Inhibition of the Phosphatidylinositol 3-Kinase/Mammalian Target of Rapamycin Pathway in Hematologic Malignancies

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Opinion statement

The phosphatidylinositol 3-kinase (PI3-K)/mammalian target of rapamycin (mTOR) signal transduction pathway integrates signals from multiple receptor tyrosine kinases to control cell proliferation and survival. Key components of the pathway are the lipid kinase PI3-K, the small guanosine triphosphate–binding protein Rheb, and the protein kinases Akt and mTOR. Important natural inhibitors of the pathway include the lipid phosphatase PTEN and the tuberous sclerosis complex. Several components of this pathway are targeted by investigational antineoplastic agents. Rapamycin (sirolimus), the prototypic mTOR inhibitor, exhibits activity in acute myeloid leukemia. Three rapamycin analogs, temsirolimus, everolimus, and AP23573, are in clinical trials for various hematologic malignancies. Temsirolimus has produced a 38% overall response rate in relapsed mantle cell lymphoma, and AP23573 has demonstrated activity in acute leukemia. Everolimus is undergoing clinical testing in lymphoma (Hodgkin and non-Hodgkin) and multiple myeloma. In addition, perifosine, an inhibitor of Akt activation that exhibits substantial antimyeloma activity in preclinical models, is being examined in relapsed multiple myeloma. Based on results obtained to date, it appears that inhibitors of the PI3-K/mTOR pathway hold promise as single agents and in combination for hematologic malignancies.

Introduction

Many of the important approaches to the treatment of neoplastic diseases have been piloted in hematologic malignancies. The development of combination chemotherapy in the 1960s and 1970s led to curative treatments for many patients with acute leukemia, Hodgkin disease, and non-Hodgkin lymphoma (NHL). The growing technical ability to synthesize monoclonal antibodies in the 1970s and 1980s led to development of rituximab, the first antineoplastic monoclonal antibody, for B-cell malignancies. Improved understanding of the molecular pathogenesis of hematologic malignancies in the late 1980s and early 1990s led to the approval of imatinib, the first highly effective signal transduction inhibitor, for chronic myelogenous leukemia.

The past decade has witnessed growing understanding of the biochemical mechanisms that contribute to survival and proliferation of neoplastic cells. Building on this expanding knowledge, investigators from a variety of disciplines are currently trying to elucidate the alterations in signal transduction pathways that contribute to neoplastic transformation, to design and test inhibitors of altered pathways in suitable cell lines,

primary cancer cells, and xenograft models, and to rapidly translate results of these studies into appropriately designed clinical trials. As illustrated in this review, extensive study of the phosphatidylinositol 3-kinase (PI3-K)/mammalian target of rapamycin (mTOR) pathway has led to promising new agents that exhibit activity in hematologic malignancies.

THE PHOSPHATIDYLINOSITOL 3-KINASE/ MAMMALIAN TARGET OF RAPAMYCIN PATHWAY

To provide a basis for discussing the preclinical and clinical studies of these agents, we first review current understanding of the PI3-K/mTOR pathway (Fig. 1). This pathway, which consists of a series of kinases, including PI3-K, Akt, mTOR and p70S6K, as well as an eclectic group of intervening signaling molecules, plays an important role in the regulation of cell growth, proliferation, and survival [1,2,3••,4,5].

The first enzyme in this pathway, PI3-K, is a lipid kinase that adds a phosphate group to phosphoinositides at the D3 position of inositol [6,7]. PI3-K is actually a family of three closely related enzymes, each composed of an 85-kDa regulatory subunit and a 110-kDa catalytic subunit. Members of this kinase family are activated when several receptor tyrosine kinases, including FLT3, epidermal growth factor receptor, HER-2/neu, and insulin-like growth factor 1 (IGF-1) receptor, bind their ligands [2,8–10]. In each case, ligand binding results in receptor automodification to generate phosphorylated sites that bind the PI3-K p85 regulatory subunit, thereby producing an activating allosteric change in p110 [3••,6]. BCR/ABL, which phosphorylates itself and adapter molecules such as Gab2, likewise generates phosphorylated motifs that bind p85 and activate p110 [11]. The p110 PI3-K catalytic subunit can also be activated by directly binding to activated Ras proteins [12] or by mutations, which are common in certain neoplasms [7].

