Dual mTORC2/mTORC1 Targeting Results in Potent Suppressive Effects on Acute Myeloid Leukemia (AML) Progenitors


Abstract

Purpose: To determine whether mTORC2 and rapamycin-insensitive (RI)-mTORC1 complexes are present in acute myeloid leukemia (AML) cells and to examine the effects of dual mTORC2/mTORC1 inhibition on primitive AML leukemic progenitors.

Experimental Design: Combinations of different experimental approaches were used, including immunoblotting to detect phosphorylated/activated forms of elements of the mTOR pathway in leukemic cell lines and primary AML blasts; cell-proliferation assays; direct assessment of mRNA translation in polysomal fractions of leukemic cells; and clonogenic assays in methylcellulose to evaluate leukemic progenitor-colony formation.

Results: mTORC2 complexes are active in AML cells and play critical roles in leukemogenesis. RI-mTORC1 complexes are also formed and regulate the activity of the translational repressor 4E-BP1 in AML cells. OSI-027 blocks mTORC1 and mTORC2 activities and suppresses mRNA translation of cyclin D1 and other genes that mediate proliferative responses in AML cells. Moreover, OSI-027 acts as a potent suppressor of primitive leukemic precursors from AML patients and is much more effective than rapamycin in eliciting antileukemic effects in vitro.

Conclusions: Dual targeting of mTORC2 and mTORC1 results in potent suppressive effects on primitive leukemic progenitors from AML patients. Inhibition of the mTOR catalytic site with OSI-027 results in suppression of both mTORC2 and RI-mTORC1 complexes and elicits much more potent antileukemic responses than selective mTORC1 targeting with rapamycin.

Introduction

Despite recent advances in the field, acute myeloid leukemia (AML) remains highly fatal. Although a large number of AML patients achieve clinical remission with current treatment regimens, the majority of patients eventually relapse and die (1, 2). Undoubtedly, there is an urgent need to develop new therapeutic approaches to overcome the resistance that AML cells develop to chemotherapy (1, 2). Given the limited efficacy of current treatment regimens, the majority of patients eventually relapse and overexpression or constitutive activation of components of the mTOR pathway may contribute to a poorer prognosis (3). Changes in transcriptional control of hematopoietic cells and mutations involving different proto-oncogenes or tumor suppressors account for the deregulation of normal myelopoiesis (3). Such changes ultimately result in the activation of cellular pathways that mediate signals, which promote cell growth and/or maintain survival, leading to leukemogenesis (4, 5).

The phosphoinositide 3-kinase (PI3K)/Akt pathway is a critical network of signals that control key cellular functions, including gene transcription, differentiation and mRNA translation, and survival (6–8). This pathway is frequently dysregulated in various tumors (6–8). Extensive work over the years has established that activation of PI3K/Akt signaling results in a growth advantage for tumor cells by promoting cell proliferation and anti-apoptotic signals (6, 7, 9). There is evidence that PI3K/Akt activation contributes to tumor cell resistance to antineoplastic therapies, and overexpression or constitutive activation of components of this pathway may contribute to a poorer prognosis in different malignancies (reviewed in refs. 10, 11).

mTOR is a kinase activated downstream of PI3K/Akt that plays critical and essential roles in the regulation of mRNA translation, cell-cycle progression, and growth of malignant cells (6, 12–14). Because mTOR mediates pro-growth signals in malignant cells, its pharmacologic targeting is currently under extensive investigation for the treatment of various malignancies, including leukemias (11, 15). In fact, 2 rapamycin-related drugs, temsirolimus and everolimus, have
significant activity in the treatment of renal cell carcinoma (16, 17) and were recently approved by the Food and Drug Administration (FDA) for the treatment of this disease.

mTOR inhibitors that are currently approved for clinical use (e.g., rapamycin, temsirolimus, everolimus) are allosteric inhibitors of mTORC1 but not of mTORC2 (18), however the precise functional roles of mTORC2 and other rapamycin-insensitive (RI) complexes in AML cells is unknown. In the present study, we provided direct evidence for the presence and activity of mTORC2 complexes in AML cell lines and primary AML cells. Furthermore, we established that mTOR-mediated phosphorylation of RI sites on the translational repressor 4E-BP1 plays a key role in mRNA translation in AML cells and regulates cyclin D1 expression. We show that activation of mTORC2 and RI-mTORC1 complexes is selectively inhibited by a novel dual mTORC1/mTORC2 inhibitor OSI-027, has greater antileukemic effects than rapamycin on AML progenitors. These findings provide the basis for the development of clinical–translational efforts involving mTORC1/mTORC2 inhibitors, such as OSI-027, or combinations of such agents with chemotherapeutic agents for the treatment of AML.

