PI3K/Akt/mTOR pathway as a target for cancer therapy

Daniel Morgensztern[a] and Howard L. McLeod[a]

The PI3K/Akt/mTOR pathway regulates several normal cellular functions that are also critical for tumorigenesis, including cellular proliferation, growth, survival and mobility. Components of this pathway are frequently abnormal in a variety of tumors, making them an attractive target for anti-cancer therapy. Inhibition of mTOR in patients with cancer became more feasible after the development of rapamycin analogs with improved pharmacologic properties. The promising activity of these agents in early clinical trials has led to the development of ongoing phase III trials in renal cell carcinoma and breast cancer. Future studies are needed to identify the patients most likely to benefit from this form of therapy, and to define its role in combination with chemotherapy, hormones and growth factor inhibitors. *Anti-Cancer Drugs* 16:797–803 © 2005 Lippincott Williams & Wilkins.

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[a]Washington University School of Medicine, Departments of Medicine and Genetics, St Louis Veteran's Administration Medical Center and Siteman Cancer Center, St Louis, Missouri, USA.

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Correspondence to H. L. McLeod, 660 S. Euclid Ave, Campus Box 8069, St Louis, Missouri 63110 USA.
Tel: +1 314 747-2060; fax: +1 314 362-3764;
e-mail: hmcleod@im.wustl.edu

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The PI3K/Akt/mTOR pathway

Signaling through the PI3K/Akt/mTOR pathway begins with the activation of receptor tyrosine kinases in response to growth factors, leading to autophosphorylation on tyrosine residues and transphosphorylation of adaptor proteins (Fig. 1). Activation of phosphatidylinositol (PI)-3-kinase (PI3K) class Ia subsequently occurs upon binding of its Src homology (SH2) domains from the p85 regulatory unit to specific phosphotyrosine residues on the activated receptor or associated adaptor proteins, bringing the enzyme to the membrane and resulting in the activation of the p110 catalytic unit [1,2]. Activated PI3K acts as a lipid kinase, adding a phosphate group to the D3-OH position of the inner membrane phosphoinositides leading to the conversion of PI-4-phosphate and PI-4,5-biphosphate (PIP_2) into PI-3,4-biphosphate and PI-3,4,5-triphosphate (PIP_3), respectively. Subsequent signaling from PI3K class Ia is mediated mainly through the recruitment of proteins containing pleckstrin homology (PH) domains, including the serine/threonine kinases Akt and PDK1, to 3′-phosphoinositides [3]. The relocation from the cytoplasm to the inner surface of the plasma membrane places Akt, the primary mediator of PI3K-initiated signaling, in close proximity to the regulatory kinases responsible for its phosphorylation and activation. Upon binding to PIP3, Akt undergoes conformation changes that allow the phosphorylation by PDK1 at Thr308 in the activation loop. Subsequent phosphorylation at Ser473 by an unidentified kinase results in full Akt activation [4]. Fully activated Akt subsequently translocates to the cytosol and nucleus where it phosphorylates its substrates.

Akt regulates several target proteins involved in the control of apoptosis and cell proliferation. Akt promotes cell survival by inhibiting the pro-apoptotic activity of BAD, caspase-9 and the forkhead family, and activating several anti-apoptotic substrates including IκB kinase (IKK) and cAMP response element binding protein (CREB) [5,6]. Inhibition of glycogen synthase kinase-3 (GSK-3), $p21^{Cip1}$ and $p27^{Kip1}$, as well as decreased proteolytic degradation of cyclin D_1, promote increased cell cycle progression through the G_1/S phase [7,8]. An additional target of Akt, the mammalian target of rapamycin (mTOR), plays a critical role in the cell cycle progression from the G_1 to the S phase. Activation of mTOR by Akt occurs through inactivation of the tuberous sclerosis complex (TSC). Unphosphorylated TSC2 (tuberin) is bound to TSC1 (hamartin) in a complex that blocks mTOR activation. This complex is disrupted by Akt-mediated phosphorylation of TSC2, which relieves the Rheb GAP activity of tuberin and allows Rheb to bind ATP. In the presence of ATP, Rheb switches from the inactive GDP state to the active GTP form and subsequently activates mTOR [9].