Once activated, PI3-K converts phosphatidylinositol-4,5-bisphosphate (PIP2) to phosphatidylinositol-3,4,5-trisphosphate (PIP3) at the inner surface of the plasma membrane. This PIP3 is recognized by lipid-binding domains that are present in a variety of molecules, including members of the Akt protein kinase family and phosphoinositide-dependent kinase 1 (PDK1). As a consequence of the interaction with PIP3, PDK1 is recruited to the plasma membrane and activated. Akt is also recruited to the plasma membrane, where PDK1 catalyzes phosphorylation of Akt on Thr^{308}, one of the two phosphorylations required for maximal Akt activation.

Similar to PI3-K, Akt is thought to play important roles in cell growth, proliferation, and survival [4,13]. After activation, Akt phosphorylates and alters the biological activity of a variety of substrates [3••,4,6,14–16]. Some of the Akt-mediated phosphorylations release brakes on proliferation (eg, Akt-mediated phosphorylation leading to cytoplasmic relocalization of the cyclin-dependent kinase inhibitor $p27^{KIP1}$), whereas others result in inactivation of proapoptotic molecules (eg, Akt-mediated phosphorylation of the transcription factor FOXO3a that ordinarily transactivates the genes for Fas ligand and the proapoptotic Bcl-2 family member Bim). In addition, Akt-mediated phosphorylation activates the transcription factor nuclear factor-κB, which transactivates genes for prosurvival molecules such as XIAP and c-FLIP [7,17].

Among the myriad Akt substrates, one that is particularly pertinent to the pathway under discussion is tuberous sclerosis (TSC) protein 2 (also called tuberin). In its unphosphorylated state, TSC2 is complexed with TSC1 (hamartin) and acts as a GTPase-activating protein that facilitates GTP turnover by (and consequence inactivation of) the small guanine nucleotide–binding protein Rheb [1,3••,5]. When TSC2 is phosphorylated by Akt, the TSC1/TSC2 complex is inactivated and Rheb, in its GTP-bound state, remains capable of interacting with and activating the mTOR kinase.

The mTOR kinase, which is encoded by a gene at 1p36, is a 289-kDa serine/threonine kinase that receives multiple upstream signals regarding the nutrient and energy status of the cell [1,5,18]. Integration of these signals by mTOR helps assure that cells enter the cell cycle only if nutrients and energy are sufficient for cell duplication. Upon activation by Rheb, mTOR facilitates cell cycle progression from G1 into S phase by phosphorylating two important cell constituents, p70S6 kinase (p70S6K) and 4E-binding protein 1 (4E-BP1) [19,20].

Mammalian target of rapamycin–mediated phosphorylation activates p70S6K. The latter kinase then phosphorylates and activates S6, a 40S ribosomal subunit polypeptide involved in initiating translation of 5′ terminal oligopyrimidine tract–containing mRNAs that encode components of the protein synthesis machinery [21,22].

Mammalian target of rapamycin–mediated phosphorylation of 4E-BP1 facilitates the translation of a different set of mRNAs. Previous studies have shown that the elongation initiation factor eIF4E is a component of a helicase complex that binds to the cap structure at the 5′ end of mRNAs and enhances the ability of ribosome-eIF complexes to scan the mRNA in search of a translation initiation site [21]. The eIF4E-binding protein 4E-BP1, in its unphosphorylated state, binds to eIF4E and inhibits the eIF4E-containing helicase complex [21,23]. mTOR-mediated phosphorylation of 4E-BP1 diminishes the stability of the 4E-BP1/eIF4E complex, facilitates eIF4E action, and thereby enhances translation of certain mRNAs.

