

Target of Rapamycin Signaling in Leukemia and Lymphoma

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Abstract

Growth factors and many oncogenes activate the lipid kinase phosphoinositide 3-kinase (PI3K), initiating a signaling cascade that includes the protein kinases AKT and target of rapamycin (TOR). The PI3K/AKT/TOR signaling pathway is a significant contributor to disease in various human cancers, including hematologic malignancies. Here we discuss different strategies to inhibit TOR for the treatment of leukemia, lymphoma, and myeloma. The TOR enzyme exists in two complexes in cells, TORC1 and TORC2. The majority of preclinical and clinical efforts to target TOR have involved using rapamycin and its analogs (rapalogs), which suppress TORC1 only partially and do not acutely inhibit TORC2. A new class of small molecules targeting the ATP-binding site of the TOR kinase, termed active-site TOR inhibitors (asTORi), achieves greater inhibition of both TOR complexes, resulting in broader suppression of the PI3K/AKT/TOR signaling network. Preclinical evidence suggests that asTORi have greater efficacy than rapalogs in Philadelphia chromosome-positive acute lymphoblastic leukemia and in T-cell lymphoma. These agents also show greater tolerability in animal models relative to rapalogs or inhibitors of PI3K. These findings encourage broader evaluation of asTORi efficacy in acute myeloid leukemia, B-cell lymphoma, myeloma, and other blood cancers. *Clin Cancer Res*; 16(22); 5374–80. ©2010 AACR.

Background

PI3K, AKT, and the two TORCs

The phosphoinositide 3-kinase (PI3K)/AKT signaling network is highly conserved and regulates cell size, number, and metabolism; thus, it is not surprising that it is one of the most frequently activated pathways in human cancer (1, 2). Binding of growth factors and cytokines to their cell-surface receptors initiates signals leading to recruitment of PI3K to cellular membranes (Fig. 1). PI3K activation can also be driven by oncogenic receptor and nonreceptor tyrosine kinases, independently of extracellular signals. Class I PI3Ks, which are the primary group of PI3Ks activated by growth factors and oncoproteins, catalyze the conversion of phosphatidylinositol-4,5-bisphosphate (PI-4,5-P₂) to phosphatidylinositol-3,4,5-trisphosphate (PIP₃). The presence of PIP₃ at the membrane allows recruitment and activation of the serine-threonine kinase AKT (Fig. 1), which has many key substrates that control various aspects of cell growth, metabolism, proliferation, and survival (3).

TOR [often called mammalian TOR (mTOR), owing to its discovery after the yeast *Tor* genes] is one of the most

important components of the PI3K/AKT signaling network in relation to carcinogenesis (4–7). In mammals, a single TOR enzyme is present in at least two distinct cellular complexes, TORC1 and TORC2 (Fig. 1). Both complexes contain mLST8 and DEPTOR; TORC1 also contains raptor and PRAS40, whereas TORC2 contains rictor, mSin1, and PROTOR. TORC1 is activated downstream of AKT and is also sensitive to nutrient availability and various cellular stresses. TORC2, which acts upstream of AKT (see below), is insensitive to nutrient status, but its activity might be increased by growth factors and PI3K.

TORC1 plays a central role in regulating mRNA translation, metabolism, and lipid biosynthesis (8, 9). The two best-characterized substrates of TORC1 are the eukaryotic initiating factor-4e (eIF4e) binding proteins (4EBP) and the S6 kinases (S6K; Fig. 1). 4EBP1 phosphorylation by TORC1 releases 4EBP1 from eIF4e, allowing eIF4G to bind and form the eIF4F complex that promotes cap-dependent mRNA translation. Hence, TORC1 inhibition increases binding of 4EBP1 to eIF4e and reduces the translation of specific mRNAs. Proteins whose translation is controlled by the 4EBP/eIF4E axis include the pro-survival factor Mcl-1, the cell-cycle regulator cyclin D3, and the proangiogenic growth factor vascular endothelial growth factor (VEGF; refs. 10, 11). Phosphorylation of S6Ks by TORC1 is an activating event that potentiates S6K-dependent phosphorylation of ribosomal S6 protein and other substrates that coordinate aspects of protein and lipid biosynthesis, while opposing autophagy. TORC2 phosphorylates a conserved hydrophobic motif found in several members of the AGC protein kinase family (12, 13). Of particular relevance to cancer, TORC2 phosphorylates AKT at Ser473, increasing AKT activity in a substrate-selective