mTOR proteins are serine/threonine kinases that control cell growth and proliferation in response to nutrient availability and growth factor stimulation [10]. The major downstream targets of mTOR appear to be the translational components ribosomal p70S6 kinase (S6K1) and eukaryotic initiation factor 4E (eIF4E)-binding protein 1 (4E-BP1). Whereas activation of S6K1 enhances the translation of mRNAs containing the 5′-terminal oligopyrimidine tract (TOP), phosphorylation of 4E-BP allows the release of eIF4E which facilitates mRNA binding to

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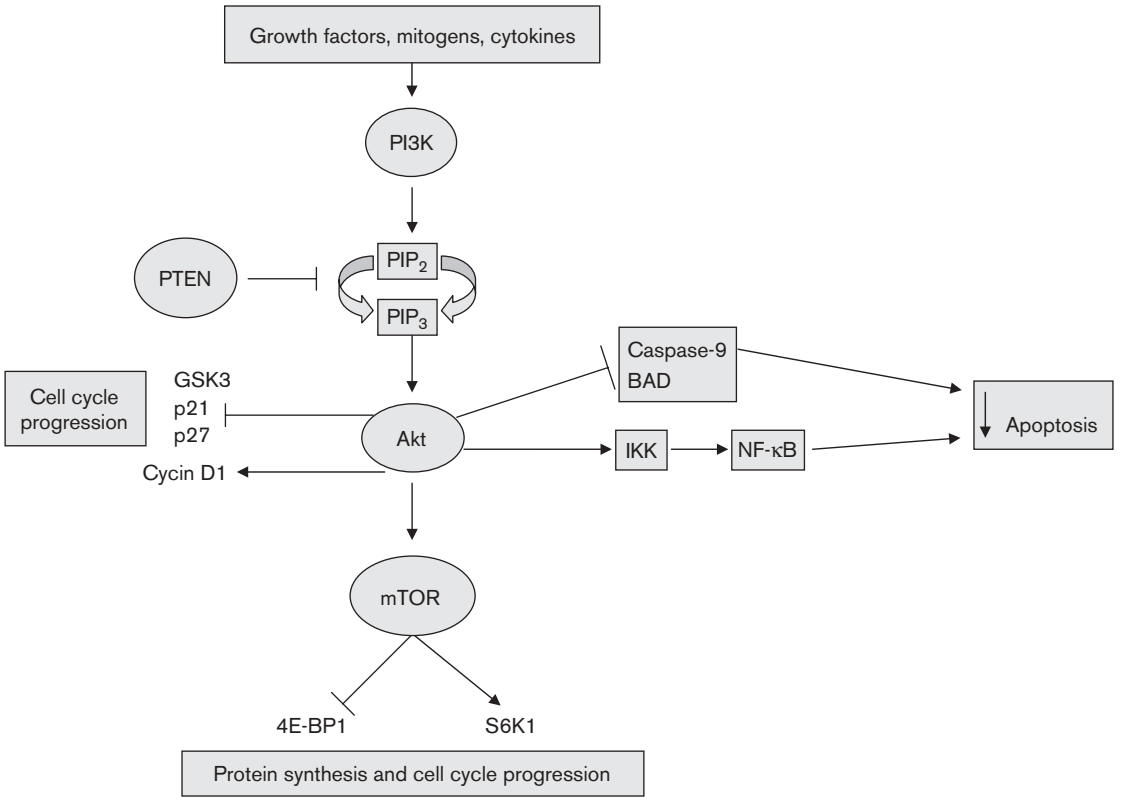
Fig. 1



Schematic of the PI3K/Akt/mTOR pathway.

the 40S ribosomal subunit. [11]. Thus, in the presence of mitogen stimulation of the PI3K/Akt pathway and sufficient nutrients, mTOR stimulates the translation of proteins required for the cell cycle progression from the G_1 to the S phase.

PI3K signaling is terminated by degradation of PIP_3, which can be mediated by two different subtypes of phosphatases. The SH2-containing inositol phosphatase (SHIP) dephosphorylates PIP_3 at position D5, producing PI-3,4-biphosphate [12]. The second phosphatase, named phosphatase and tensin homolog (PTEN), directly antagonizes the effects of class I PI3Ks by removing the phosphate group on the position D3 and converting PIP_3 back to its original PI-4,5-biphosphate substrate [13]. The PTEN function is frequently attenuated in tumors due to deletions or mutations, leading to constitutive activation of AKT and upregulation of mTOR-dependent pathways.

