# Recent developments in targeting the mammalian target of rapamycin (mTOR) kinase pathway

Piotr Smolewski

**The mammalian target of rapamycin (mTOR) is a threonine kinase involved in intracellular pro-survival signaling. Its activation leads to progression from the $G_1$ to S phase of the cell cycle. Constitutive activation of the mTOR-related messengers, including phosphatidylinositol 3-kinase, Akt kinase, ribosomal p70S6 kinase and eukaryotic translation initiation factor 4E-binding protein kinase, was found in numerous malignancies. Recent data indicate that the mTOR kinase pathway can be an attractive target for anti-cancer drug development. A well-known mTOR inhibitor is rapamycin (RAPA), previously applied as an immunosuppressive agent in transplant studies. Recently, analogs of RAPA, such as CCI-779, RAD001 and AP23573, have been developed. All of those agents are currently being tested in patients with solid or hematological tumors in several clinical trials. This review presents recent developments in targeting the mTOR kinase pathway. *Anti-Cancer Drugs* 17:487–494 © 2006 Lippincott Williams & Wilkins.**

**Anti-Cancer Drugs** 2006, **17**:487–494

**Keywords: cancer, mammalian target of rapamycin, mTOR, mTOR inhibitor, mTOR kinase, leukemia, treatment**

Department of Hematology, Medical University of Lodz, Lodz, Poland.

Correspondence to P. Smolewski, Department of Hematology, Medical University of Lodz, Copernicus Memorial Hospital, Ciolkowskiego 2, 93-510 Lodz, Poland. Tel: +48 42 689-51-91; fax: +48 42 689-51-92; e-mail: piotr_smolewski@wp.pl

Sponsorship: This work was supported by grant 507-11-248 from the Ministry of Health (KBN), Warsaw, Poland.

**Received** 23 January 2006 **Accepted** 28 January 2006

## Mammalian target of rapamycin (mTOR) kinase as a target for anti-cancer therapy

### The structure and activation of mTOR

mTOR is a serine/threonine-specific protein kinase, downstream of the phosphatidylinositol 3-kinase (PI3K)/Akt (protein kinase B) pathway. It is also known as sirolimus effector protein (SEP), rapamycin-associated protein (FRAP), FK506-binding protein (FKBP12) or rapamycin target (RAPT1). The structure of mTOR kinase, highly conserved during evolution, is shown in Fig. 1. mTOR consists of up to 20 tandemly repeated 'HEAT' motifs at the N-terminus, including Huntington, elongation factor 3, the A subunit of protein phosphatase 2A (PP2A) and TOR. At the C-terminus there are FRAP-ataxia-teleangiectasia mutated, transformation/transcription domain-associated protein (FAT), catalytic kinase and FKPB12–rapamycin binding (FRB) domains [1–3]. In the cytoplasm of mammalian cells mTOR kinase is co-localized with three peptides: regulatory-associated protein of mTOR (raptor), GbetaL and mLST8. Raptor is a scaffold protein, presenting substrates to the mTOR kinase domain for optimal phosphorylation of downstream targets [4]. GbetaL binds to the catalytic kinase domain of mTOR and stabilizes the raptor–mTOR interaction, increasing activity of mTOR kinase. Under nutrient-deplete conditions raptor inhibits its function and reduces the impact of GbetaL [5]. The role of mLST8 remains unclear.

mTOR kinase is involved in the regulation of cell growth and proliferation, controlling these processes at the translational level (Fig. 2). There are two downstream messengers of mTOR: ribosomal p70S6 kinase (p70S6K) and eukaryotic translation initiation factor 4E (eIF4E)-binding protein (4E-BP1). Phosphorylation of S6K1 enhances translation of mRNA that carry a 5′-terminal oligopyrimidine tract (TOP). Activation of 4E-BP1 results in dissociation from the RNA cap-binding protein eIF4E and formation of the eIF4F complex. The complex, consisting of the cap-binding protein eIF4E, the scaffold protein eIF4G and the RNA helicase eIF4A, enhances cap-dependent protein translation [2].

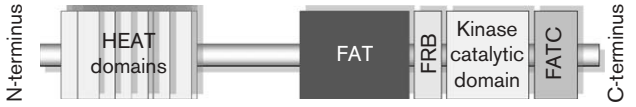
In mammalian cells, activation of mTOR signaling to S6K1 and 4E-BP1 depends on signal transmission through the phosphatidylinositol 3-kinase (PI3K)/Akt pathway. PI3K and Akt lie upstream of mTOR, and are activated by growth factors or mitogenic stimuli, such as cytokines (Fig. 2). Thus, mTOR kinase can be defined as a key element of the PI3K/Akt signaling pathway.

### Aberrant mTOR signaling in malignant disorders

Aberrantly activated mTOR signaling plays an essential role in the growth of different types of tumors, leading to an uncontrolled proliferation of malignant clones. Activation of the PI3K/Akt pathway allows T and B cell proliferation. Increased activity of PI3K in transgenic mice extends T cell survival *in vivo* [6], affects T cell homeostasis and contributes to the early development of T cell lymphomas or autoimmunological disorders [7]. p65-PI3K, a truncation mutant of the regulatory PI3K subunit, was isolated from thymic lymphoma [8].

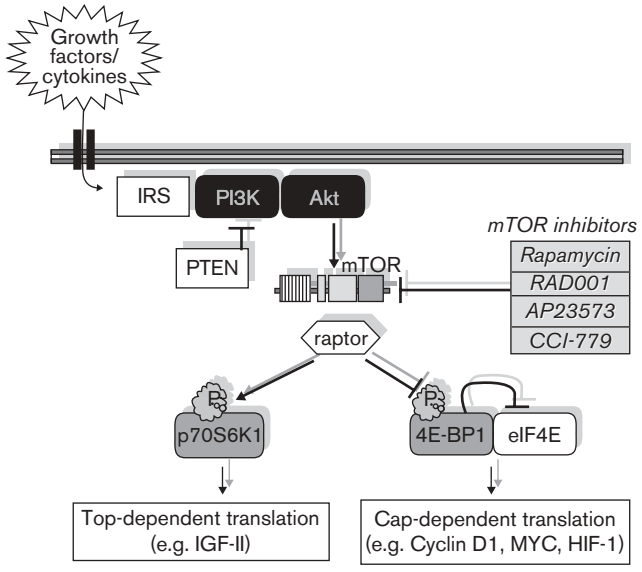
0959-4973 

**Fig. 1**




Structure of mTOR kinase. mTOR consists of up to 20 tandemly repeated HEAT motifs at the N-terminus, which include Huntington, elongation factor 3, PP2A and TOR. From the C-terminus there are the FAT domain, catalytic kinase domain and FRB domain (see main text). Structurally, the mTOR kinase is a member of a big PI3K-related kinase family; the catalytic kinase domain in the C-terminus is highly homologous to the lipid kinase domain of PI3K.