Collectively, activation of S6 and eIF4E by mTOR enhances translation of mRNAs that have extensive

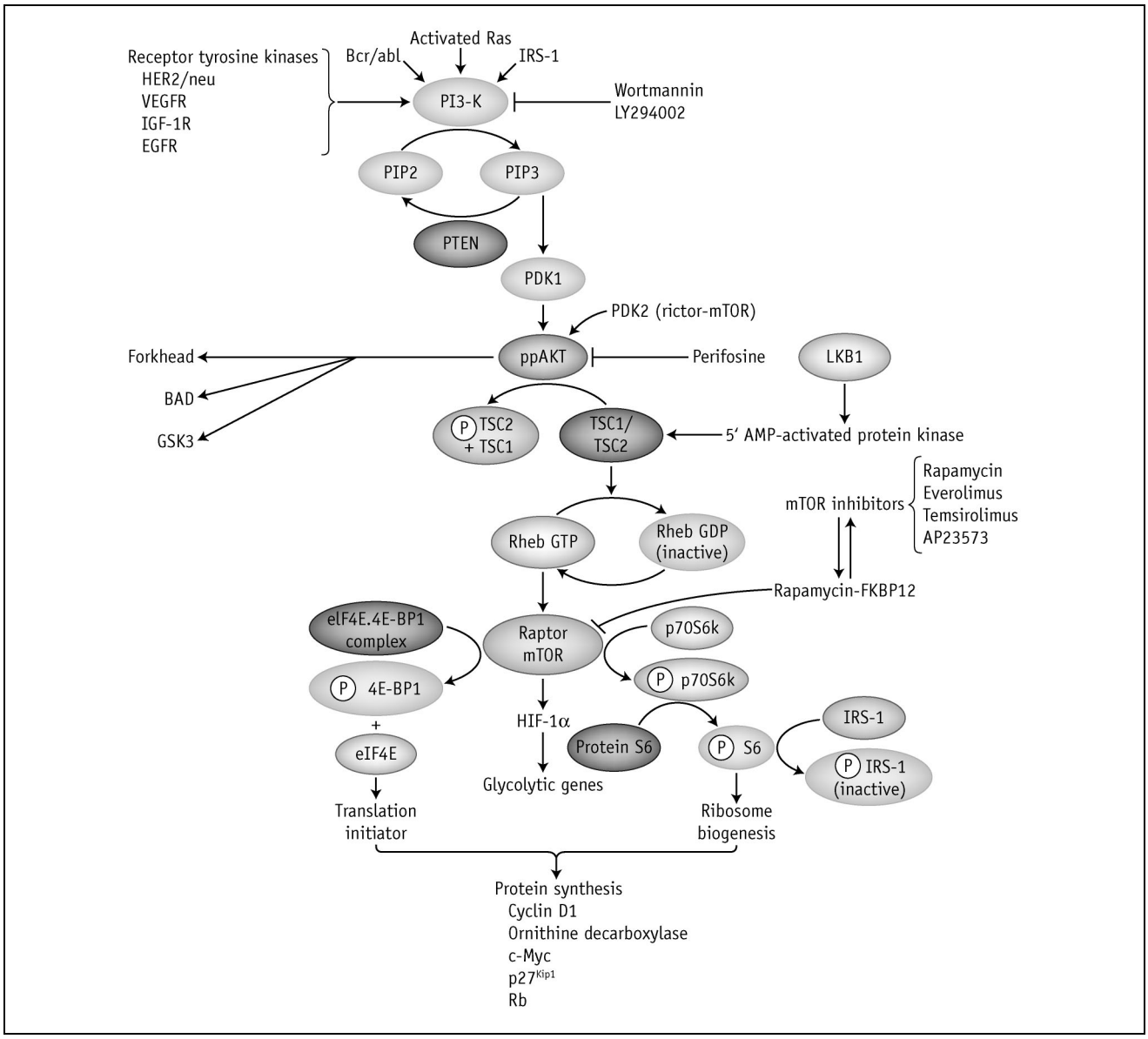

Figure 1. Current understanding of the phosphatidylinositol 3-kinase (PI3-K)/mammalian target of rapamycin (mTOR) pathway and mechanism of action of inhibitors. In this diagram, arrows pointing downward or curved arrows pointing to the right indicate activation, whereas curved arrows pointing left or lines ending in crossbars indicate inhibition (see text for discussion). 4E-BP1—4E-binding protein 1; AKT—α-serine/threonine-protein kinase (protein kinase B); AMP—adenosine monophosphate; BAD—Bcl-xL/Bcl-2–associated death promoter; EGFR—epidermal growth factor receptor; GDP—guanosine 5′-diphosphate; GSK3—glycogen synthase kinase 3; GTP—guanosine 5′-triphosphate; HIF-1α—hypoxia-inducible transcription factor 1α; IGF-1R—insulin-like growth factor 1 receptor; IRS-1—insulin-receptor substrate-1; PDK—phosphoinositide-dependent kinase; PIP2—phosphatidylinositol-4,5-bisphosphate; PIP3—phosphatidylinositol-3,4,5-trisphosphate; TSC—tuberous sclerosis; VEGFR—vascular endothelial growth factor receptor.

secondary structure in their 5′ untranslated regions. Included in this class of transcripts are messages encoding cyclin D1, the transcription factors c-myc, hypoxia-inducible transcription factor 1α (HIF-1α), and signal transducer and activator of transcription 3, ornithine decarboxylase, the growth factors vascular endothelial growth factor and fibroblast growth factor, and ribosomal proteins themselves [1,5,7,21,22,24,25].