The PI3K/Akt/mTOR pathway regulates several normal cellular functions that are also critical for tumorigenesis. This pathway can be aberrantly activated in a variety of tumors due to amplification of the PI3KC gene encoding for the p110α catalytic subunit of PI3K [14–19], gene amplification of Akt [20–25] and PTEN mutations [26–31].

Inhibition of the PI3K/Akt/mTOR pathway

Although the PI3K inhibitors wortmannin and LY294002 have been extensively evaluated in cultured cells as research tools, the lack of selectivity of these compounds within the PI3K family and the instability of wortmannin or insolubility of LY294002 have limited their clinical use [32,33].

Rapamycin, a natural product derived from *Streptomyces hygroscopicus*, was initially developed as a fungicidal [34] and immunosuppressant agent for prevention of allograft rejection after organ transplantation [35]. Subsequent studies from the natural products program at the National Cancer Institute revealed the anti-proliferative activity of rapamycin in several murine tumor systems. In order to inhibit mTOR, rapamycin must first bind to the intracellular receptor, the ubiquitous immunophilin FKBP-12. The molecular complex FKBP12–rapamycin subsequently inhibits mTOR function [36,37]. Despite its significant anti-tumoral activity, rapamycin was not developed as a cancer therapy due to poor aqueous solubility and chemical stability. The subsequent synthesis of

rapamycin analogs with improved pharmaceutical properties and comparable efficacy led to the development of clinical trials. There are currently three rapamycin analogs in different stages of development. The water-soluble rapamycin ester cell cycle inhibitor (CCI)-779 is the most extensively studied agent with completed phase I and II studies, and ongoing phase III trials. The two other agents, the orally bioavailable RAD001 (everolimus) and AP23573, are in the early phase of development.

Phase I trials

Two recent studies evaluating the toxicity profile and pharmacokinetics of escalating doses of CCI-779 given in intermittent schedules have been reported. The intermittent administration was selected in order to minimize the potential immunosuppressive effects of CCI-779, as observed and desired with rapamycin. Raymond and colleagues treated 24 patients using a weekly 30-min i.v. infusion of CCI-779 with doses ranging from 7.5 to 220 mg/m^2. The treatment resulted in no immunosuppressive effects and the maximally tolerated dose (MTD) was not reached, as there was no clear relationship between dose and toxicity. The most frequent adverse events were maculopapular rash and mucositis or stomatitis. The main dose-limiting toxicity was reversible thrombocytopenia. At the dose of 220 mg/m^2, three patients developed reversible neuropsychiatric manifestations. Two patients had a partial response (PR). One of the six patients with renal cell carcinoma (RCC) achieved a PR lasting 6.5 months following progression of disease under treatment with interferon (IFN)-α and interleukin (IL)-2. The second patient achieving a PR had breast cancer previously treated with anthracycline and taxane. Her remission lasted 5.4 months. Two additional patients with RCC achieved minor responses (MRs), with 34 and 39% tumor reduction, lasting 3 and 4.9 months [38]. In a second study, Hidalgo and colleagues administered CCI-779 at doses ranging from 0.75 to 24 mg/m^2 to 51 patients as a 30-min infusion daily for 5 days every 2 weeks. There was no evidence of immunosupression and the MTD was 19.1 mg/m^2/day for minimally treated patients and 15 mg/m^2/day for heavily treated patients. Dose-limiting grade 3 toxicities included asymptomatic hypocalcemia, reversible increase in hepatic transaminases and thrombocytopenia. One patient with non-small-cell lung cancer (NSCLC) achieved a PR. Minor anti-tumor responses or disease stabilization longer than 4 months were observed in several patients, including four patients with RCC, two patients with soft tissue sarcoma, one patient with cervical cancer, one patient with uterine cancer and one patient with NSCLC. The inconsistent pattern of lymphocyte subsets and mitogen proliferation assays precluded their use as useful pharmacodynamic surrogates [39]. A subsequent study identified the peripheral blood mononuclear cell p70S6 kinase assay as a feasible and valid method for pharmacodynamic analysis of CCI-779. This assay is currently being incorporated in further studies with CCI-779 to determine its relation with dose and plasma concentration, as well as its value as a predictor of treatment efficacy [40].

