RESEARCH & REVIEW
Targeting PI3K/AKT/mTOR signaling in Acute Myeloid Leukemia

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The phosphatidylinositol 3-kinase (PI3K), AKT, mammalian target of rapamycin (mTOR) signaling pathway (PI3K/AKT/mTOR) is involved in a diverse number of cellular functions and has emerged as one of the most deeply investigated cell signaling networks. This pathway is frequently deregulated in many types of human cancers including acute myeloid leukemia (AML) at multiple points, providing a compelling rationale to consider PI3K/AKT/mTOR as an effective target for therapeutic treatment of AML. This discussion highlights the activation of PI3K/AKT/mTOR and its positive effects on AML. Moreover, we will discuss the development of inhibitors that are used to target PI3K/AKT/mTOR in clinical trials. Targeting the PI3K/AKT/mTOR signaling network with small molecule inhibitors, employed either alone or in combination with other drugs, may result in less toxic and more efficacious treatment of AML patients.

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ABSTRACT

The phosphatidylinositol 3-kinase (PI3K), AKT, mammalian target of rapamycin (mTOR) signaling pathway (PI3K/AKT/mTOR) is involved in a diverse number of cellular functions and has emerged as one of the most deeply investigated cell signaling networks. This pathway is frequently deregulated in many types of human cancers including acute myeloid leukemia (AML) at multiple points, providing a compelling rationale to consider PI3K/AKT/mTOR as an effective target for therapeutic treatment of AML. This discussion highlights the activation of PI3K/AKT/mTOR and its positive effects on AML. Moreover, we will discuss the development of inhibitors that are used to target PI3K/AKT/mTOR in clinical trials. Targeting the PI3K/AKT/mTOR signaling network with small molecule inhibitors, employed either alone or in combination with other drugs, may result in less toxic and more efficacious treatment of AML patients.
Acute myeloid leukemia (AML) is comprised of a group of clonal malignant diseases characterized by deregulated proliferation of immature myeloid cells (1). AML accounts for approximately 80% of all adult leukemias and most patients will relapse and die from the disease (2). In AML, there is deregulation of many signaling pathways that include the PI3K/Akt/mTOR, ERK/MAPK, STAT3/5, Wnt/β-catenin and NF-κB pathways (3-8). Between 50 and 70% of AML patients have Akt activation by phosphorylation at Thr308 and Ser473 (9). The overall survival of patients who have Akt activation is significantly shorter than that of patients without Akt activation (10). Moreover, alterations in the PI3K/Akt axis are known to be both associated with and causal of oncogenesis (11, 12). Therefore, the PI3K/Akt/mTOR signaling pathway appears to represent a valid target for innovative treatments of AML patients.

The phosphatidylinositol 3-kinase (PI3Ks) are a large family of kinases that function as intracellular messengers to orchestrate an array of important cellular processes, such as growth, survival, and metabolism. Their primary biochemical function is to phosphorylate the 3’-OH group in inositol lipids (13). The PI3K family is comprised of three classes (I, II, III), each of which has a distinct structure, distribution in the cell, and mechanism of action. The roles of class II and III PI3Ks are not well-characterized, while class I PI3Ks are widely implicated in the development of cancer. The class I PI3Ks are composed of a regulatory (p85) and a catalytic (p110) subunit (14, 15). There are several isoforms of each subunit. For example, p85 is encoded by the PIK3R1 (p85α), PIK3R2 (p85β) or PIK3R3 (p85γ) genes; p110 is encoded by the PIK3CA (p110α), PIK3CB (p110β) or PIK3CG (p110δ) genes. Class I PI3Ks are divided into class Iα and class Iβ based on whether they are activated by receptor tyrosine kinases (RTKs) or G-protein coupled receptors (GRCPs) (13, 16). The class I PI3Ks are responsible for the conversion of the membrane lipid phosphatidylinositol-4,5-biphosphate (PIP2) into phosphatidylinositol-3,4,5-triphosphate (PIP3) through the addition of a phosphate group to the 3’ position of its inositol ring. PIP3 recruits phosphoinositide-dependent protein kinase 1 (PDK1) and AKT to the plasma membrane (17), followed by Akt phosphorylation and activation by PDK1 (13, 16). The S6K1 and phosphate and tensin homologue deleted on chromosome 10 (PTEN) negatively regulates PI3K/Akt activation by converting PIP3 product to PIP2 (18) (Figure 1).