**Fig. 2**




Simplified scheme of the mTOR signaling pathway. PI3K, phosphatidylinositol 3-kinase; mTOR, mammalian target of rapamycin; raptor, regulatory-associated protein of mTOR; ASK1, apoptosis signal-regulating kinase 1; p70 S6K1, ribosomal p70 S6 kinase 1; eIF4E, eukaryotic translation initiation factor 4E; 4E-BP1, eIF4E-binding protein 1; IRS, insulin-receptor substrates; IGF-II, insulin growth factor II; P, phosphorylation.

Several mutations upstream and downstream of mTOR kinase were detected in malignant cells. These include amplification of a catalytic subunit of PI3K, loss of PTEN (phosphatase and tensin homolog deleted on chromosome 10), a tumor-suppressor gene which downregulates expression of either PI3K or Akt kinases, amplification of Akt, as well as overexpression or amplification of eIF4E or S6K1 [9–16].

Somatic PTEN mutations appear in numerous malignancies. They were most frequently described in glioblastomas (approximately 70% of patients). Moreover, PTEN mutations were found in 25–60% of tumor cells in lung, skin, thyroid, prostate or endometrial cancers [9]. Loss of PTEN leads to constitutive activation of Akt and an increased activity of mTOR, which may sensitize cells to mTOR inhibitors. Overexpression of Akt was detected in more than 50% of colon adenomas and colorectal cancers. It may indicate a role for this kinase as a protooncogene during tumorigenesis [10]. Recently, increased activity of Akt kinase was found in both human and mouse mesotheliomas [17]. Moreover, overexpression or hyperactivation of Akt correlating with sensitivity to treatment has been found in non-small cell lung and breast cancer cells [11,12].

Proteins lying downstream of mTOR are also altered in several malignancies. Namely, overexpression of eIF4E appeared to be a common event in solid tumors, especially in breast, colon and neck cancers [13]. Amplification of the eIF4E gene and eIF4E protein overexpression is associated with progression of those cancers [14]. High eIF4E levels correlated with a higher rate of relapses and cancer-related deaths. In contrast to eIF4E, overexpression of 4EBP inhibits cell proliferation. 4EBP-1 expression levels correlate inversely with tumor progression [15]. Moreover, activation of the mTOR/p70S6K pathway was found in a pancreatic cancer cell line [18,19].

Similarly, various data indicate a crucial role of sustained hyperactivation of the PI3K/Akt/mTOR pathway in the development of hematological tumors. Permanently overexpressed PI3K/Akt kinases as well as increased levels of eIF4E protein were found in malignant B cell proliferations, including B cell non-Hodgkin's lymphomas (NHL) [20,21] or multiple myeloma [22,23]. Activation of mTOR signaling contributes to tumor cell survival in ALK-positive anaplastic large cell lymphoma [24]. Inhibition of the Akt/mTOR pathway results in cell cycle arrest and apoptosis in mantle cell lymphoma (MCL) cells [25]. Overexpression of eIF4E and subsequent deregulation of eIF4E-dependent mRNA transport can be responsible for inhibition of granulocyte and monocyte differentiation. This may contribute to leukemogenesis in acute myeloid leukemia (AML) or chronic myelogenous leukemia (CML) [26]. The constitutive activation of PI3K/Akt is necessary for the survival of AML tumor cells [27] and is associated with a poor prognosis in AML patients [28,29]. In turn, inhibition of this pathway leads to cell cycle arrest and apoptosis of AML cells [30]. Similarly, sustained activation of the PI3K kinase pathway is probably crucial for escape from apoptosis and accumulation of mature $CD5^+/CD19^+$ lymphocytes in B cell chronic lymphocytic leukemia (B-CLL). Inhibition of this pathway increases dexamethasone- and fludarabine-induced ex-vivo apoptosis of B-CLL cells [31,32].

### Inhibition of the mTOR kinase pathway

Thus, targeting of the mTOR kinase may lead to cell growth inhibition, which induces an anti-tumor effect in several types of malignant disorders. Since mTOR is a downstream component of PI3K/Akt signaling, tumor cells with PI3K/Akt activation or harboring mutations of this pathway should be more prone to mTOR-targeting treatment. Therefore, a significant effort has been made in recent years to define and develop specific inhibitors of this pathway. One of them is an agent used for many years for other indications. This is rapamycin (RAPA, sirolimus), a macrolide antibiotic discovered as an anti-fungal agent in the 1970s [33]. In 1990, RAPA was approved by the FDA as an immunosuppressive agent for use after renal transplantation (Rapamune; Wyeth-Ayerst, Collegeville, Pennsylvania, USA). Furthermore, an anti-proliferative and anti-tumor activity of RAPA has been discovered. More recently, three analogs of RAPA with superior pharmacokinetic and biological properties have been synthesized and introduced to clinical trials in different malignant disorders. They are CCI-779 (cell cycle inhibitor-779, temsirolimus; Wyeth; a soluble ester analog of sirolimus [34]), RAD001 (40-*O*-[2-hydroxyethyl]-rapamycin, everolimus; Novartis Pharma, Basel, Switzerland; an orally bioavaliable derivative of RAPA previously approved also as an immunosuppressant agent) and AP23573 (Ariad Pharmaceuticals, Cambridge, Massachusetts, USA; a non-pro-drug RAPA analog).

In general, inhibition of mTOR kinase blocks the signal to two downstream messengers, S6K1 and 4E-BP1, and prevents translation of key mRNAs required for cell cycle progression from the $G_1$ to S phase [35,36]. As a consequence, it prevents activation of cyclin-dependent kinase (CDK), accelerates the turnover of cyclin D1, inhibits phosphorylation of retinoblastoma protein (pRB) and leads to a deficiency of active CDK4/cyclin D1 complexes. All these events lead to $G_1$ arrest [35]. Another mode of action of mTOR inhibitors, which increases its anti-tumor activity *in vivo*, is an anti-angiogenic effect related to decreased production of vascular endothelial growth factor (VEGF) and reduced response of endothelial cells to VEGF stimulation [37–39].

