice; which is a well-known inhibitor of the P450 enzyme system and can result in decreased metabolism of rapamycin and its analogs. Thus, drug-drug interactions can significantly affect serum levels of rapamycin and its analogs, and specific attention should be paid to this issue in designing clinical trials using these drugs.

CONCLUSIONS

Rapamycin and its analogs are versatile drugs with proven efficacy in cardiovascular and transplant medicine and with promising results in early cancer clinical trials. In specific tumor types, a select minority of patients likely will benefit from mono-therapy. The challenge for the future will be to dissect further the molecular signaling pathways modulated by rapamycin in order to appreciate fully the

molecular mechanisms underpinning sensitivity or resistance to mTOR inhibition. This understanding will provide insight into rational combinations of mTOR inhibitors with classic cytotoxic agents, radiation and other molecularly targeted therapies.

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