In addition to the importance of these molecules for cell survival and proliferation, at least one of the transcripts also has potential importance in terms of monitoring therapy. As is the case with other rapamycin-regulated targets, mTOR regulates the translation of HIF-1α mRNA but not the turnover of the HIF-1α protein [26,27]. Because HIF-1α regulates the glycolytic pathway, fluorodeoxyglucose positron emission tomography, which detects tumors by their increased rate of glycolysis, can potentially be used to assess inhibition of this pathway after treatment with mTOR inhibitors.

REGULATION OF THE PHOSPHATIDYLINOSITOL 3-KINASE/MAMMALIAN TARGET OF RAPAMYCIN PATHWAY

The PI3-K/mTOR pathway is regulated at several critical junctures. One of these involves degradation of PIP3 by lipid phosphatases [3••,6]. The phosphatase and tensin homolog deleted on chromosome 10q23 (PTEN) molecule serves as a negative regulator by converting PIP3 back to PIP2, whereas SHIP2 removes phosphate from the D5 position of inositol. In both cases, destruction of PIP3 is thought to dampen signaling through the pathway and suppress tumor cell proliferation [6]. Conversely, PTEN is deleted, mutated, or silenced by epigenetic changes in more than 50% of carcinomas, thus permitting prolonged transduction of signals through the PI3-K/mTOR pathway [3••,28].

Another important regulatory node occurs at the level of the tumor suppressor complex consisting of TSC1 and TSC2. This complex is inactivated when TSC2 is phosphorylated by activated Akt, protein kinase C, or extracellular signal-regulated kinase (ERK). The ability of all three of these kinases to inactivate the TSC1-TSC2 complex, thereby activating Rheb and mTOR, allows "crosstalk" between important proliferation-inducing pathways. Conversely, the energy-sensing kinase 5′ adenosine monophosphate–activated protein kinase (AMPK), which senses increased levels of 5′ AMP when cells encounter hypoxia or limited energy sources, phosphorylates TSC2 on a different site, activating the TSC1-TSC2 complex and inhibiting mTOR activity [18].