## Targeting mTOR: clinical application of mTOR inhibitors

### RAPA

RAPA is a product of *Streptomyces hygroscopicus*, a bacteria isolated from a soil sample from Easter Island (*Rapa Nui*). Its molecule is a mixture of two conformational isomers due to *cis–trans* rotation about an amidic bond in its 31-membered macrolide ring [1]. Formation of the active complex, consisting of RAPA and the FK506-binding protein 12 (FKBP12), mediates an anti-proliferative effect via inhibiting mTOR kinase.

The main effect of RAPA is induction of growth arrest in the $G_1$ phase of the cell cycle. In higher concentrations, however, RAPA exerts a pro-apoptotic effect in several types of tumor cells [25,30,40,41]. RAPA was also shown to induce apoptosis in non-malignant B and T lymphocytes as well as in 'double-null' lymphocytes in the mouse model of autoimmune lymphoproliferative syndrome [42].

Due to problems with RAPA stability, development of its parenteral formulation has failed. Therefore, for several years RAPA has not been introduced into clinical trials in oncology. Only recently have Mayerhofer *et al.* tested RAPA in a pilot study as a second-line treatment in seven imatinib-resistant patients with CML [43]. The drug was administrated orally at a dose of 2 mg for 14 consecutive days, with dose adjustment to maintain the serum concentration at 10–20 ng/ml. The authors observed a major decrease in leukocyte numbers in three patients and minor transient responses in two patients. Two patients did not respond to RAPA. No significant cytogenetic improvement was observed. The drug was well tolerated and no severe side-effects were reported.

### CCI-779

CCI-779 showed high anti-tumor activity, inhibiting malignant cell growth in a wide range of cancer types either *in vitro* or in animal models. This includes glioblastoma, melanoma, and prostate, breast, renal cell and pancreatic cancers [44]. Treatment of nude mouse xenografts with CCI-779, however, resulted in growth inhibition and a significant delay of tumor progression rather than in regression of tumor mass [45]. Cytotoxic activity of CCI-779, however, has been shown in human neuroectodermal tumor models [46].

#### *Determining doses, toxicity and pharmacodynamic effects in phase I clinical trials for solid tumors*

Based on the preclinical data, CCI-779 has been introduced into first clinical trials in patients with solid tumors such as renal, breast and lung cancers. As a result, two schedules of CCI-779 administration, once weekly [47] or 5 days every 2 weeks infusions [48], were established. The main dose-limiting, mild and reversible toxicities of CCI-779 treatment were mucositis and skin reactions [47] or hypocalcemia, liver tests elevation and thrombocytopenia [48]. No immunosuppressive effects were observed. Recently, the pharmacokinetic parameters of CCI-779 relating to safety and clinical activity have been carefully estimated in patients with advanced renal cancer [49]. Assessment of p70S6K expression in peripheral blood mononuclear cells (PBMCs) was described as a good parameter determining the pharmacodynamic effect of CCI-779 [50].

#### *Renal cell carcinoma*

Another randomized phase I study comparing CCI-779 and interferon-$\alpha$ used alone or in combination in patients

with advanced renal cell carcinoma has been performed, with 13% partial remissions (PRs) and 71% stable diseases (SDs) after CCI-779 [51]. A phase II clinical trial with CCI-779 alone in advanced refractory renal cell carcinoma has also been conducted [52]. Among the 111 patients assigned to the trial, 6% of patients obtained PRs and 26% SDs, with the median time to tumor progression 5.8 months and median survival 15 months.

#### *Lung cancer*

A preliminary report from a phase II study showed significant activity of CCI-779 in 87 patients with small cell lung cancer in remission after induction chemotherapy [53]. The median survival after two infusions of both CCI-779 doses, 25 (arm A) and 250 mg (arm B) (16.5 and 22.9 months, respectively), was superior to median survival in historical groups of patients treated with chemotherapy only (8.9 months). Interestingly, the rate of grade 3 and 4 drug-related toxicities was similar in arms A and B (21 versus 26 patients, respectively).

#### *Breast cancer*

One hundred and nine patients with advanced and metastatic breast cancer were enrolled to another phase II clinical trial with CCI-779 [54]. Clinical benefit was observed in almost half of the patients, with an overall response rate (ORR) of 9.2% (10 patients with PRs), with efficacy for two doses, 75 and 250 mg, administrated as a 30-min i.v. infusion. Median time to disease progression was 12 weeks. Both doses had tolerable safety profiles. Mucositis, maculopapular rash and nausea were the most common drug-related side-effects of all grades.

#### *Glioblastoma multiforme*

Two phase II clinical trials of CCI-779 in recurrent glioblastoma multiforme were performed [55,56]. In both studies the basic CCI-779 dose schedule was 250 mg i.v. weekly. Galanis *et al.* observed evidence of radiological improvement in 20 out of 65 (36%) examined patients [55]. This response was associated with significantly longer progression-free survival and median overall survival when compared to non-responsive patients. In the most recent, updated report, development of a grade 2 hyperlipidemia on treatment appeared to be a surrogate marker of radiographic improvement [57]. High levels of phosphorylated p70S6K, determined by immunohistochemistry in baseline tumor samples, allowed a better definition of the responsive patient population. In another study, Chang *et al.* observed a rapid disease progression after initial stabilization, which had been initially achieved in approximately 50% of patients [56]. Among the 43 eligible patients, only one was free of progression at 6 months of observation. Thus, CCI-779 seems not to be efficient enough to warrant further trials in recurrent glioblastoma multiforme.

#### *Malignant melanoma*

CCI-779 was also tested in patients with metastatic melanoma; however, no significant effectiveness was observed [58]. Only one out of 33 patients treated achieved a PR lasting 2 months. The median time to disease progression and overall survival were 10 weeks and 5 months, respectively.