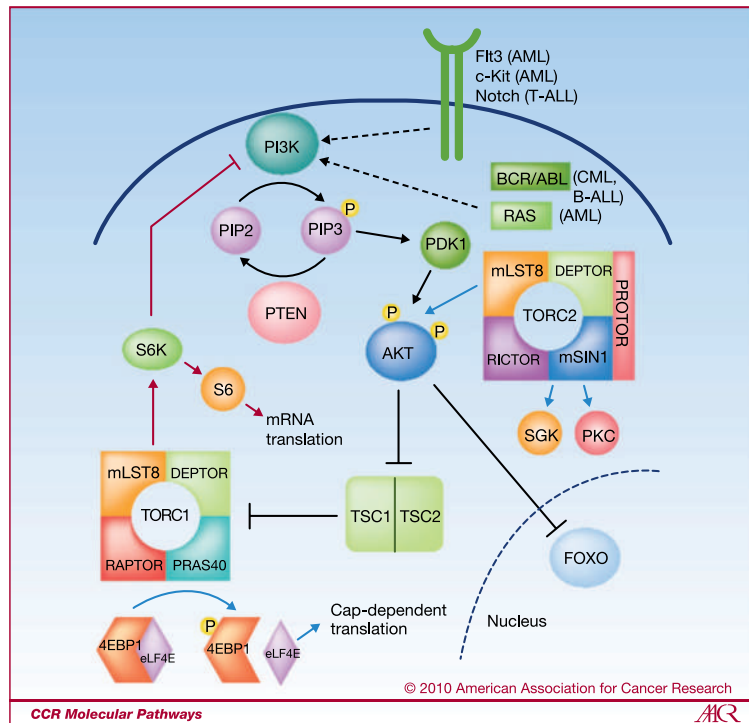
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Fig. 1. Diagram of key features of the PI3K/AKT/TOR signaling network. The lipid kinase PI3K is activated downstream of oncogenic receptors or intracellular proteins (dashed arrows) in various hematological diseases, with examples shown. PI3K generates the second messenger PIP₃. The TOR serine-threonine kinase is present in two distinct multiprotein complexes, termed TORC1 and TORC2. Key substrates are shown, and their activities are either increased (pointed arrows) or decreased (block arrows) by TOR-mediated phosphorylation. Rapalogs inhibit the processes shown in red. asTORi block these events, as well as those shown in blue, though it should be noted that rapalogs have partial effects on 4EBP1 phosphorylation in a context-dependent manner. Some molecular details and functional outcomes have been omitted for simplicity.



manner. TORC2 can also phosphorylate certain protein kinase C isoforms as well as members of the serum- and glucocorticoid-induced kinase (SGK) family (Fig. 1).

Activation of the PI3K/AKT/TOR signaling network is opposed by several mechanisms (Fig. 1). The tumor suppressor phosphatase and tensin homolog (PTEN) is a lipid phosphatase that converts PIP₃ to PI-4,5-P₂. The tuberous sclerosis complex tumor suppressors (TSC1/TSC2) form a dimer that restrains TORC1 activity. In addition, S6K1 exerts strong negative feedback control on PI3K activity and on the RAS/extracellular signal-regulated kinase (ERK) pathway. An important consequence of this feedback loop is that selective inhibition of TORC1 results in “rebound” increases in PI3K/AKT, as well as RAS/ERK activity. Adding to this complexity, the activities of both TORC1 and TORC2 are modulated by regulated gene expression controlled by FOXO transcription factors, whose activity is suppressed by AKT (14, 15).

PI3K/AKT/TOR activation in hematologic malignancy

PI3K/AKT signaling output is elevated in a large fraction of human malignancies (1, 16, 17). This elevated output can occur through several mechanisms: activating mutations in genes encoding PI3K or AKT, loss of function of the tumor suppressor PTEN, or activation of upstream oncoproteins such as epidermal growth factor receptor (EGFR) or RAS. In hematologic malignancies (leukemias, lymphomas, and myeloma), a number of genetic lesions have been shown to elevate PI3K/AKT activity and TOR signaling (Fig. 1).