A third important level of regulation occurs at mTOR itself. This kinase exists in mutually exclusive complexes with raptor (regulatory-associated protein of TOR) or rictor (rapamycin-insensitive companion of TOR) [1,5,29]. Importantly, the raptor-mTOR complex is rapamycin sensitive and is responsible for phosphorylation of p70S6K and 4E-BP1, whereas the rictor-mTOR complex is rapamycin insensitive and is one of the enzymes that can catalyze the activating phosphorylation of Akt at Ser473. Prolonged treatment of cells with rapamycin can shift the equilibrium from raptor-mTOR to rictor-mTOR, thus upregulating Akt activity by this feedback mechanism.

INHIBITORS OF THE PHOSPHATIDYLINOSITOL 3-KINASE/MAMMALIAN TARGET OF RAPAMYCIN PATHWAY

Alterations in the PI3-K/mTOR pathway are observed in a wide range of malignancies. These changes include amplifications or activating mutations of PI3-K [7,30], mutation or silencing of PTEN [3••,31], and amplification or activation of Akt [1,30]. Less commonly, loss of TSC1, TSC2, or LKB1 (a kinase upstream of AMPK) occurs. In each case, these changes result in increased or prolonged propagation of signals through the PI3-K/mTOR pathway, thereby enhancing cell survival [7] and chemoresistance [32]. These observations have prompted considerable interest in components of the PI3-K/mTOR pathway as potential antineoplastic targets.

Over the past decade substantial effort has gone into developing inhibitors of this pathway [1,30]. The following discussion emphasizes agents currently being tested in hematologic malignancies. For a more comprehensive review, the reader is referred to the article by Granville et al. [33•].

Phosphatidylinositol 3-kinase inhibitors LY294002 is a synthetic flavonoid that reversibly inhibits PI3-K by competing with adenosine triphosphate for binding to the enzyme [34]. Uddin et al. [35] demonstrated that LY294002 could induce apoptosis in primary effusion lymphoma cell lines that contained constitutively activated Akt. Wortmannin is also a PI3-K inhibitor that has undergone extensive testing in vitro. Although very useful in the laboratory, LY294002 and wortmannin are unsuitable for human testing because of their chemical instability and hepatotoxicity. In addition, the importance of PI3-K in insulin signaling and cerebral function makes it likely that treatment with these agents would be associated with substantial side effects.

Akt inhibitors A variety of Akt inhibitors have been examined in preclinical studies. In many cases these are lipid mimetics and exhibit little selectivity between PI3-K, PDK1, and Akt. Abbott Laboratories (North Chicago, IL) has developed A443564, a nonlipid-based molecule that inhibits Akt signaling at submicromolar concentrations in cells [36]. Preclinical studies of this compound have been reported in solid tumor cell lines but not in models of hematologic malignancies.

Perifosine is a synthetic alkylphospholipid that binds plasma membranes and inhibits Akt activation without any direct effect on related kinases such as PI3-K or PDK1. Hideshima et al. [37] have recently reported that perifosine is able to completely inhibit the constitutive phosphorylation of Akt in multiple myeloma (MM) cells in vitro, as well as basal and interleukin-6–stimulated phosphorylation of the Akt target glycogen synthase kinase 3β. These changes are accompanied by increased mitogen-activated protein extracellular kinase and ERK phosphorylation. At concentrations in which peripheral blood mononuclear cells from normal volunteers are unaffected, perifosine kills plasma cells from myeloma patients. Further studies have demonstrated that perifosine induces typical apoptotic biochemical changes in myeloma cell lines in vitro. Perifosine is also able to block the proliferative response typically observed in myeloma cells after adherence to stroma in vitro and reduce tumor growth in a murine plasmacytoma model in vivo.

Although there are no reports of perifosine being tested in patients with hematologic malignancies, phase I

studies in patients with solid tumors have been completed. Because of hemolysis after intravenous administration, this agent is administered orally. When perifosine was taken for 21 of every 28 days, major toxicities included nausea, vomiting, and fatigue, and a maximum tolerated dose (MTD) of 200 mg/day was identified [38]. When perifosine was administered using a loading dose over 4 days and then daily maintenance without interruption, the gastrointestinal toxicity and fatigue were again dose-limiting. The MTD was defined as 150 mg loading dosage for 4 to 6 days followed by a maintenance dosage of 100 mg/day [39].