mTOR provides an attractive cellular target for the development of new therapeutic approaches for hematologic malignancies, but a major limitation of the classic mTOR inhibitor rapamycin and related rapalogues is their selectivity for mTORC1 and their inability to effectively inhibit mTORC2. In the present study, we provide evidence for constitutive formation of mTORC2 and rapamycin-insensitive (RI)-mTORC1 complexes in AML cells and establish that dual mTORC2/mTORC1 inhibition using a novel catalytic inhibitor of mTOR, OSI-027, has greater antileukemic effects than rapamycin on AML progenitors. These findings provide the basis for the development of clinical–translational efforts involving mTORC1/mTORC2 inhibitors, such as OSI-027, or combinations of such agents with chemotherapeutic agents for the treatment of AML.

**Materials and Methods**

**Cells and reagents**

The KG-1, HL-60, KBM-3B, and U937 human leukemia cell lines were cultured in RPMI 1640 supplemented with 10% FBS and 1% L-glutamine. U937, KG-1, and HL60 cells were obtained from the American Type Culture Collection (ATCC). ML-1 and KBM-3B cells were generously provided by E. Harlow, Cold Spring Harbor Laboratories. Rapamycin was purchased from Calbiochem/EMD. Cytarabine (AraC) was purchased from Sigma. All antibodies were purchased from Cell Signaling Technologies except the antibody against PDCD4, which was purchased from Rockland, and the antibody against glyceraldehyde 3-phosphate dehydrogenase (GAPDH), which was purchased from Millipore.

**Measurement of proliferation**

Inhibition of proliferation was measured using the Cell Titer Glo Assay (Promega Corporation), as noted in figure legends. To generate dose–response curves, cell lines were seeded at a density of 5,000 cells per well in a 96-well plate. After 24 hours of plating, cells were dosed with varying concentrations of either OSI-027 or rapamycin. The signal for Cell Titer Glo Assay was determined 72 hours after dosing and normalized to that of vehicle-treated controls. Inhibition of proliferation, relative to vehicle-treated controls, was expressed as a fraction of 1 and graphed using PRISM software (Graphpad Software).

**Cell lysis and immunoblotting**

For the immunoblotting experiments, cells were treated with rapamycin (20 nmol/L), OSI-027, or dimethyl sulfoxide (DMSO), used as control for untreated cells, for the indicated times and lysed in phosphorylation lysis buffer. Immunoblotting using an enhanced chemiluminescence (ECL) method was done as in our previous studies (20, 21).

**Isolation of polysomal RNA and quantitative reverse transcriptase PCR in polysomal fractions**

U937 cells were treated with DMSO, rapamycin, or OSI-027, and polysomal fractionation and quantitative reverse transcriptase PCR (RT-PCR) were carried out with slight modifications of the protocol used in our previous studies (22, 23).

**Studies with primary AML samples and hematopoietic progenitor cell assays**

Peripheral blood was obtained from patients with AML after obtaining informed consent, following approval by the Institutional Review Board of the Northwestern University. Cells were separated over Ficoll–Hypaque and cultured with the indicated concentrations of rapamycin, OSI-027, and/or AraC, and leukemic progenitor (CFU-L) colony formation was assessed in clonogenic assays in methylcellulose (21, 24–27).