Disease-specific phase II studies

RCC

mTOR inhibition may be particularly useful in patients with RCC where there is activation of the hypoxia-inducible factor (HIF) pathway. HIF-1 is a heterodimer consisting of a constitutively expressed HIF-1β and a highly regulated HIF-1α subunit. HIF-1α synthesis is stimulated by growth factors and cytokines through the PI3K/Akt/mTOR and mitogen-activated kinase (MAPK) pathways, and inhibited by von Hippel–Lindau (VHL) product [41].

Under normal conditions, VHL product forms stable complexes with other proteins, which are capable of directing the covalent attachment of polyubiquitin tails to specific targets for subsequent proteasome-dependent degradation. One of these targets is HIF, which is hydroxylated and recognized by VHL in the presence of oxygen, but not under hypoxic conditions [42]. The loss of VHL function, a common event in the clear cell subtype of RCC, leads to the accumulation of HIF-1α and HIF-2α with subsequent increased transcription of HIF-regulated proteins including vascular endothelial growth factor (VEGF), platelet-derived growth factor and transforming growth factor-α [43]. RCC tumors have been shown to produce particularly high levels of VEGF and a recent study showed a significant prolongation of time to disease progression in patients with metastatic RCC treated with the anti-VEGF monoclonal antibody bevacizumab [44].

In a phase II study, Atkins and colleagues treated 111 patients with metastatic RCC who were either previously treated or not considered appropriate candidates for first-line therapy with IL-2. Patients were randomly assigned to treatment with i.v. CCI-779 at doses of 25, 75 or 250 mg weekly as a 30-min infusion. Treatment was continued until progression or unacceptable toxicity. Similarly to phase I studies, the most common adverse effects were maculopapular rash and mucositis, occurring in 76 and 70% of patients, respectively. The most frequent grade 3 or 4 toxic effects were hyperglycemia, hypophosphatemia, anemia and hypertriglyceridemia. The overall response rate observed in eight patients was 7%. One patient achieved a complete remission (CR) and remained disease free continuing into the third year of treatment. Seven additional patients achieved a PR and 29 had MRs. Clinical benefits, including CRs, PRs, MRs and stable disease (SD) lasting more than 24 weeks, were seen in 51% of patients. Median time to progression (TTP) and overall survival (OS) were 5.8 and 15 months,

respectively. Toxicity and efficacy were not significantly influenced by the CCI-779 dose [45]. An additional stratification of patients was performed based on the prognostic model developed by Motzer and colleagues in RCC patients treated with IFN-α. Prognostic factors included Karnofsky performance status less than 80%, lactate dehydrogenase greater than 1.5 times the upper normal limit, calcium more than 10 mg/dl, decreased hemoglobin and time from diagnosis to starting therapy less than 1 year. Patients with none, one to two, and three or more risk factors were classified as good, intermediate or poor risk, respectively [46]. Median survivals for intermediate and poor-risk patients treated with CCI-779 were 1.6- to 1.7-fold longer than those in the original study by Motzer. Although no advantage was observed in good-risk patients, a possible explanation is the limited number of patients receiving CCI-779 in this prognostic category [45].

The combination of IFN and CCI-779 in the treatment of RCC has been evaluated in a phase I/II study. In phase I of the study, IFN-α was given as 6×10^6 U s.c. 3 times a week and CCI-779 was given weekly on escalating doses ranging from 5 to 25 mg. The dose of 15 mg was selected as the MTD and used in the phase II part of the study. In a preliminary evaluation of tumor responses in 55 patients, seven PRs (13%) and 39 SDs (71%) were seen. The favorable toxicity profile and encouraging responses observed in the phase I study led to the development of an ongoing phase III trial comparing CCI-779, IFN-α or a combination of both in RCC patients [47].

Although the initial combination study in RCC was performed with the addition of IFN, it would be worthwhile to attempt the combination of CCI-779 and bevacizumab. Both agents appear to have a good toxicity profile and may act synergistically against the highly manifested tumor-induced angiogenesis.