#### *Hematological malignancies*

CCI-779 is also being introduced into clinical trials for patients with hematological tumors. Namely, in a phase II clinical trial, 35 patients with relapsed and refractory MCL have been treated with a weekly dose of 250 mg CCI-779 [59]. The ORR was 38%, including one CR and 12 PRs. Twenty six patients progressed, with a median time to progression of 6.5 months. Thrombocytopenia was frequently observed and was dose limiting. Recently, the same group tested whether lower doses of CCI-779 (25 mg) could produce a similar ORR [60]. As was shown in the interim analysis, among the 13 eligible patients, four achieved PRs and another four patients had SDs. So far, no grade 4 toxicities have been noted, and one each grade 3 neutropenia and thrombocytopenia have been observed.

Moreover, CCI-779 has been evaluated in seven patients with advanced leukemias. Five patients were diagnosed with AML, one with myeloid blast crisis in the course of CML and one with acute lymphocytic leukemia (ALL) [61]. No patient achieved a CR. The transient reduction of blasts was observed in a patient with heavily pretreated ALL. Six out of seven patients have discontinued treatment, including four who had progressive disease (PD).

### RAD001

#### *Phase I clinical trials in solid tumors: establishing doses, a toxicity profile and biological effectiveness*

The phase I dose-escalation study with orally administered RAD001 in patients with solid tumors showed that weekly doses of 5–30 mg were well tolerated. The moderate side-effects included grade 1 or 2 fatigue, anorexia, rash, headache, mucositis or hyperlipidemia [62]. The weekly doses of 20–30 mg have been established as sufficient to achieve inhibition of the signal transduction pathway. Inhibition of S6K1 in PBMCs and has been suggested as a valuable surrogate marker [63].

Thirty-three patients with advanced solid tumors were treated in a phase I study reported by Tabernero *et al.* [64]. Grade 3 dose-limiting toxicity (DLT) occurred in five patients comprising stomatitis, neutropenia and hyperglycemia. One patient achieved PR (colon cancer) and two patients SDs lasting for more than 4 months (renal and breast cancer). The doses and schedules studied (weekly 20, 50 and 70 mg or daily 5 and 10 mg RAD001) resulted in an inhibition of mTOR signaling in

tumor cells. A dose-related decrease in 4E-BP1 expression and an increase in phosphorylated Akt levels were observed, with maximal effect at the doses of 10 mg daily and more than 50 mg weekly. According to the authors, a dose of 10 mg daily can be recommended for further phase II–III development with RAD001 as a single agent.

Lerut *et al.* evaluated the molecular pharmacodynamics and a dosage schedule of RAD001 in 15 patients with newly diagnosed localized prostate cancer [65]. The patients were treated for 4 weeks prior to radical prostatectomy. RAD001 was administered as either weekly (30, 50 or 70 mg) or daily (5 or 10 mg) doses. Changes in expression of phosphorylated S6K, Akt and 4E-BP1 were assessed using immunohistochemistry. RAD001 showed good tolerability in at the doses tested.

#### *Currently open RAD001 clinical trials for patients with solid tumors*

RAD001 is being evaluated in a phase II clinical trial in patients with progressive or recurrent endometrial cancer. This type of malignancy is characterized by deactivating mutations of PTEN in up to 50% of cases. The study is currently recruiting patients (NCT00087685 trial). Moreover, the phase I/II trial of RAD001 in children with recurrent or refractory solid tumors and brain tumors, with phase II limited to recurrent or refractory rhabdomyosarcomas and non-rabdomyosarcomatous soft tissue sarcomas, is currently active (recurrentmalignancies@stjude.org).

#### *Hematological malignancies*

Several reports confirmed the anti-tumor activity of RAD001 in hematopoietic tumor cells. In Epstein–Barr virus (EBV)-transformed lymphoblastoid B cell lines and in a mouse model, RAD001 was found to inhibit cell growth, induce accumulation in the $G_1$ phase of the cell cycle and also to increase the apoptotic rate of the cells [66]. RAD001 has shown effectiveness in post-transplant lymphoproliferative disorders caused by EBV transformation [67].

A phase I/II study with RAD001 in patients with advanced hematologic malignancies has already been closed (NCT00081874). Patients with relapsed/refractory AML, ALL, CML in blastic phase, B-CLL and T cell leukemia or MCL were enrolled. As mTOR-related messengers are upregulated in hematological malignancies, the new mTOR inhibitor is a promising agent in these disorders. According to the available initial results, RAD001 showed minor effectiveness, with two out of the 14 eligible patients with B-CLL having 33 and 65% lymphadenopathy, and one patient with myelodysplastic syndrome (MDS) showing decreased transfusion requirements.

### AP23573

#### *Solid tumors: assessment of pharmacodynamic effects, administration schedule and tumor response in preclinical and phase I clinical trials*

AP23573 has showed anti-proliferative activity *in vitro* in a variety of PTEN-deficient tumor cell lines, including glioblastoma, and prostate, breast, pancreatic, lung and colon cancers [36]. AP23573 completely inhibited in-vivo activity of mTOR in PBMCs as measured by decreased phosphorylation levels of mTOR messenger proteins. In the phase I study by Rivera *et al.*, 32 patients with different malignancies received AP23573 in doses ranging from 3 to 28 mg [68]. Inhibition of S6K phosphorylation was assessed in skin biopsies as a surrogate for the tumor. AP23573 exerted a pharmacodynamic effect in more than 85% of examined patients, showing a decrease of phosphorylated S6K expression in the skin, with the stable expression of total p70S6K, which confirmed good tissue penetration of the drug.

Recently, Desai *et al.* described an AP23573 pharmacokinetic model for optimization of AP23573 dosage [69]. The study was based on two dose-escalation trials evaluating the drug as a 30-min infusion i.v. administered either once weekly or once daily for 5 days. AP23573 appeared to exhibit non-linear pharmacodynamic behavior. It was consistent with saturation of the red blood cell compartment, which contains an abundance of AP23573-binding FKBP protein.

AP23573 has been tested in two phase I studies in patients with refractory or advanced solid tumors. Mita *et al.* administered AP23573 daily for 5 days every 2 weeks for 4-week cycles [70]. Among the eight eligible patients, one PR has been achieved in a patient with metastatic renal cell cancer. One patient with metastatic sarcoma had SD lasting more than 6 months. In the trial conducted by Desai *et al.*, AP23573 was administered weekly on 4-week cycles. Among five eligible patients with medullary thyroid cancer, one patient had SD for over 2 months [71]. The drug was generally well tolerated. The severe DLT was oral mucositis.