Mammalian target of rapamycin inhibitors Rapamycin (sirolimus) is a macrolide antibiotic derived from the bacteria *Streptococcus hygroscopicus* [40–42]. The compound was isolated from a soil sample from Easter Island (Rapa Nui, hence the name rapamycin) and was approved as an oral immunosuppressant to prevent acute rejection in 1999 [1,5]. Rapamycin is unable to directly inhibit mTOR. Instead, it binds to FK506 binding protein 12 (FKBP12), an abundant cytoplasmic protein, and the rapamycin/FKBP12 complex then binds to and inhibits mTOR [1,5]. Although rapamycin has been extensively studied as an immunosuppressant, there has been only very limited testing of this drug as an antineoplastic agent for hematologic malignancies.

Currently there are three rapamycin analogs in clinical trials: temsirolimus, everolimus, and AP23573 (Ariad Pharmaceuticals, Cambridge, MA) [1,43]. Temsirolimus (CCI-779; Wyeth Pharmaceuticals, Madison, NJ) is an ester of rapamycin that is available as an intravenous or oral formulation. Everolimus (40-O-[2-hydroxyethyl]-rapamycin, RAD001; Novartis, Basel, Switzerland) is an oral mTOR inhibitor that is approved in Europe as an immunosuppressive agent for solid organ transplantation. A third mTOR inhibitor, AP23573, can be delivered orally or intravenously. All three of these agents have been tested extensively in patients with solid tumors [43]. The following sections review pertinent preclinical and clinical studies performed in hematologic malignancies.

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Treatment

Disease-specific activity of mammalian target of rapamycin inhibitors

Acute leukemia

- The activity of rapamycin against acute myelogenous leukemia (AML) has been studied in vitro and in one small clinical trial. Recher et al. [44] demonstrated that rapamycin upregulated the CDK inhibitor p27 and inhibited proliferation of AML cell lines in vitro. Analysis of blasts from 22 patients revealed phosphorylation of S6 kinase at Thr389 in 77% (17/22) of specimens and phosphorylation of 4E-BP1 in 100%, documenting mTOR activation in clinical AML. Subsequent treatment of nine relapsed AML patients with oral rapamycin at a dose of 6 mg on day 1 followed by 2 mg daily for 28 days resulted in four partial remissions (PRs), with a median duration of response of 38 days (range 35–120 days). In vitro studies have also demonstrated activity of temsirolimus in acute lymphoblastic leukemia cells [45], but no trials of this drug or everolimus in acute leukemia patients have been reported to date.
- AP23573 was tested in a phase II study in patients with relapsed or refractory hematologic malignancies. In this open-label nonrandomized parallel study of five disease-specific cohorts, patients received a fixed dose of AP23573 intravenously over 30 minutes daily for 5 days every 2 weeks. Preliminary results demonstrated minor responses in some patients with AML [46]. The toxicity of mTOR inhibitors in this setting was primarily mucositis, rash, nausea, and myelosuppression. Thrombocytopenia was more common than neutropenia [46,47•].

Lymphoma

- Several rapamycin analogs have been examined for potential antilymphoma activity. In vitro studies with the human mantle cell lymphoma (MCL) line M0258 demonstrated constitutive phosphorylation of p70S6

kinase and its substrate S6, indicating constitutive of mTOR activation. With rapamycin treatment, inhibition of S6 phosphorylation was readily detectable at 0.1 nM rapamycin and essentially complete at 1 nM [47•]. Consistent with these observations, rapamycin was observed to inhibit proliferation of MCL lines and exhibit cytotoxicity in clinical MCL samples in vitro [48]. No clinical trials of oral sirolimus as a potential antilymphoma agent have been reported to date.