**Short hairpin RNA-mediated knockdown**

U937 cells were transduced with lentiviral particles (28–30), expressing either control-shRNA or S6K1 (p70 S6 kinase α) short hairpin RNAs (shRNA; Santa Cruz Biotechnology, Inc.) according to the manufacturer’s instructions. Briefly, 5 × 10⁴ cells/mL were infected with the abovementioned lentviruses, and selected in puromycin, as recommended.
Results

In initial studies, we determined whether functional mTORC1 and mTORC2 complexes are present in AML cells and evaluated the effects of OSI-027 or rapamycin on their activation. Using the AML cell line U937 or circulating primary leukemic AML blasts, we examined the phosphorylation of Akt on serine 473 (Ser473), a marker of mTORC2/Akt activity. As shown in Figure 1, Akt phosphorylation was detectable in AML cells, and it was completely inhibited by treatment of cells with OSI-027 (Fig. 1A and B). On the other hand, rapamycin did not inhibit such phosphorylation (Fig. 1A and B) and, in fact, it had a slight stimulatory effect in U937 cells (Fig. 1A), a finding consistent with the previously described rapamycin-mediated inhibition of the S6K–IRS negative feedback loop, which results in enhanced signaling through the mTORC2/Akt node (reviewed in ref. 31). Furthermore, we examined the phosphorylation of mTOR on Ser2448, a marker for mTORC1 activity, as well as the phosphorylation of the protein on Ser2481, a marker for mTORC2 activity (32). Phosphorylation of mTOR on both Ser2448 and Ser2481 was detectable in U937 cells (Fig. 1C and D), reflecting the presence of both mTORC1 and mTORC2 complexes. Ser2448 phosphorylation was inhibited by treatment of cells with either rapamycin or OSI-027 (Fig. 1C), consistent with the expected sensitivity of mTORC1 complexes to both inhibitors. However, only OSI-027 inhibited phosphorylation on Ser2481, showing mTORC2 targeting (Fig. 1D). Similar results were obtained when the AML cell line, KG-1 (Fig. 1E), or primary leukemic blasts from a patient with AML (Fig. 1F) were studied. mTORC1 kinase activity could be also directly shown in in vitro kinase assays on anti-Raptor immunoprecipitates from U937 cell lysates by using S6K as a substrate (Supplementary Fig. S1). These data clearly established the presence of mTORC1 kinase activity and showed decreased phosphorylation of S6K substrate upon treatment with OSI-027 (Supplementary Fig. S1A). Notably, in these studies it...
was also shown that, in contrast to rapamycin, the suppression of mTOR kinase activity occurs without disruption of the protein–protein interactions in the mTORC1 complex, a finding consistent with ATP-competitive catalytic inhibition of mTOR by OSI-027 (Supplementary Fig. S1B).

A major effector of activated mTORC1 complexes is S6K, which, in turn, phosphorylates and activates several downstream effectors, including the S6 ribosomal protein (rpS6) and the eukaryotic initiation factor 4B (eIF4B; ref. 6, 13). When different AML cell lines or primary AML blasts were treated with rapamycin or OSI-027, there was complete suppression of phosphorylation of S6K on threonine 389 (Thr389), a site whose phosphorylation correlates with activation of its kinase domain (Fig. 2A–C). Consistent with the suppressive effects of both rapamycin and OSI-027 on S6K activity (Fig. 2A–C), there was upregulation of PDCD4 expression in response to treatment of Ph+ cells (35). Consistent with the suppressive effects of both rapamycin and OSI-027 on S6K activity (Fig. 2A–C), there was upregulation of PDCD4 expression in response to treatment of AML cells with either inhibitor (Fig. 3A and B). Thus, both

![Figure 2](image-url)

Figure 2. Phosphorylation/activation of S6K and S6 ribosomal protein in AML cells. A–C, U937 cells (A), KG-1 cells (B), or primary leukemic blasts from an AML patient (C) were incubated with OSI-027 (10 μmol/L) or rapamycin (20 nmol/L) for the indicated times. Cell lysates were resolved by SDS-PAGE and immunoblotted with an antibody against the phosphorylated form of S6K on Thr389, or with antibodies against S6K or GAPDH, as indicated. D–I, U937 cells (D, G) KG-1 cells (E, H), or primary AML blasts (F, I) were incubated in the absence or presence of rapamycin (20 nmol/L) or OSI-027 (10 μmol/L) as indicated. Cell lysates were resolved by SDS-PAGE and immunoblotted with an antibody against the phosphorylated form of rpS6 on Ser235/236 (D–F), against the phosphorylated form of rpS6 on Ser240/244 (G–I), or against rpS6, as indicated.