Acute myeloid leukemia (AML) is a disease caused by clonal proliferation of a cell of myeloid lineage. Despite optimal care, 5-year survivals are as low as 14% with poor risk AML (18), indicating a need for better therapies. Most AML samples display constitutive activation of PI3K/AKT signaling (19–24); this is usually associated with oncogenic mutations of upstream targets, such as receptor tyrosine kinases (FLT3, c-KIT; up to 35 to 40%) or mutated N-RAS or K-RAS (up to 20%). Of these, activation mutations in FLT3 are the most frequently observed molecular abnormalities in AML and are associated with very poor prognosis (25). AKT activity is increased in mutant FLT3-positive AML (26). In addition, elevated PI3K/AKT pathway activity is associated with lower overall survival and disease-free survival (27). Notably, retroviral transduction of hematopoietic stem cells with constitutively active AKT resulted in AML-like disease in mice (28), providing direct evidence that AKT activity can directly drive myeloid transformation.

B-precursor acute lymphoblastic leukemia (B-ALL) cells carrying the Philadelphia chromosome (Ph⁺) translocation show constitutive BCR-ABL tyrosine kinase activity that drives elevated PI3K/AKT and TOR signaling (29, 30). Patients with Ph⁺ B-ALL show lesser clinical responses to ABL tyrosine kinase inhibitors (TKI; imatinib, dasatinib), than do patients with Ph⁺ chronic myeloid leukemia (CML) patients in chronic phase (31). Transformation of mouse B-cell progenitors with the p190 isoform BCR-ABL is profoundly impaired by genetic ablation of class IA PI3K, illustrating the importance of PI3K activation for leukemogenesis (32). However, rare PI3K-deficient cells can emerge, and these maintain considerable TORC1 activity (32).

Malignancies arising from more mature B cells may also be rational targets for TOR inhibitors, especially subtypes with elevated activity of the PI3K/AKT pathway. Diffuse large B-cell lymphoma (DLBCL), an aggressive variant of non-Hodgkin's lymphoma, can be categorized into an activated B-cell like (ABC) and germinal center B-cell like (GCB), on the basis of unique gene expression profiles (33, 34). ABC lymphomas have a poorer prognosis. Interestingly, survival of ABC lymphoma lines *in vitro* depends on chronic, active signaling through the B-cell receptor, which is upstream of PI3K/AKT/TOR and NF- κ B activity (35).

In multiple myeloma, a malignancy of B-cell-derived plasma cells, some cell lines and primary human samples display constitutively active AKT and might, therefore, be rational targets for active-site TOR inhibitors (asTORi) or panPI3K/TOR inhibitors (36). However, in a subset of myelomas, the elevated AKT activity occurs concurrently with reduced TORC1 activity. Mechanistically, this phenomenon appears to result from elevated expression of DEPTOR, which inhibits TOR kinase activity (37). Loss of TORC1-mediated feedback inhibition seems to dominate over reduced TORC2 activity in these cells, resulting in greater AKT activity (37).

A subset of T-cell malignancies exhibits dependence on PI3K/AKT/TOR signaling. In T-cell acute lymphoblastic leukemia (T-ALL), this pathway is often activated (38, 39), and resistance to NOTCH1 inhibitors is associated with mutational loss of PTEN (40). In mouse models, expression of constitutively active AKT in T cells can drive thymocyte survival and transformation through a TORC1-dependent mechanism (11).

Rapamycin, an allosteric TOR inhibitor

The first discovered TOR inhibitor was rapamycin, originally isolated from bacteria discovered on Easter Island (also called Rapa Nui). Initial interest in the drug was due to its antifungal potential, but its immunosuppressive properties prevented rapamycin from being developed in this arena. Rapamycin works by binding to FKBP12, an abundant intracellular chaperone protein. The rapamycin/FKBP12 complex affects TORC1 activity and stability through an allosteric mechanism, binding to a surface of TOR distant from the active site (41). Although the detailed molecular structure of TOR has not been determined, an elegant electron microscopy approach has uncovered important aspects of TORC1 structure and regulation by rapamycin (42). TORC2 is considered rapamycin-insensitive because rapamycin/FKBP12 complexes do not bind directly to TORC2 (12, 13). Indeed, acute treatment with rapamycin generally increases phosphorylation of AKT on both the TORC2 site and the PDK-1 site, owing to loss of negative feedback on PI3K. This feature can be seen in patient tumors treated with rapalogs, which show increased AKT phosphorylation as predicted by this model (43). However, prolonged treatment with rapamycin can decrease TORC2 activity by disrupting *de novo* assembly of the TORC2 complex (44). The kinetics and potency of