Figure 1: Targeting the PI3K/AKT/mTOR signaling in Acute Myeloid Leukemia.

RTKs: Receptor Tyrosine Kinases; PTEN: Phosphate and Tensin homologue deleted on chromosome 10; PIP2: Phosphatidylinositol-4,5-biphosphate; PIP3: Phosphatidylinositol-3,4,5-triphosphate; PDK1: Phosphoinositide-dependent protein kinase 1; Akt: Protein kinase PKB/Akt.

Akt, a serine/threonine protein kinase also known as protein kinase B (PKB), is recruited to the inner surface of plasma membrane through the interaction of its pleckstrin homology (PH) domain with phospholipid products of PI3K. At the plasma membrane, activation of Akt is dependent on phosphorylation, which is achieved at least in part by the protein kinase PDK1 (16, 19) (Figure 1). Akt was originally identified as a retroviral oncogene product, v-Akt, that can transform rodent cells (20, 21). There are three isoforms of Akt which are encoded from distinct genetic loci: Akt1/α; Akt2/β and Akt3/γ (22). Overexpression of constitutively activated Akt mutants in many cell types promotes cellular transformation by stimulating proliferation and inhibiting apoptosis (23). Recently, mounting evidence indicates that Akt overexpression is observed in diverse cancers and Akt perturbations play an important role in human malignancy.

Activation of the target of Rapamycin (TOR) has emerged as a major effector of the PI3K/Akt pathway’s ability to govern protein synthesis (Figure 1), a function that is conserved from yeast to mammals (24 - 26). The mTORC1 complex is composed of FRAP, Raptor, PRAS40 and LST8 (27) and its activation is mostly regulated by Akt/tuberous sclerosis 2 (TSC2) axis (28). Akt inhibits the tuberous sclerosis complex-2 (TSC2) regulated mTORC1 activity through direct phosphorylation of TSC2 at S939 and T1462 (29). mTORC1 is also directly phosphorylated and activated as a substrate of Akt (30). mTORC1 regulates protein synthesis by activating p70S6K and inactivating 4EBP1 (31). The mTORC1 complex is sensitive to rapamycin and inhibits Akt via a negative feedback loop (32).
The PI3K/AKT/mTOR signaling cascade is crucial to many widely divergent physiological processes that include cell cycle progression, transcription, translation, differentiation, apoptosis, motility and metabolism. As a result, this pathway has received considerable attention as a potential therapeutic target in malignancy since a gain of function is present in many types of cancers including AML (34 - 38). Furthermore, activation of this pathway confers leukemogenic potential to mouse hematopoietic cells (39). There are promising preclinical data demonstrating activity of different PI3K/Akt/mTOR targeted agents against AML (40 - 43). For a number of these malignances, the real promise of these inhibitors is their ability to overcome chemotherapy resistance and synergize with existing cytotoxic therapies. Therefore, this signal transduction cascade may represent a valuable addition to the current regimens for AML. Drugs that target mTOR were the first to be studied, showing remarkable efficacy in a number of conditions. Subsequently, drugs were developed to target PI3K and AKT as well as a number of intermediates in the PI3K/AKT/mTOR signaling pathway, including agents that target individual protein kinases and drugs that target multiple kinases in the pathway (44, 45).

Following are several pharmaceutical inhibitors that selectively target the PI3K/Akt/mTOR signaling pathway and have therapeutic potential (Table 1).

**PI3K p110 inhibitors:** Wortmannin and LY294002 are classical PI3K inhibitors that induce apoptosis in leukemic progenitors. Wortmannin irreversibly inhibits PI3K by covalent modification of Lys802 while LY294002 competes with ATP for the ATP binding site of PI3K (4, 46). However, both Wortmannin and LY294002 are not specific for the PI3K/Akt pathway (47). IC87114 is a potent selective inhibitor of PI3K p110δ which down-regulates Akt phosphorylation and cell proliferation in AML blast cells with elevated PI3K p110δ but not in normal hematopoietic precursor cells (4). There are also more potent inhibitors currently under development by pharmaceutical companies such as Piramed, Novartis and Calistoga. For example, CAL-101 (Calistoga) treatment inhibits ribosomal RNA synthesis and cell proliferation in AML cells through inhibition of Akt activation (48). Results obtained with these inhibitors indicate that selectively targeted PI3K inhibitors are less toxic than those compounds that inhibit all PI3K activities and they may offer greater clinical benefit.