Published OnlineFirst March 17, 2011; DOI: 10.1158/1078-0432.CCR-10-2285
rapamycin and OSI-027 upregulate PDCD4, suggesting that this S6K target is a mediator of the antileukemic responses elicited by targeting mTORC1 complexes. To define the functional consequences of inhibition of S6K phosphorylation in leukemic progenitors, experiments were conducted in which S6K1 was knocked down using S6K1-shRNA in a lentiviral vector (Fig. 3C). These studies showed substantial inhibition in leukemic CFU-L colony formation by S6K1 knockdown (Fig. 3D), suggesting an important role for S6K1 activity and suppression of PDCD4 in the growth of AML progenitors.

A key functional cellular response downstream of the mTOR pathway is the regulation of the initiation of cap-dependent mRNA translation, which occurs primarily via regulation of the 4E-BP1 repressor of mRNA translation (6, 13, 14). 4E-BP1 is phosphorylated in a hierarchic manner on Thr37/46, Ser65, and Thr70; and such phosphorylation is essential for the deactivation of the protein and its subsequent dissociation from the eukaryotic initiation factor 4E (eIF4E), so that cap-dependent mRNA translation can proceed (36, 37). Significant baseline levels of phosphorylation of 4E-BP1 on Thr37/46 and Ser65 were detectable in both U937 and KG-1 cells (Fig. 4A and B). This type of phosphorylation was completely blocked by treatment of cells with OSI-027, whereas treatment with rapamycin had no effect (Fig. 4A and B). Similar results were also seen when the phosphorylation of the protein on Thr70 was studied (Fig. 4C). OSI-027 was used at a maximal concentration of 10 μmol/L, which inhibits both mTORC1 and mTORC2 (19) and is readily secreted in plasma in preclinical rodent models (38). Rapamycin was dosed at a maximal concentration of 20 nmol/L, which is consistent with multiple published studies and exceeds the dose required to inhibit mTORC1 function (38, 39). In dose–response experiments, we found that rapamycin failed to block Thr37/46 4E-BP1 phosphorylation even at very high, supra-normal concentrations (Fig. 4D), thus definitively establishing that 4E-BP1 phosphorylation at Thr37/46 is an RI event. A similar pattern of 4E-BP1 dephosphorylation in response to OSI-027 or rapamycin was seen in studies using primary AML leukemic blasts (Fig. 4E and F; Supplementary Fig. S2). Consistent with the complete suppression of 4E-BP1 phosphorylation, OSI-027 treatment resulted in the formation of 4E–BP1–eIF4E complexes that suppress cap-dependent mRNA translation, while it inhibited the formation of eIF4E–eIF4G complexes that are required for initiation of translation (Fig. 4G). On the other hand, rapamycin had much weaker effects in promoting formation of 4E–BP1–eIF4E complexes and it did not disrupt formation of eIF4E–

---

**Figure 3.** Effects of mTOR inhibition on the expression of the S6K-dependent PDCD4 tumor suppressor in AML cells and functional consequences of S6K knockdown. A and B, U937 (A) or KG-1 (B) cells were incubated in the absence (lane 1) or presence of OSI-027 at concentrations of either 5 μmol/L (lane 2) or 10 μmol/L (lane 3) or rapamycin (20 nmol/L; lane 4) for 24 hours. Cell lysates were resolved by SDS-PAGE and immunoblotted with an antibody against PDCD4. The same blots were re-probed with an antibody against tubulin, to control for protein loading. C, cell lysates from lentivirus transduced control (ctrl)-shRNA or S6K1-shRNA U937 cells were resolved by SDS-PAGE and immunoblotted with an anti-S6K1 antibody. The same blot was then re-probed with an anti-GAPDH antibody to control for protein loading. D, leukemic CFU-L colony formation derived from control-shRNA and S6K1-shRNA U937 cells is shown. Data are expressed as percentages of colony formation of untreated control-shRNA transduced cells and represent means ± SE of 5 independent experiments.
eIF4G complexes (Fig. 4G). Thus, OSI-27 is a potent inhibitor of mTOR activity in AML cells and suppresses formation of complexes that regulate cap-dependent mRNA translation.