Breast cancer

In breast cancer, activation of the PI3K/Akt/mTOR pathway may occur through activation of membrane receptors, including growth factors and the estrogen receptor. This pathway has been linked to promotion of survival in breast cancer cells, and resistance to chemotherapy, trastuzumab and tamoxifen [48–50]. Approximately 50% of patients with breast cancer have a mutation or loss of at least one copy of the PTEN gene, resulting in activation of PI3K signaling [51]. Preclinical studies have shown that in breast cancer cells with reduced PTEN expression, the PI3K/Akt/mTOR pathway becomes a fundamental pathway for tumor proliferation and survival. These cells consequently display increased sensitivity to LY294002 and rapamycin compared with PTEN-positive cells [52]. Inhibition of mTOR has also been shown to restore tamoxifen sensitivity in breast cancer cells with aberrant Akt activity [53]. A recent study showed that trastuzumab, a monoclonal antibody directed against ErbB2, stabilizes PTEN with a consequent decrease in the activity of the PI3K signaling. Consequently, the loss of PTEN function may predict resistance to trastuzumab [54].

In a multicenter phase II study, 106 women with advanced breast cancer refractory to anthracyclines and taxanes were treated with weekly i.v. CCI-779 at doses of 75 or 250 mg. Response rates were seen in nine patients (8%), including one CR and eight PRs. An additional 43 patients achieved SD for at least 8 weeks for a total clinical benefit of 49%. Although activity was seen at both dose levels, therapy was better tolerated in the 75-mg arm [55].

Based on preclinical findings suggesting an association between hormone resistance and activation of the PI3K/Akt/mTOR pathway, a phase II trial evaluating the combination of CCI-779 and the aromatase inhibitor letrozole was initiated. In this study, 55 post-menopausal patients with hormone-positive locally advanced or metastatic breast cancer were randomized to treatment with letrozole alone or in combination with oral CCI-779 in two different schedules, i.e. daily or intermittent. Although both schedules required dose reduction, therapy was overall well tolerated. The most common side-effects were stomatitis and diarrhea. The intermittent schedule using 30 mg of CCI-779 oral daily for 5 days every 2 weeks was chosen to be used in combination with letrozole 2.5 mg daily in a phase III study comparing this therapy with letrozole alone [56].

Prostate cancer

The frequent presence of PTEN abnormalities in prostate cancer may render this tumor particularly sensitive to mTOR inhibition. Although the PTEN mutations are relatively infrequent in androgen-dependent prostate cancer, the likelihood of PTEN inactivation becomes considerably higher in androgen-refractory cases [57–59]. Resistance to androgen deprivation and progression to androgen-refractory prostate cancer have been attributed to overexpression of Bcl-2 [60]. Therefore, phosphorylation and inactivation of the pro-apoptotic molecule BAD by Akt with subsequent increased activity of Bcl-2 may account for androgen resistance in PTEN-deficient tumors. In patients with hormone-refractory prostate cancer, particularly in cases of PTEN-deficient tumors, the addition of mTOR inhibitors to androgen blockade in an attempt to restore hormone sensitivity may prove useful.

Mantle cell lymphoma

Tumors characterized by increased expression of cyclin D_1 may have increased sensitivity to mTOR inhibition. Cyclin D_1 degradation occurs mainly after phosphorylation at residue Thr286 followed by proteasome

degradation. GSK-3 is the main kinase responsible for phosphorylation at this residue [61]. Therefore, phosphorylation and inactivation of GSK by Akt increases cyclin D_1 stabilization.

CCI-779 has recently been evaluated as monotherapy in patients with mantle cell lymphoma, a malignancy characterized by overexpression of cyclin D_1 in the majority of cases. Eighteen previously treated patients received 250 mg of CCI-779 i.v. weekly. The overall response rate was 44% with one CR and seven PRs. However, treatment was poorly tolerated with grade 3 or 4 myelosuppression occurring in all patients. Due to encouraging responses, the protocol was modified to allow dose reduction to as low as 50 mg weekly in the second stage of the trial [62].

Finding the ideal candidates for therapy

Although several preclinical studies have identified PTEN mutation as a predictor for response to mTOR inhibition [63–65], the complexity of this pathway indicates that a more comprehensive approach is required to allow better patient selection. In a recent study, Xu and colleagues used tissue array technology to evaluate the expression of the PI3K/Akt/mTOR pathway components in eight common human tumor types. A total of 124 cases were evaluated for the expression of pAKT, PTEN, mTOR and its downstream molecules. The prediction for sensitivity to mTOR inhibition was based on the criteria of low PTEN, higher pS6K1 and pAkt expression in tumors. Thirty-two tumors analyzed (26%) met the above criteria, including 41% of ovarian adenocarcinomas, 33% of lung cancers, 31% of colonic adenocarcinomas, 28% of lymphomas, 23% of breast adenocarcinomas, 14% of prostate cancers, 13% of brain tumors and none of the cases of melanoma (Table 1) [66].