Based on the preclinical studies that described 40% growth reduction in glioblastoma cells treated with AP23573, an additional phase I trial has been evaluating patients with relapsed or refractory glioblastoma. Antiproliferative activity was observed in both cells overexpressing epidermal growth factor receptor (EGFR) gene and cells without EGFR overexpression [72,73].

#### *Sarcomas: phase II clinical trials*

Preliminary data from phase II study of 25 patients with advanced sarcomas, including bone sarcomas, leiomyosarcoma or liposarcoma, has recently been reported [74]. AP23573 was administered at doses used in the phase I trial [71]. [$^{18}$F]2-Fluoro-2-deoxy-D-glucose positron

emission tomography (PET) performed before and 5 days after starting AP23573 dosing was used for pharmacodynamic and biomarker endpoints in 23 out of 25 treated patients. Among them, a decrease in overall uptake on PET imaging has been observed in 18 patients during the first course of AP23573. Improvement in disease-related clinical symptoms was observed in 13 patients. The main drug-related side-effects were mucositis, anemia, thrombocytopenia and skin rash. Most of those events were mild or moderate in severity. Of note, one patient with advanced abdominal liposarcoma died of rapidly progressing disease after receiving one course of AP23573. The overall effects of treatment, however, warranted continuation of the study.

PET appeared to be valuable in an initial evaluation of response to AP23573 treatment. Sankhala *et al.* described the feasibility of early response evaluation by PET imaging in 25 patients with sarcoma [75]. In five out of nine responding patients the PET evidence of partial metabolic response correlated with clinical improvement as demonstrated by reduced symptoms of pain, shortness of breath and cough.

#### Hematological malignancies: phase II clinical trials

Most recently, results of the phase II clinical trial with AP23573 in 51 patients with relapsed or refractory hematological malignancies were reported [76]. The study group consisted of 35 patients with acute leukemias, nine with CLL and eight with lymphomas (six MCL and two T cell lymphoma patients). Among all 46 evaluable patients, an anti-tumor activity of AP23573 determined as at least SD was shown in 19 (41%) cases. The drug given at the dose of 12.5 mg i.v. daily for 5 consecutive days showed good tolerability. The acceptable side-effect profile included mild/moderate diarrhea, mucositis, skin reactions, hyponatremia, hypokalemia, hypertriglyceridemia and neutropenia complicated with pneumonia, pleural effusion or sepsis.

Another phase II clinical trial showed safety and efficacy of AP23573 treatment (12.5 mg i.v. daily $\times$ 5 days, every 2 weeks) in relapsed or refractory hematological malignancies [77]. In this preliminary report, 11 patients (six AML, three agnogenic myeloid metaplasia, one ALL and one MDS) were eligible for the end-of-first cycle response assessment. Among them, three achieved minor hematological response; three other patients had SDs and four PDs. Treatment-related adverse events included hypertriglyceridemia, neutropenic sepsis and mucositis. Patient enrollment to this study is continuing.

## Conclusions

The mTOR kinase pathway is a crucial for cell survival and therefore it has been explored as an attractive target for anti-tumor treatment. Special benefits of such treatment can be expected in tumors with constitutionally activated elements of the PI3K/Akt/mTOR pathway. Clinical trials showed that mTOR inhibitors, including RAPA analogs CCI-779, RAD001 and AP23573, were well tolerated and can produce SDs or even substantial responses in relapsed or resistant solid tumors and some hematological malignancies. Several clinical trials are underway and it will require time to assess any real benefit of this novel therapeutic approach.

## References


1 Mita MM, Mita A, Rowinsky EK. The molecular target of rapamycin (mTOR) as a therapeutic target against cancer. *Cancer Biol Ther* 2003; **2**: 169–177.

2 Bjornsti MA, Houghton PJ. The TOR pathway: a target for cancer therapy. *Nat Rev Cancer* 2004; **4**:335–348.

3 Panwalkar A, Verstovsek S, Giles FJ. Mammalian target of rapamycin as therapy for hematologic malignancies. *Cancer* 2004; **100**:657–666.

4 Kim DH, Sarbassov DD, Ali SM, King JE, Latek RR, Erdjument-Bromage H, *et al.* mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. *Cell* 2002; **110**:163–175.

5 Kim DH, Sarbassov DD, Ali SM, King JE, Latek RR, Erdjument-Bromage H, *et al.* GbetaL, a positive regulator of the rapamycin-sensitive pathway required for the nutrient-sensitive interaction between raptor and mTOR. *Mol Cell* 2003; **11**:895–904.

6 Borlado LR, Redondo C, Alvarez B. Increased phosphoinositide 3-kinase activity induces a lymphoproliferative disorder and contributes to tumor generation *in vivo*. *FASEB J* 2000; **14**:895–903.

7 Vose JM, Chiu BCH, Cheson D, Dancey J, Wright J. Update on epidemiology and therapeutics for non-Hodgkin's lymphoma. *Hematology (Am Soc Hematol Educ Program)* 2002; 241–262.

8 Jimenez C, Jones DR, Rodrguez-Viciana P, Gonzalez-Garcia A, Leonardo E, Wennsrtom S, *et al.* Identification and characterization of a new oncogene derived from the regulatory subunit of phosphoinositide 3-kinase. *EMBO J* 1998; **17**:743–753.

9 Raftopoulou M, Etienne-Manneville S, Self A, Nicholls S, Hall A. Regulation of cell migration by the tumor suppressor PTEN. *Science* 2004; **303**: 1179–1181.

10 Royl HK, Olusolal BF, Clemens DL, Karolski WJ, Ratashak A, Lynch HT, *et al.* AKT proto-oncogene overexpression is an early event during sporadic colon carcinogenesis. *Carcinogenesis* 2002; **23**:201–205.

11 David O, Jett J, LeBeau H, Dy G, Hughes J, Friedman M, *et al.* Phospho-Akt overexpression in non-small cell lung cancer confers significant stage-independent survival disadvantage. *Clin Cancer Res* 2004; **10**:6865–6871.

12 Zhou X, Tan M, Stone Hawthorne V, Klos KS, Lan KH, Yang Y, *et al.* Activation of the Akt/mammalian target of rapamycin/4E-BP1 pathway by ErbB2 overexpression predicts tumor progression in breast cancers. *Clin Cancer Res* 2004; **10**:6779–6788.