The suppressive effects of OSI-027 on formation of eIF4E–eIF4G complexes prompted us to directly examine the effects of OSI-027 on mRNA recruitment to polysomal fractions in AML cells. OSI-027 treatment resulted in suppression of mRNA recruitment to polysomes, reflecting potent inhibitory effects on translation (Fig. 5A and B). Notably, the effects of OSI-027 were much more pronounced than the effects of rapamycin analyzed in parallel (Fig. 5A and B). In addition, when mRNA translation for cyclin D1 was directly analyzed in polysomal fractions from U937 cells, we found suppression by OSI-027, but not rapamycin (Fig. 5C). Consistent with the effects seen in the suppression of cyclin D1 mRNA translation, time-dependent suppression of cyclin D1 protein expression after OSI-027 treatment was documented (Fig. 5D). Thus, mTORC1-mediated phosphorylation of 4E-BP1 is an R1 event that plays a key role in mRNA translation for cyclin D1 and possibly other genes whose products control cell-cycle progression in AML cells. These findings prompted us to directly examine the effects of dual mTORC1/mTORC2 inhibition in AML cells. Treatment of U937 cells with OSI-O27 resulted in G0/G1 arrest (Supplementary Fig. S3), consistent with the suppressive effects of this agent on cyclin D1 mRNA translation and protein expression.

In subsequent studies, the effects of dual mTORC2/mTORC1 inhibition on cell proliferation of several AML cell lines were examined. As shown in Figure 6A, OSI-027 (µmol/L) 0.02   1   5       0     0.5    1    5

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>OSI-027</th>
<th>Rapamycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>90</td>
<td>90</td>
<td>90</td>
</tr>
</tbody>
</table>

Blot: anti-phospho rpS6 S240/244
Blot: anti-phospho 4E-BP1 Thr37/46
Blot: GAPDH

Figure 4. OSI-027, but not rapamycin, inhibits phosphorylation of the 4E-BP1 repressor of mRNA translation and blocks formation of complexes required for cap-dependent mRNA translation. A and B, U937 or KG-1 cells were incubated in the absence or presence of OSI-027 (10 µmol/L) or rapamycin (20 nmol/L) for 90 minutes. Cell lysates were resolved by SDS-PAGE and immunoblotted with an antibody against the phosphorylated form of 4E-BP1 on Thr37/46 (A) or Ser65 (B). Subsequently, the same blots were re-probed with an antibody against 4E-BP1 or against GAPDH, to control for protein loading. C, KG-1 cells were incubated in the absence or presence of OSI-027 (10 µmol/L) or rapamycin (20 nmol/L) for 60 minutes. Cell lysates were resolved by SDS-PAGE and immunoblotted with a antibody against the phosphorylated form of 4E-BP1 on Thr70. The same blot was re-probed with an antibody against 4E-BP1 or against GAPDH. D, U937 cells were treated with increasing concentrations of rapamycin or OSI, as indicated for 90 minutes. Cell lysates were resolved by SDS-PAGE and immunoblotted with antibodies against the phosphorylated form of rpS6 at Ser240/244, the phosphorylated form of 4E-BP1 on Thr37/46, or against GAPDH as indicated.
inhibited the growth of several acute leukemia cell lines of myeloid/megakaryocytic origin in a dose-dependent manner, including U937, KG-1, KBM-3B, ML-1, HL-60, and MEG-01 cells (Fig. 6A). On the other hand, rapamycin treatment of such AML cell lines had minimal effects on proliferation (Fig. 6B).

To determine the effects of OSI-027 in a more functionally relevant system, experiments were done in which its effects on primary leukemic progenitor colony formation (CFU-L) from patients with AML were examined in a clonogenic survival assay in methylcellulose, using standard approaches (24–27). OSI-027 exhibited very potent dose-dependent suppressive effects on primary leukemic progenitors (Fig. 6C), and such effects were clearly more potent than the effects of rapamycin (Fig. 6C). Furthermore, we assessed whether OSI-027 exhibits enhancing effects on the antileukemic properties of very low concentrations of AraC; OSI-027 was combined with very low concentrations of AraC that have minimal antileukemic effects when used alone. As shown in Figure 6D, combined addition of low-dose AraC and OSI-027 resulted in more suppressive effects than did each agent alone (Fig. 6D), suggesting that combinations of OSI-027 with chemotherapeutic agents may ultimately provide an approach to improve generation of antileukemic responses.