- In a recently reported phase II trial [47•], temsirolimus was administered to patients with relapsed/refractory MCL as a 250-mg weekly intravenous bolus. MCL was chosen for this study because of the critical role of dysregulated cyclin D1 expression in the pathogenesis of this disorder. The overall response rate was 38% (13/34; 90% CI = 24% to 54%), with one complete response and 12 PRs. The median time to progression and overall survival were 6.5 months (95% CI = 2.9–8.3 months) and 12 months (95% CI = 6.7 months to not yet reached), respectively. The median duration of response for the 13 responders was 6.9 months (95% CI = 5.2–12.4 months).
- In this trial, temsirolimus dose reductions were necessary in all but four patients, and the median dose received per month on study was 564 mg in responding patients and 525 mg in nonresponders [47•]. Thrombocytopenia was the cause of most dose reductions and was rapidly reversible, with drug delays of typically only 1 week. Only three patients required platelet transfusions, and four patients required red blood cell transfusions. Other toxicities commonly observed were hyperglycemia, hyperlipidemia, fatigue, and rash. No grade 5 events (deaths on treatment) were reported.
- The frequency of dose reductions after administration of temsirolimus 250 mg intravenously weekly led to a subsequent study in which patients with the same eligibility criteria received a dosage of 25 mg temsirolimus intravenously weekly. Preliminary results suggest a similar response rate with less hematologic toxicity in this follow-up study [49].
- It is clear from these studies that temsirolimus exhibits substantial single-agent activity in MCL. An international randomized phase III trial comparing two different doses of temsirolimus with control chemotherapy in relapsed MCL is ongoing. In addition, there are several ongoing phase II trials in the United States assessing possible activity of this agent in other types of NHL.
- Consistent with these observations, other mTOR inhibitors also appear to have antilymphoma activity, although the available data are less complete. Everolimus inhibits the growth of lymphoma cell lines and induces cell death in clinical lymphoma specimens in vitro [50,51]. Its clinical antilymphoma activity is being assessed in an ongoing trial in patients with NHL and Hodgkin disease, but no results have been reported to date. In a trial of fixed-dose intravenous AP23573 in patients with lymphoid malignancies, one of nine patients with chronic lymphocytic leukemia had a PR [46].

Multiple myeloma

- Rapamycin has demonstrated activity in vitro against MM cell lines as a single agent and in combination with the immunomodulatory drug CC-5013 [52]. Frost et al. [53] studied the effect of parenteral temsirolimus in a mouse model of MM. Temsirolimus was shown to inhibit the growth of human myeloma cell lines by not only inducing G1 cell cycle arrest and apoptosis, but also by reducing tumor angiogenesis. Clinical trials of single-agent temsirolimus and everolimus for patients with relapsed MM are ongoing.

Current treatment options

- At this time, none of the PI3-K/mTOR pathway inhibitors are approved for the treatment of cancer. Although oral sirolimus is available in the United States and everolimus is available in Europe, these agents are only approved as immunosuppressants for transplantation. Clinical trials of the mTOR inhibitors temsirolimus, everolimus, and AP23573 as well as the Akt activation inhibitor perifosine as potential treatments for various hematologic malignancies are currently ongoing.

Comparison of treatment options

- It is premature to speculate about the activity of these agents and their ultimate role in the treatment of hematologic malignancies. As outlined earlier, rapamycin and AP23573 exhibit activity in AML, and temsirolimus exhibits activity against MCL. Because there are many different subtypes of lymphoma, further studies are required to define the clinical setting where mTOR inhibitors will provide the greatest benefit. After these initial single-agent studies, rational combination studies will need to be completed to maximize the efficacy of this class of novel agents.