TORC2 inhibition by rapamycin appears to be cell-type dependent. One study found strong TORC2 inhibition by rapamycin in AML specimens (45).

An important point to emphasize about rapamycin is that the compound does not completely inhibit TORC1 activity (6, 7, 46). In particular, rapamycin has little effect on phosphorylation of 4EBP1 on key threonine residues (T37/46), even under conditions in which S6K phosphorylation is abrogated. A consequence of incomplete inhibition of 4EBP1 phosphorylation is that rapamycin only weakly attenuates cap-dependent translation and overall protein synthesis.

In most cellular systems examined, rapamycin delays cell-cycle progression through G₁ phase, but rarely has cytotoxic effects as a single agent. Although rapamycin can synergize with chemotherapeutics in some settings, in other contexts the drug can promote chemoresistance through the PI3K/AKT rebound effect (4, 6, 7, 41).

Active-site TOR inhibitors

The evidence for rapamycin-resistant TORC1 and TORC2 outputs has prompted the development of competitive "active-site" inhibitors that target the ATP-binding site of the TOR kinase domain, thus fully inhibiting the enzyme in all cellular complexes (6, 7, 47). The kinase domains of TOR and PI3K enzymes share significant homology, and the first class of competitive TOR inhibitors to be identified were found to cross-inhibit PI3Ks and are, thus, termed "panPI3K/TOR inhibitors" (6, 16, 17). Screening and medicinal chemistry efforts have yielded a new class of small molecule ATP-competitive TOR inhibitors with high selectivity for TOR compared to PI3Ks and other cellular enzymes (29, 48–53). This compound class has been termed TORKinibs (49), TORC1/2 kinase inhibitors (29), or TKIs (7); we endorse the nomenclature asTORi as coined by Sonenberg and colleagues (10). Compounds of this class include the preclinical "tool" compounds PP242, Ku-0063794, Torin-1, and WYE-354; clinical candidate compounds include AZD8055, OSI-027, and INK-128 (Table 1). The impact of asTORi on TORC1/TORC2 signaling is remarkably similar across chemotypes, and clearly distinct from the effects of rapamycin. Specifically, asTORi strongly inhibit phosphorylation of 4EBP1 and suppress phosphorylation and activity of TORC2 substrates (29, 48–53). Accordingly, asTORi have a more profound impact than rapamycin on cap-dependent translation, protein synthesis, metabolism, proliferation, and survival (29, 48–53). Genetic studies have shown that the stronger antiproliferative potential of asTORi in fibroblasts results from greater TORC1 inhibition, and appears to be independent of TORC2 status (49, 50). Similarly, in a lymphoma model driven by constitutively active AKT, the proapoptotic potency of PP242 compared to rapamycin was associated with greater TORC1 inhibition (11). However, it remains possible that TORC2 inhibition contributes to the biological impact of asTORi in other cellular contexts. To date, the development of TORC2-specific inhibitors has not been achieved (13).