13 De Benedetti A, Harris AL. eIF4E expression in tumors: its possible role in progression of malignancies. *Int J Biochem Cell Biol* 1999; **31**:59–72.

14 De Benedetti A, Graff JR. eIF4E expression and its role in malignancies and metastases. *Oncogene* 2004; **23**:3189–3199.

15 Jiang H, Coleman J, Miskimins R, Miskimins WK. Expression of constitutively active 4EBP-1 enhances $p27^{Kip1}$ expression and inhibits proliferation of MCF7 breast cancer cells. *Cancer Cell Int* 2003; **3**:2.

16 Altomare 2005. Perturbations of the Akt signaling pathway in human cancer. *Oncogene* 2005; **24**:7455–7464.

17 Altomare DA, You H, Xiao GH, Ramos-Nino ME, Skele KL, De Rienzo A, *et al.* Human and mouse mesotheliomas exhibit elevated AKT/PKB activity, which can be targeted pharmacologically to inhibit tumor cell growth. *Oncogene* 2005; **24**:6080–6089.

18 Asano T, Yao Y, Zhu J, Li D, Abbruzzese JL, Reddy SA. The rapamycin analog CCI-779 is a potent inhibitor of pancreatic cancer cell proliferation. *Biochem Biophys Res Commun* 2005; **331**:295–302.

19 Ito D, Fujimoto K, Mori T, Kami K, Koizumi M, Toyoda E, *et al. In vivo* antitumor effect of the mTOR inhibitor CCI-779 and gemcitabine in xenograft models of human pancreatic cancer. *Int J Cancer* 2005; [Epub ahead of print]

20 Slupianek A, Nieborowska-Skorska M, Hoser G, Morrione A, Majewski M, Xue L, *et al.* Role of phosphatidylinositol 3-kinase–Akt pathway in nucleophosmin/anaplastic lymphoma kinase-mediated lymphomagenesis. *Cancer Res* 2001; **61**:2194–2199.

21 Ruggero D, Montanaro L, Ma L, Xu W, Londei P, Cordon-Cardo C, *et al.* The translation factor eIF-4E promotes tumor formation and cooperates with c-Myc in lymphomagenesis. *Nat Med* 2004; **10**:484–486.

22 Pene F, Claessens YE, Muller O, Viguie F, Mayeux P, Dreyfus F, *et al.* Role of the phosphatidylinositol 3-kinase/Akt and mTOR/P70S6-kinase pathways in the proliferation and apoptosis in multiple myeloma. *Oncogene* 2002; **43**:6587–6597.

23 Lentzsch S, Chatterjee M, Gries M, Bommert K, Gollasch H, Dorken B, *et al.* PI3-K/AKT/FKHR and MAPK signaling cascades are redundantly stimulated by a variety of cytokines and contribute independently to proliferation and survival of multiple myeloma cells. *Leukemia* 2004; **18**:1883–1890.

24 Vega F, Mediros LJ, Atwell C, Cho JH, Tian L, Claret F-X, *et al.* Activation of mTOR signaling pathway contributes to lymphoma cell survival in ALK-positive anaplastic large cell lymphoma. *Blood* 2005; **106**:abstr 2419.

25 Peponi E, Drakos E, Reyes G, Leventaki V, Medeiros LJ. Inhibition of Akt/mTOR signalling pathway induces cell cycle arrest and apoptosis in mantle cell lymphoma. *Blood* 2005; **106**:abstr 2415.

26 Topisirovic I, Guzman ML, McConnel MJ, Licht JD, Culjkovic B, Neering SJ, *et al.* Aberrant eukaryotic translation initiation factor 4E-dependent mRNA impedes hematopoietic differentiation and contributes to leukemogenesis. *Mol Cell Biol* 2003; **23**:8992–9002.

27 Xu Q, Simpson SE, Scialla TJ, Bagg A, Carroll M. Survival of acute myeloid leukemia cells requires PI3 kinase activation. *Blood* 2003; **102**:972–980.

28 Min YH, Eom JI, Cheong JW, Maeng HO, Kim JY, Jeung HK. Constitutive phosphorylation of AktPKB protein in acute myeloid leukemia: its significance as a prognostic variable. *Leukemia* 2003; **17**:995–997.

29 Kubota Y, Ohnishi H, Kitanaka A, Ishida T, Tanaka T. Constitutive activation of PI3K is involved in the spontaneous proliferation of primary acute myeloid leukemia cells:direct evidence of PI3K activation. *Leukemia* 2004; **18**:1438–1440.

30 Chen W, Grammatikakis I, Li J, Leventaki V, Medieros LJ, Rassidakis GZ. Inhibition of Akt/mTOR signalling pathway induces cell cycle arrest and apoptosis in acute myelogenous leukaemia. *Blood* 2005; **106**:abstr 2355.

31 Cuni S, Perez-Aciego P, Perez-Chacon G, Vargas JA, Sanchez A, Martin-Saavedra FM, *et al.* A sustained activation of PI3K/NF-kappaB pathway is critical for the survival of chronic lymphocytic leukemia B cells. *Leukemia* 2004; **18**:1391–1400.

32 Barragan M, Bellosillo B, Campas C, Colomer D, Pons G, Gil J. Involvement of protein kinase C and phosphatidylinositol 3-kinase pathways in the survival of B-cell chronic lymphocytic leukemia cells. *Blood* 2002; **15**:2969–2976.

33 Vezina C, Kudelski A, Sehgal SN. Rapamycin (AY-22,989) a new antifungal antibiotic. Taxonomy of the producing streptomycyte and isolation of the active principle. *J Antibiot (Tokyo)* 1975; **10**:721.

34 Gu J, Ruppen ME, Cai P. Lipase-catalyzed regioselective esterification of rapamycin: synthesis of temsirolimus (CCI-779). *Org Lett* 2005; **7**:3945–3948.

35 Hidalgo M, Rowinsky EK. The rapamycin-sensitive signal transduction pathway as a target for cancer therapy. *Oncogene* 2000; **27**:6680–6686.

36 Rowinsky EK. Targeting the molecular target of rapamycin (mTOR). *Curr Opin Oncol* 2004; **16**:564–575.

37 Gambacorti-Passerini CB, Gunby RH, Piazza R, Galietta A, Rostagno R, Scapozza L. Molecular mechanisms of resistance to imatinib in Philadelphia-chromosome-positive leukaemias. *Lancet Oncol* 2003; **4**:75–85.