**Discussion**

Because of its critical role in regulation of malignant cell growth and survival, the Akt/mTOR pathway has been identified as an attractive target for the treatment of various malignancies. Over the last few years, there has been a significant effort to precisely define the role of this pathway in leukemogenesis and to incorporate its targeting in the development of novel approaches for the treatment of AML. Previous work has shown that there is constitutive phosphorylation and activation of Akt in primary AML blasts, whereas normal human CD34+ cells have only low levels of Akt activation (40, 41). A role for the PI3K in such regulation of Akt activity has been suggested based on the fact that pharmacologic inhibition of PI3K using LY294002 blocks growth of primary leukemic blasts (40). Interestingly, phosphorylation of Akt on the PDK1 phosphorylation site, Thr308, has been associated with high-risk cytogenetics and poor overall survival in AML (42). Other studies have shown that mTOR inhibition using rapamycin increases the cytotoxicity of etoposide chemotherapy against AML blasts in vitro (43). On the other hand, more recent work has shown that mTORC1 targeting using the rapamycin derivative everolimus (RAD001) fails to inhibit eIF4F assembly and c-Myc mRNA translation in AML cells and does not induce apoptosis of leukemic blasts (44). A phase I, dose-escalation study of rapamycin in combination with the chemotherapy regimen MEC (mitoxantrone, etoposide, and AraC) in AML was recently reported (45). The study showed that combination of rapamycin and chemotherapy was a feasible regimen, but failed to show synergistic effects between that mTOR inhibitor and chemotherapy in vivo (45).

Although the role of mTORC1-mediated signaling in AML is well established, the precise functional relevance of mTORC2 complexes has been unknown. As rapalogues have only limited antileukemic effects, this has suggested that mTORC2 or other RI-mTOR signals may be of importance in the regulation of leukemic cell growth and survival. mTORC1 and mTORC2 are distinct protein complexes,
both of which have mTOR as their central catalytic subunit (46). In the case of mTORC1, mTOR is associated with Raptor and mLST8, whereas in mTORC2 it is associated with Rictor, mLST8, and SIN1 (45, 47). There have been some reports that rapamycin or related rapalogues are capable of inhibiting mTORC2 activity under certain conditions in vitro and in vivo (48, 49). However, it appears that such effects occur in a cell type-, time-, and dose-dependent manner (48), although, in general, rapamycin exhibits specificity toward mTORC1 (46, 47). Similarly, other FDA-approved rapalogues (e.g., temsirolimus, everolimus) selectively block mTORC1, but have minimal activity against mTORC2 (46, 47). This is highly relevant, as engagement of mTORC2 during inhibition of mTORC1 leads to Akt activation and generation of anti-apoptotic signals (46, 47). In addition, other negative feedback regulatory mechanisms are induced during treatment of AML cells with classic mTOR inhibitors, resulting in Akt activation. For instance, inhibition of mTOR in AML cells by RAD001 also upregulates expression of IRS2 (50), providing an alternative mechanism for activation of Akt and induction of anti-apoptotic responses.

In the present study, we provided evidence for the existence of mTORC2 complexes in AML cells, and showed that such mTORC2 activity is selectively blocked by the novel dual mTORC2/mTORC1 inhibitor OSI-027 (19, 38). In addition, our studies establish the presence of RI-mTORC1 complexes that regulate phosphorylation of the translational repressor 4E-BP1 at sites required for its deactivation and dissociation from the elf4E. In contrast to rapamycin, OSI-027 acts as a potent inhibitor of phosphorylation of 4E-BP1 on Thr37/46, Ser65, and Thr70, and this results in dissociation of elf4E–elf4G complexes as well as enhanced formation of elf4E–4E-BP1 complexes that negatively control mRNA translation. Consistent with this, OSI-027 treatment results in potent suppression of polysomal assembly and mRNA translation and this correlates with generation of much more potent antileukemic responses than treatment with rapamycin. It should be noted that a previous study had suggested that rapalogues can inhibit phosphorylation of 4E-BP1 in AML cells and also reduce mTORC2 signaling (49). However, in that study (49), the concentrations of CCI-779 that were used for the in vitro experiments showing inhibition of 4E-BP1....
phosphorylation were very high, when taken in context with previous pharmacokinetic studies of CCI-779 in patients with cancer (51).