Clinical-Translational Advances

Rapalogs in hematologic diseases

Rapamycin (sirolimus) is currently U.S. Food and Drug Administration (FDA)-approved as an immunosuppressant and anti-inflammatory agent, but it is not used in standard oncology practice. Since the discovery of rapamycin, second generation (temsirolimus, everolimus, deferolimus) rapalogs with improved drug properties have been developed and tested in preclinical and clinical models of cancer. Temsirolimus and everolimus are FDA approved for use in metastatic renal cell carcinoma with modest benefits (54, 55). Although rapalogs are currently not FDA approved for any hematologic malignancy, clinical studies have shown efficacy in some lymphomas and leukemia and thus made rapalogs a valid treatment option. Phase III studies in mantle cell lymphoma showed increased progression-free survival and response rate with temsirolimus, versus "investigators choice" regimens (i.e., fludarabine, thalidomide, lenalidomide, chlorambucil, etc.; ref. 56). *In vitro*, rapamycin enhanced the growth-suppressive effect of an inhibitor of NF- κ B signaling in ABC-type DLBCL cells (49). In chronic lymphocytic leukemia, everolimus resulted in partial remissions in a minority (18%) of patients (57). Rapamycin decreased the clonogenicity of primary human AML samples (58), yet phase I-II trials of rapalogs in AML have again shown modest effects. Rapamycin induced a partial response in 4 out of 9 AML patients in the relapsed or refractory setting (58). Further, everolimus showed no responses for AML (59), and deferolimus showed activity in only 1 out of 22 AML patients (60).

Studies from our laboratory have suggested that rapalogs will have limited efficacy in the Ph⁺ subset of B-ALL (29, 32). *In vitro*, the growth inhibitory effect of rapamycin on mouse and human BCR-ABL⁺ cell lines reached a plateau (approximately 60% inhibition), with cell-cycle analysis confirming that this was mainly due to cell-cycle arrest. In colony assays with primary Ph⁺ ALL specimens, rapamycin had little effect as a single agent or in combination with BCR-ABL TKIs. In mouse models of Ph⁺ ALL, rapamycin had limited effects on tumor expansion at a dose that was strongly immunosuppressive. On the other

hand, rapamycin can inhibit survival of Ph⁺, but imatinib-resistant, CML specimens (29, 61).

In myeloma models, rapalogs can suppress proliferation and potentiate dexamethasone-induced apoptosis (62). Additional trials are in progress (~39 registered on ClinicalTrials.gov) to study rapalogs as single agents and in combination with chemotherapy for refractory lymphomas, leukemias, and myeloma.

ATP-competitive TOR inhibitors in leukemia and lymphoma

The efficacy of asTORi in preclinical models of AML or myeloma has not yet been reported. However, panPI3K/TOR inhibitors have been tested in these settings. LY294002 reduced the ability of AML cells to engraft in immunocompromised mice (23). Similarly, PI-103 caused potent cell-cycle arrest in AML blasts and reduced the clonogenic capacity of AML samples (63). PI-103 also seemed to induce apoptosis in the CD34⁺/CD38^{low/neg} population that is the putative stem cell compartment, an effect not seen in samples treated with the rapalog RAD001. In addition, the effect of PI-103 on AML blasts and the leukemic stem-cell compartment was potentiated by the addition of etoposide, a traditional chemotherapeutic agent. In myeloma models, BEZ235 showed efficacy as a single agent and was either additive or synergistic with current treatments including doxorubicin and bortezomib (64). These findings raise the question of where panPI3K/TOR inhibitors would fit in the treatment schema for AML and myeloma. Although, undoubtedly, the first clinical trials will involve relapsed or refractory disease, the evidence above suggests that the optimum placement of these medications may be in the first-line setting with traditional chemotherapy, to strengthen responses and increase cure rates.

Our laboratory has investigated the efficacy of panPI3K/TOR inhibitors and asTORi in Ph⁺ B-ALL. These studies were prompted initially by our finding that rapamycin could be converted from cytostatic to cytotoxic by blocking PI3K/AKT activity (32), suggesting that inhibiting both TOR complexes would provide added efficacy. We found that both PI-103 and BEZ235 reduce the clonogenic potential

Table 1. List of asTORi in preclinical and clinical studies

Chemical Name	Status*	References
PP242	Preclinical tool compound	29, 49
Torin-1	Preclinical tool compound	37, 51
Ku-0063794	Preclinical tool compound	50
WYE-354	Preclinical tool compound	53
OSI-027	Phase I	http://www.osip.com/OSI_027
AZD8055	Phase II	48
INK128	Phase I	http://www.intellikine.com/pipeline/ink128.html
WYE-132	??	52

*Trials information was obtained from <http://ClinicalTrials.gov>.