Potential for synergy

- Current understanding suggests that signal transduction inhibitors will usually be most effective in combination with other signal transduction inhibitors or with traditional chemotherapy [30]. There are at least two approaches to developing these combinations. One involves combining agents that inhibit several steps in a single critical pathway to achieve greater overall pathway inhibition (so-called vertical combinations). The other involves administering agents that affect different pathways implicated in neoplastic transformation (so-called horizontal combinations). In order to develop combinations involving mTOR inhibitors, it will be important to understand the alterations of various pathways in neoplastic cells, which undoubtedly will vary among different types of malignancies and even within the B-cell neoplasms.
- In developing combinations involving the mTOR inhibitors, it will also be important to recognize that blocking one pathway may cause the cell to signal through an alternate pathway, thus overcoming the effect of the block. Examples of this phenomenon have already been described in conjunction with PI3-K/mTOR pathway inhibitors. For instance, prolonged treatment of cells with rapamycin can shift the equilibrium from raptor-mTOR to rictor-mTOR, which can then activate Akt. As another example, activated p70S6 kinase normally phosphorylates insulin-receptor substrate-1 (IRS-1), causing the latter to dissociate from the IGF-1 receptor and thereby dampening IGF-1–induced signaling through the PI3-K/mTOR pathway [5]. When rapamycin inhibits mTOR, p70S6 kinase activity is diminished, IRS-1 phosphorylation is decreased, and signaling from IGF-1 receptor through PI33-K to Akt is enhanced. Consistent with these observations, temsirolimus has been shown to induce activation of Akt in myeloma cells [36] and solid tumor cell lines [9], providing a rationale for using combinations of mTOR inhibitors (such as temsirolimus or everolimus) with an Akt activation inhibitor such as perifosine. The full extent and implications of various feedback loops in the PI3-K/mTOR pathway are still in the process of being examined.

- Several preclinical studies have identified potentially interesting effects when mTOR inhibitors are combined with other agents. For example, the recently licensed B-Raf inhibitor sorafenib synergizes with rapamycin when tested against melanoma cell lines in vitro [54]. Likewise, recent in vitro experiments demonstrate synergy between rapamycin and imatinib in Bcr-Abl–expressing cells [11]. Rapamycin and/or its analogs also synergize with DNA-damaging agents such as cisplatin [55] and etoposide [56] in vitro and with cyclophosphamide in a murine lymphoma model in vivo [57].
- Some of the future combinations might involve classes of drugs that are not considered antineoplastic agents at present. Through a pathway that remains poorly understood, 3′,5′-cyclic AMP (cAMP) elevation is associated with inhibition of the PI3-K/Akt pathway as well as induction of cell cycle arrest and apoptosis in diffuse large cell lymphoma (DLCL) cells [58]. Importantly, overexpression of the phosphodiesterase PDE4B, which degrades cAMP, was shown to activate PI3-K/Akt signaling in lymphoma cell lines and to be associated with a poor prognosis in clinical DLCL. Further studies have suggested that PDE4B inhibitors, which are currently in clinical trials, may synergize with PI3-K inhibitors [58].
- Although the preceding studies suggest several novel combinations for possible future testing, it is important to emphasize that not all of the recently identified drug-drug interactions are favorable. Retinoids have been demonstrated to have important antitumor activity in the acute promyelocytic (M3) type of AML. Lal et al. [59] demonstrated that all-trans retinoic acid, one of the mainstays of therapy for M3 AML, activates the PI3-K/mTOR pathway, resulting in p70S6 kinase phosphorylation. This activation could be blocked at the mTOR level by rapamycin and at the PI3-K level by LY294002. The authors appropriately caution that combining all-trans retinoic acid and PI3-K/mTOR pathway inhibitors may not be a wise choice in this type of AML.

Future directions

- The PI3-K/mTOR pathway plays an important role in many hematologic malignancies. Promising data have already emerged from the trials of mTOR inhibition in MCL. Definitive trials of these drugs as single agents in all of the common hematologic malignancies—leukemias, lymphomas, and myeloproliferative disorders—seem to be warranted. If these new drugs demonstrate single-agent activity, rational combinations with other signal transduction inhibitors and with conventional antineoplastic agents should be examined. We hope that this approach will lead to much needed improvements in the treatment of one or more of the hematologic malignancies.

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