38 Blagosklonny MV, Darzynkiewicz Z. Four birds with one stone: RAPA as potential anticancer therapy. *Cancer Biol Ther* 2002; **1**:359–361.

39 Zhong H, Chiles K, Feldser D, Laughner E, Hanrahan C, Georgescu MM, *et al.* Modulation of hypoxia-inducible factor 1alpha expression by the epidermal growth factor/phosphatidylinositol 3-kinase/PTEN/AKT/FRAP pathway in human prostate cancer cells: implications for tumor angiogenesis and therapeutics. *Cancer Res* 2000; **15**:1541–1545.

40 Smolewski P, Janus A, Cebula B, Linke A, Jamroziak K, Robak T. Inhibition of mTOR kinase pathway selectively sensitizes acute myeloid leukemia cells to cytarabine-induced apoptosis. *Blood* 2005; **106**:abstr 2474.

41 Teachey DT, Obzut DA, Cooperman J, Fang J, Carroll M, Choi JK, *et al.* The mTOR inhibitor CCI-779 induces apoptosis and inhibits growth in preclinical models of primary adult human ALL. *Blood* 2005; **107**:1149–1155.

42 Teachey DT, Axsom K, Brown VI, Obzut D, Grupp A. Murine ultrasonography confirms that rapamycin is effective against autoimmune lymphoproliferative syndrome (ALPS). *Blood* 2005; **106**:abstr 2386.

43 Mayerhofer M, Boehm A, Aichberger KJ, Esterbauer H, Derdak S, Piekl WF, *et al. In vitro* and *in vivo* antileukemic effects of the mTOR-targeting drug rapamycin in patients with imatinib-resistant CML. *Blood* 2005; **106**:abstr 4834.

44 Dudkin L, Dilling MB, Cheshire PJ, Harwood FC, Hollingshead M, Arbuck S, *et al.* Biochemical correlates of mTOR inhibition by the rapamycin ester CCI-779 and tumor growth inhibition. *Clin Cancer Res* 2001; **7**:1758–1764.

45 Gibbons JJ, Discafani C, Peterson R, Hernandez R, Skotnicki J, Frost J. The effect of CCI-779, a novel macrolide antitumor agent on the growth of human tumor cells *in vitro* and in nude mouse xenograft *in vivo*. *Proc Am Assoc Cancer Res* 1999; **40**:abstr 2000.

46 Georger B, Kerr K, Tang CB, Fung KM, Powell B, Sutton LN, *et al.* Antitumor activity of the rapamycin analog CCI-779 in human primitive neuroectodermal tumor/medulloblastoma models as single agent and in combination chemotherapy. *Cancer Res* 2001; **61**:1572–1532.

47 Raymond E, Alexandre J, Faivre S, Vera K, Materman E, Boni J, *et al.* Safety and pharmacokinetics of escalated doses of weekly intravenous infusion of CCI-779, a novel mTOR inhibitor, in patients with cancer. *J Clin Oncol* 2004; **15**:2336–2347.

48 Hidalgo M, Rowinsky E, Erlichman C, Drengler R, Marshall B, Marks R, *et al.* Phase 1 and pharmacologic study of CCI-779, a cell cycle inhibitor. Presented at: 11th NCI–EORTC–AACR Symposium on New Drugs in Cancer Therapy, Amsterdam; 2000. abstr 545.

49 Boni JP, Leister C, Bender G, Fitzpatrick V, Twine N, Stover J, *et al.* Population pharmacokinetics of CCI-779: correlations to safety and pharmacogenomic responses in patients with advanced renal cancer. *Clin Pharmacol Ther* 2005; **77**:76–89.

50 Peralba JM, DeGraffenried L, Friedrichs W, Fulcher L, Grunwald V, Weiss G, *et al.* Pharmacodynamic evaluation of CCI-779, an inhibitor of mTOR, in cancer patients. *Clin Cancer Res* 2003; **9**:2887–2892.

51 Smith JW, Ko Y-J, Duthcer G, Hudes B, Escudier R, Motzer S, *et al.* Update of a phase 1 study of intravenous CCI-779 given in combination with interferon-$\alpha$ to patients with advanced renal cell carcinoma. *J Clin Oncol* 2004; **22**:abstr 4513.

52 Atkins MB, Hidalgo M, Stadler WM, Logan TF, Dutcher JP, Hudes GR, *et al.* Randomized phase II study of multiple dose levels of CCI-779, a novel mammalian target of rapamycin kinase inhibitor, in patients with advanced refractory renal cell carcinoma. *J Clin Oncol* 2004; **22**:909–918.

53 Pandya KJ, Levy DE, Hidalgo M, Cohen RB, Lee MW, Schiller JH, *et al.* A randomized, phase II ECOG trial of two dose levels of temsirolimus (CCI-779) in patients with extensive stage small cell lung cancer in remission after induction chemotherapy. *J Clin Oncol* 2005; **23**:abstr 7005.

54 Chan S, Scheulen ME, Johnston S, Mross K, Piccart M, Hess D, *et al.* Phase 2 study of two dose levels of CCI-779 in locally advanced or metastatic breast cancer (MBC) failing prior anthracycline and/or taxane regimens. *J Clin Oncol* 2005; **23**:5314–5322.

55 Galanis E, Buckner JC, Maurer MJ, Kreisberg JI, Ballman K, Boni J, *et al.* Phase II trial of temsirolimus (CCI-779) in recurrent glioblastoma multiforme: a North Central Cancer Treatment Group Study. *J Clin Oncol* 2005; **23**:5294–5304.

56 Chang SM, Wen P, Cloughesy T, Greenberg H, Schiff D, Conrad C, *et al.* North American Brain Tumor Consortium and the National Cancer Institute. Phase II study of CCI-779 in patients with recurrent glioblastoma multiforme. *Invest New Drugs* 2005; **23**:357–361.

57 Galanis E, Buckner JC, Maurer MJ, Hidalgo M, Kreisberg JI, Peralba J, *et al.* N997B: phase II trial of CCI-779 in recurrent glioblastoma multiforme (GBM): updated results and correlative laboratory analysis. *J Clin Oncol* 2005; **23**:abstr 1505.