of primary human Ph⁺ B-ALL cells when combined with ABL TKIs (29, 32). These effects were greater than those obtained with TKIs alone or with TKIs in combination with rapamycin (29, 32). In a mouse syngeneic B-ALL model, imatinib plus PI-103 suppressed leukemic proliferation more strongly than imatinib plus rapamycin (29, 32). Surprisingly, we found that PI3K inhibition was dispensable for the strong antileukemic effects of ATP-competitive TOR inhibitors. Thus, selective asTORi (PP242 and Ku-0063794) had effects equivalent to PI-103 and BEZ235 in each of the *in vitro* and *in vivo* assays for Ph⁺ B-ALL proliferation and survival (29). PP242 reduced the clonogenic capacity of primary Ph⁺ B-ALL specimens from newly diagnosed subjects, and was effective in combination with dasatinib in relapse samples (29). asTORi showed great selectivity toward leukemic cells compared to normal bone marrow and peripheral blood lymphocytes (29). In mouse models, combinations of ABL TKIs with PP242 strongly suppressed proliferation of leukemic cells, while boosting the fraction of normal bone marrow cells in the cell cycle. Furthermore, asTORi had little effect on lymphocyte numbers or immune responses at doses showing profound antileukemic efficacy. In contrast, rapamycin and panPI3K/TOR inhibitors showed considerable hematotoxicity and/or immunosuppression. These data imply that Ph⁺ B-ALL cells have a greater "addiction" to elevated TOR kinase activity compared to normal hematopoietic cells responding to physiological stimuli (discussed further in ref. 6). The mechanism by which rapamycin selectively affects nontransformed lymphocytes is not yet clear (6).

Currently, the standard of care for Ph⁺ B-ALL is induction chemotherapy in addition to ABL TKI (imatinib, dasatinib), with consideration for allogeneic stem-cell transplant in patients with high-risk disease. In the relapse setting, single agent TKIs have limited efficacy (duration of response of months; refs. 65, 66), and although asTORi might potentiate efficacy, it is unclear whether TKI/asTORi combinations will be effective against heavily treated B-ALL that may harbor BCR-ABL mutations such as T3151. Indeed, a T3151-mutated Ph⁺ B-ALL sample displayed resistance to dasatinib plus PP242 *in vivo* (29). As with AML and myeloma, the optimal setting for asTORi (or panPI3K/TOR) in Ph⁺ B-ALL may be in newly diagnosed patients as an adjunct to induction and consolidation therapy.

PanPI3K/TOR inhibitors and asTORi have shown anti-tumor efficacy in T-cell lymphoma (PI-103, ref. 67; PP242, ref. 11), Waldenstrom's macroglobulinemia (BEZ-235; ref. 68), and anaplastic large cell lymphoma (LY294002; ref. 69).

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Future Directions

Several panPI3K/TOR inhibitors are in phase I or phase II trials (BEZ235, BGT226, XL675). In addition, clinical trials are now underway to investigate the safety and efficacy of asTORi (Table 1). OSI-027 is in phase I studies open to solid tumors and lymphoma (clinical trial identifier NCT00698243). AZD8055 has three open studies enrolling advanced solid tumor patients and hepatocellular carcinoma patients (NCT00999882, NCT00973076, NCT00999882). INK128 is in a phase I trial for advanced solid tumors (NCT01058707), with an additional phase I trial planned for Waldenstrom's macroglobulinemia and multiple myeloma (NCT01118689). Additional compounds of the asTORi class are in late preclinical development and are likely to enter trials soon. The pharmaceutical development community is eagerly awaiting the results of these trials, which will begin to reveal whether the impressive efficacy and safety profiles of these compounds in animals will translate into a viable therapeutic window in humans. We hope that sufficient attention will be given to hematologic malignancies, which are less common than many solid tumors but might be particularly sensitive to TOR inhibition. While these human studies proceed, it will be important to continue basic research studies of the mechanism of action of asTORi in different tumor types, and on the tumor microenvironment and cancer stem cells. In order to optimize the design and interpretation of clinical trials, it will also be crucial to identify biomarkers of sensitivity and resistance, and to validate efficacious drug combinations in preclinical models. Combinations to be tested might include MEK inhibitors and TKIs targeting FLT3 or BCR-ABL.

Disclosure of Potential Conflicts of Interest

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