Summary and conclusions

Rapamycin analogs represent the first class of agents for the inhibition of the PI3K/Akt/mTOR pathway in clinical use. Results from clinical trials have shown good tolerability and promising response rates. Ongoing phase III trials will help in defining the role of this therapy in RCC and breast cancer.

Table 1 Modeling of putative sensitivity to mTOR inhibitors based on the criteria of lower PTEN, high pS6K1 and pAkt in 124 samples from eight human tumor types

Tumor	Total no. cases	Potential sensitive cases (%)	Potential non-sensitive cases (%)
Ovarian	22	9 (41)	13 (59)
Lung	9	3 (33)	6 (67)
Colon	16	5 (31)	11 (69)
Lymphoma	32	9 (28)	23 (72)
Breast	13	3 (23)	10 (77)
Prostate	14	2 (14)	12 (86)
Brain	8	1 (13)	7 (87)
Melanoma	10	0 (0)	10 (100)

Future studies are required to define the optimal dose, ideal candidates for therapy and the potential for combination with chemotherapy, hormones or growth factor inhibitors.

The possibility of synergism with the combined use of the two currently available classes of drugs capable of inhibiting the PI3K/Akt/mTOR pathway, i.e. rapamycin analogs and epidermal growth factor receptor (EGFR) inhibitors, should be further explored.

The combination of rapamycin analogs with growth factor inhibitors appears to be a promising area of research, particularly in NSCLC, where the PI3K/Akt/mTOR pathway has been shown to play a critical role in the anti-tumor effects of the EGFR tyrosine kinase inhibitor gefinitib.

The role of the PI3K/Akt pathway in the sensitivity to gefitinib has been recently demonstrated in two preclinical studies and a retrospective clinical study. The first study showed that in tumors with overexpression of either EGFR or HER2, the dephosphorylation of these receptors by gefitinib is followed by downregulation of PI3K and inactivation of Akt. This downregulation of Akt was found to correlate with gefitinib response in the MDA-468 breast cancer cell line. In this study, resistance to gefitinib was caused by PTEN loss, which caused hyperactivation of Akt and uncoupling of the Akt pathway from EGFR. Restoration of PTEN in these cells restored sensitivity to gefitinib, by decreasing Akt activity without any reduction in EGFR-stimulated activity [67]. The second study, performed in nine different NSCLC cell lines, showed that the PC9 adenocarcinoma cell line was the most sensitive to gefitinib. This increased sensitivity was attributed to a higher dependency on the extracellular signal-regulated kinase 1/2 (ERK1/2) and Akt pathway for its survival and proliferation [68]. Further support for the role of the Akt pathway was provided by a trial performed on 106 patients receiving gefitinib on a compassionate-use basis. Tumor specimens were stained for P-Akt and P-MAPK. From a total of 97 patients with evaluable tumors, responses were seen in 14 cases (14.4%). Patients with Akt-positive tumors had a significantly better response rate (26.1 versus 3.9%), clinical benefit (60.9 versus 23.5%) and median TTP (5.5 versus 2.8 months). P-MAPK was not associated with the patients' characteristics or response to therapy [69].

It is possible that inhibition of the PI3K/Akt/mTOR pathway is necessary for the anti-tumor effects of EGFR inhibitors and increased Akt activity, caused by Akt overexpression or PTEN mutation, may prevent responses to growth factor inhibition alone.

A recent preclinical study evaluated the effects induced by the combination of the rapamycin analog RAD001

(Everolimus) and gefitinib in different cell lines. Simultaneous treatment induced additive anti-proliferative effects in the BT-474 and DU-145 cell lines, whereas sequential treatment with gefitinib followed by RAD001 induced synergistic effects in both cell lines. Different effects were observed in MDA-MB-145 cells, which are relatively resistant to gefitinib. In this case, simultaneous treatment induced synergistic effects, whereas the sequential approach with gefitinib followed by RAD001 produced additive effects [70]. Therefore, mTOR inhibition appears to potentiate the effects of the EGFR inhibitor gefitinib in a schedule-dependent fashion.

The combined inhibition of mTOR and EGFR appears logical, and should be tested in clinical trials.

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