The precise relationship of pathways targeted by dual mTORC1/mTORC2 inhibition with other cellular events that may contribute to cap-dependent mRNA translation in AML cells remains to be precisely defined. A recently published study (44) showed that phosphorylation of 4E-BP1 on Ser65 and assembly of eIF4F complexes is resistant to RAD001 in AML cells. In this study, it was also shown that Pim-2 regulates phosphorylation of 4E-BP1 on Ser65 and may ultimately control cap-dependent mRNA translation in an mTORC1-independent manner (44). In the study, siRNA knockdown of Pim-2 blocked phosphorylation of 4E-BP1 on Ser65, but not Thr37/46 (44). It is possible that OSI-027 also affects the function of Pim-2 in AML cells, and this should be examined in future studies. However, as OSI-027 blocks phosphorylation of all key phosphorylation sites of 4E-BP1 (e.g., Thr37/46, Ser65, Thr70), its major effects on cap-dependent translation are consistent with inhibition of RI-mTORC1 complexes (52).

Our data establish that dual targeting of mTORC2 and mTORC1 with a catalytic site inhibitor is much more effective than treatment with rapamycin in blocking the growth of AML cell lines and primitive leukemic progenitors from AML patients in vitro. In addition, we show that OSI-027 enhances the suppressive effects of AraC on leukemic precursors, raising the possibility of future clinical-translational efforts involving OSI-027, or combinations of chemotherapy with OSI-027, for the treatment of AML. Notably, recently published studies showed potent antileukemic effects of other dual mTORC2/mTORC1 inhibitors, PP242 (53) or OSI-027 (54), on BCR-ABL–expressing leukemia cells including Ph+ acute lymphoblastic leukemia (ALL) cells. This suggests that, beyond AML, dual mTORC2/mTORC1 inhibition may provide an approach for the treatment of other leukemias as well. Future studies should examine the antileukemic potential of combined AML cell targeting using OSI-027 together with other
agents that target cap-dependent translation or with inhibitors of pathways activated in a negative feedback regulatory manner to counteract the antileukemic effects of mTOR inhibition. For instance, it would be interesting to test combinations of OSI-027 with the 4E-BP1 mimetic 4EGI-1 that blocks mRNA translation in AML cells by competing with 4E-BP1 binding (44). There is also evidence that, in certain solid tumor cells, mTORC1 inhibition results in activation of Mek/Erk via an S6K–PI3K–Ras feedback loop (55, 56). Based on this, studies to determine whether the Mek/Erk pathway is activated in AML cells and whether it can be exploited to further enhance the antileukemic effects of mTORC2/mTORC1 inhibition are warranted.

Disclosure of Potential Conflicts of Interest
S. Barr and S. Russo are employees of OSI Pharmaceuticals.

Grant Support
This work was supported in part by NIH grants R01CA121192, R01CA77816, and KL2RR025740-01, a VA Merit review grant, and the Leukemia and Lymphoma Society grant LLS-6166-09.

References

30. Fritz RD, Varga Z, Radzivilov G. CNK-1 is a novel Akt interaction partner that promotes cell proliferation through the Akt-FoxO signalling axis. Oncogene 2010;29:3575–82.
32. Copp J, Manning G, Hunter T. TORC-specific phosphorylation of mammalian target of rapamycin (mTOR): phospho-Ser 2481 is a

www.aacrjournals.org
Clin Cancer Res; 17(13) July 1, 2011 4387

Published OnlineFirst March 17, 2011; DOI: 10.1158/1078-0432.CCR-10-2285

Downloaded from clincancerres.aacrjournals.org on May 4, 2017. © 2011 American Association for Cancer Research.
Clinical Cancer Research

Dual mTORC2/mTORC1 Targeting Results in Potent Suppressive Effects on Acute Myeloid Leukemia (AML) Progenitors


Clin Cancer Res  Published OnlineFirst March 17, 2011.

Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-10-2285

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2011/06/30/1078-0432.CCR-10-2285.DC1

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.