58 Margolin K, Longmate J, Baratta T, Synold T, Christensen S, Weber J, *et al.* CCI-779 in metastatic melanoma: a phase II trial of the California Cancer Consortium. *Cancer* 2005; **104**:1045–1048.

59 Witzig TE, Geyer SM, Ghobrial I, Inwards DJ, Fonseca R, Kurtin P, *et al.* Phase II trial of single-agent temsirolimus (CCI-779) for relapsed mantle cell lymphoma. *J Clin Oncol* 2005; **23**:5347–5356.

60 Witzig TE, Ansell SM, Geyer SM, Kurtin PJ, Rowland KM, Flynn PJ, *et al.* Anti-tumor activity of low-dose single agent CCI-779 for relapsed mantle cell lymphoma: a phase II trial in the North Central Cancer Treatment Group. *J Clin Oncol* 2005; **23**:abstr 6504.

61 Yee KWL, Garcia-Manero G, Thomas D, Ravandi-Kashani F, Verstovsek S, Andreeff M, *et al.* A phase II study of temsirolimus (CCI-779) in patients with advanced leukemias. *Blood* 2004; **104**:abstr 4523.

62 O'Donnell S, Faivre I, Judson C, Delbado C, Brock H, Lane N, *et al.* 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 tumors. *Proc Am Soc Clin Oncol* 2003; **74**:200 (abstr 803).

63 Boulay A, Zumstein-Mecker S, Stephan C, Beuvink I, Zilbermann F, Haller R, *et al.* Antitumor efficacy of intermittent treatment schedules with the rapamycin derivative RAD001 correlates with prolonged inactivation of ribosomal protein S6 kinase 1 in peripheral blood mononuclear cells. *Cancer Res* 2004; **64**:252–261.

64 Tabernero J, Rojo F, Burris H, Casado E, Macarulla T, Jones S, *et al.* A phase I study with tumor molecular pharmacodynamic (MPD) evaluation of dose

and schedule of the oral mTOR-inhibitor Everolimus (RAD001) in patients (pts) with advanced solid tumors. *J Clin Oncol* 2005; **23**:abstr 3007.

65 Lerut E, Roskams T, Goossens E, Bootle D, Dimitrijevic S, Stumm M, *et al.* Molecular pharmacodynamic (MPD) evaluation of dose and schedule of RAD001 (everolimus) in patients with operable prostate carcinoma (PC). *J Clin Oncol* 2005; **23**:abstr 3071.

66 Majewski M, Korecka M, Kossev P, Li S, Goldman J, Moore J, *et al.* The immunosuppressive macrolide RAD inhibits growth of human Epstein–Barr virus-transformed B lymphocytes *in vitro* and *in vivo*: a potential approach to prevention and treatment of posttransplant lymphoproliferative disorders. *Proc Natl Acad Sci U S A* 2000; **97**:4285–4290.

67 Yee KWL, Wierda W, O'Brien S, Thomas D, Kurzrock R, Fayad LE, *et al.* A phase I/II study of the oral mTOR inhibitor RAD001 in patients with advanced hematologic malignancies. *Blood* 2004; **104**:abstr 4818.

68 Rivera VM, Kreisberg JI, Mita MM, Goldston M, Knowles HL, Herson J, *et al.* Pharmacodynamic study of skin biopsy specimens in patients (pts) with refractory or advanced malignancies following administration of AP23573, an mTOR inhibitor. *J Clin Oncol* 2005; **23**:abstr 3033.

69 Desai AA, Mita M, Fetterly GJ, Chang C, Netsch M, Knowles HL, *et al.* Development of a pharmacokinetic (PK) model and assessment of patient (pt) covariate effects on dose-dependent PK following different dosing schedules in two phase I trials of AP23573 (AP), a mTOR inhibitor. *J Clin Oncol* 2005; **23**:abstr 3043.

70 Mita MM, Rowinsky EK, Goldston ML, Mita AC, Chu Q, Syed S, *et al.* Phase I, pharmacokinetic (PK), and pharmacodynamic (PD) study of AP23573, an mTOR Inhibitor, administered IV daily $\times 5$ every other week in patients (pts) with refractory or advanced malignancies. *J Clin Oncol* 2004; **22**:abstr 3076.

71 Desai AA, Janisch LL, Berk R, Knowles HL, Rivera VM, Bedrosian CL, *et al.* A phase 1 trial of a novel mTOR inhibitor AP23573 administered weekly in patients with refractory or advanced malignancies: a pharmacokinetic and pharmacodynamic analysis. *J Clin Oncol* 2004; **22**:abstr 3150.

72 Clackson T, Metcalf CA, Rivera VM, Konwles HL, Tang H, Burns KD, *et al.* Broad anti-tumor activity of AP23573, an mTOR inhibitor in clinical development. [abstract] *Proc Am Soc Clin Oncol* 2003; **22**:882A.

73 Nelms JL, Goldlust SA, Kanner A, Vogelbaum MA. A novel mTOR inhibitor (AP23573) produces cell loss and apoptosis in glioblastoma multiforme independent of EGFR status. [abstract] *Neuro-Oncology* 2003; **5**:29A.

74 Chawla SP, Sankhala KK, Chua V, Menendez LR, Eilber FC, Eckardt JJ, *et al.* A phase II study of AP23573 (an mTOR inhibitor) in patients (pts) with advanced sarcomas. [abstract] *J Clin Oncol* 2005; **23**:9068A.

75 Sankhala KK, Chawla SP, Iagaru A, Dellamaggiora R, Chua V, Daly S, *et al.* Early response evaluation of therapy with AP23573 (an mTOR inhibitor) in sarcoma using [$^{18}$F]2-fluoro-2-deoxy-D-glucose (FDG) positron emission tomography (PET) scan. *J Clin Oncol* 2005; **23**:abstr 9028.

76 Rizzieri DA, Feldman E, Moore JO, Roboz GJ, DiPersio J, Gabrail N, *et al.* A phase 2 clinical trial of AP23573, an mTOR inhibitor, in patients with relapsed or refractory hematologic malignancies. *Blood* 2005; **106**:abstr 2980.

77 Feldman E, Giles F, Roboz G, Yee K, Curcio T, Rivera VM, *et al.* A phase 2 clinical trial of AP23573, an mTOR inhibitor, in patients with relapsed or refractory hematologic malignancies. *J Clin Oncol* 2005; **23**:abstr 6631.