**mTORC1 inhibitors:** mTOR inhibitors are the most developed class of compounds that target the PI3K/Akt pathway including rapamycin, CCI-779, RAD001 and AP23573. Rapamycin has shown activity against many types of cancer in phase I and II clinical trials (30). Moreover, combination treatment of rapamycin with other inhibitors may be synergistic. For examples, combined treatment with rapamycin and etoposide dramatically increases cytotoxicity in AML blast (49); adding UCN-01 to rapamycin results in marked apoptosis in U937 cells (50); and treatment with rapamycin and the cell permeable glycolytic inhibitor 3-bromo-2-oxoproprionate-1-propyl ester has synergistic effects on HL60 leukemia cell cytotoxicity (42). Although some patients do not respond to rapamycin treatment (51) and mechanisms of resistance to rapamycin have been identified (52), it is still considered to be of particular interest due to its ability to inhibit the growth of cancer cells and induce apoptosis. Another mTOR inhibitor, CCI-779, has been used in clinical trials with metastatic renal carcinoma patients. Both RAD001 and AP23573, two additional mTOR inhibitors, are also in clinical trials as antitumor drugs (53).

**TABLE 1: Therapeutic targets in the PI3K/Akt/mTOR signaling pathways**

<table>
<thead>
<tr>
<th>Molecules</th>
<th>Molecular targets</th>
<th>Drug sponsors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wortmannin</td>
<td>PI3K</td>
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</tr>
<tr>
<td>LY294002</td>
<td>PI3K</td>
<td>generic</td>
</tr>
<tr>
<td>IC87114</td>
<td>PI3K</td>
<td>ICOS</td>
</tr>
<tr>
<td>CAL-101</td>
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<td>Calistoga</td>
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<tr>
<td>Rapamycin</td>
<td>mTORC1</td>
<td>Wyeth/Pfizer</td>
</tr>
<tr>
<td>CCI-779</td>
<td>mTORC1</td>
<td>Wyeth</td>
</tr>
<tr>
<td>RAD001</td>
<td>mTORC1</td>
<td>Novartis</td>
</tr>
<tr>
<td>AP23573</td>
<td>mTORC1</td>
<td>Ariad</td>
</tr>
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<td>Akt</td>
<td>AETema Zentaris</td>
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<td>Deguelin</td>
<td>Akt</td>
<td>generic</td>
</tr>
<tr>
<td>AEZS-127</td>
<td>Akt</td>
<td>AETema Zentaris</td>
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<tr>
<td>AZD8055</td>
<td>mTORC1/2</td>
<td>AstraZeneca</td>
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Akt inhibitors: a new class of Akt inhibitors are in the phosphatidylinositol ether analog (PIAs) family and include perifosine, deguelin and AEZS-127. PIAs are inhibitors that bind to the PH domain of Akt and prevent its translocation to the plasma membrane, thereby inhibiting the phosphorylation and activation of Akt. These inhibitors sensitize leukemic cells to the effects of other chemotherapies (54). Perifosine dephosphorylates Akt and ERK 1/2 and activates caspases, inducing apoptosis in leukemic cells (41). Deguelin inhibits Akt through an unknown mechanism and increase AML sensitivity to cytarabine (40). AEZS-127 is an alkylphospholipid and has been tested in phase I for relapsed hematologic malignancies (55). The compound AZD8055, an inhibitor of both Akt and mTOR, shows more efficacy than rapamycin in decreasing ribosomal RNA synthesis and inhibiting the growth of leukemic cells in vitro and in vivo (48, 56). Akt inhibitors have the potential to provide greater efficacy and less toxicity to patients.

The PI3K/AKT/mTOR signaling pathway is aberrantly activated in a number of malignant and non-malignant diseases. It represents one of the major survival pathways that contribute to both cancer pathogenesis and drug resistance. As a result, preclinical studies and clinical trials have been designed to investigate compounds that target the various components of the pathway. Here, we described the efficacy and toxicity of agents that target the PI3K/AKT/mTOR signaling pathway in AML. The development of compounds directed against PI3K/Akt and mTORC and their modulators as novel and potent agents for treating AML is expanding and additional work is needed to determine the potential of PI3K/Akt/mTOR inhibitors in the treatment of AML.

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leukemia cells through a process that is regulated by the Raf-1/MEK/ERK, Akt, and JNK signal transduction pathways. Molecular cancer therapeutics. 4:457-470.


