Targeting the Phosphatidylinositol 3-Kinase Pathway in Multiple Myeloma

Hashem Younes,¹ Xavier Leleu,² Evdoxia Hatjiharissi,² Anne-Sophie Moreau,² Teru Hideshima,² Paul Richardson,² Kenneth C. Anderson,² and Irene M. Ghobrial²

Abstract
Multiple myeloma is a plasma cell neoplasm with a median survival of 3 to 5 years. Recent advances have improved patient outlook, but the disease remains incurable. Therefore, continued efforts to develop new therapies that target aberrant signaling pathways are needed. The phosphatidylinositol 3-kinase pathway regulates apoptosis, cell cycle regulation, and tumor proliferation. This pathway is constitutively activated in multiple myeloma and its inhibition induces apoptosis. Advances in understanding the signaling cascades mediating proliferation and survival of multiple myeloma cells have markedly improved the treatment of this disease. In this article, we review the role of the phosphatidylinositol 3-kinase/Akt pathway in the pathogenesis of multiple myeloma and the potential therapeutic implications of targeting this pathway in the treatment of multiple myeloma.

Background
Multiple myeloma is the second most common hematologic malignancy, with 15,000 new cases per year (1), and remains incurable with a median survival of 3 to 5 years (2). In multiple myeloma, plasma cells localize in the bone marrow, a microenvironment which supports their survival and growth (3). One of the signaling pathways whose deregulation may play an oncogenic role in multiple myeloma and several other cancers is the phosphatidylinositol 3-kinase (PI3K) pathway (4–7). The PI3K pathway regulates apoptosis, cell cycle regulation, and tumor proliferation (6). This cascade is constitutively activated in multiple myeloma and its inhibition induces apoptosis (8). In addition, the PI3K pathway also plays a major role in migration of plasma cells (9). In this article, we review the role of the PI3K/Akt pathway in the oncogenesis of multiple myeloma and the potential therapeutic implications of targeting this pathway in the novel treatment strategies.

The PI3K Pathway
PI3Ks are a large and complex family of lipid kinases that phosphorylate the 3′-OH group of the inositol ring in inositol phospholipids (4). The family contains three classes, with multiple subunits and isoforms (10). Class I PI3Ks catalyze the phosphorylation of multiple phosphatidylinositols, mainly PtdIns(4,5)P₂ (sometimes referred to as PIP₂), which is converted to PtdIns(3,4,5)P₃ (called PIP₃; ref. 10). Based on the receptor used to transmit their signals, class I PI3Ks are subdivided into two subgroups: IA and IB. Subgroup IA is activated by tyrosine kinase–coupled receptors, whereas subgroup IB uses G protein–coupled receptors. Because subgroup IA is clearly involved in oncogenesis, our discussion will be restricted to this subgroup (10). At the molecular level, class I PI3Ks are heterodimers which consist of a regulatory p85 subunit, a phosphoprotein substrate for many cytoplasmic and receptor tyrosine kinases (RTK), and a catalytic p110 subunit. Both subunits are encoded by a different set of three genes (α, β, and γ), which are also subject to alternate splicing, thereby yielding multiple variants (4, 10).

In normal cells, the PI3K pathway is under tight regulation by various mechanisms (11). The inactive p85-p110 complex present in the cytoplasm of resting cells is activated through ligands binding a RTK on the plasma membrane. This interaction drives the p85-p110 complex to the cytoplasmic tail of this RTK receptor and leads to activation of the PI3K kinase, either by a direct interaction between an SH2 domain of p85 regulatory subunit of the complex with consensus phosphorysotyrosine residues on RTK or by indirect interaction through IR51/IR52 signaling intermediates. The net result is activation of the PI3K complex due to proximity of the p110 catalytic subunit with its lipid substrates in the cell membrane and relief of an inhibitory effect of p85 on p110 associated with the RTK-p85 interaction and the resulting PIP₃ generation, which functions as a second messenger activating multiple downstream pathways, is the main consequence of PI3K activation. The basic function of PIP₃ is the recruitment to the inner membrane and activation of pleckstrin homology domain–containing proteins. Most important among these proteins is Akt, the cellular homologue...
of the retroviral oncogene v-Akt, which is also known as protein kinase B (12, 13). Akt encodes a serine/threonine kinase composed of an NH₂-terminal pleckstrin homology domain, a central catalytic domain, and a short COOH-terminal regulatory domain (14, 15). Akt activation involves two steps: translocation to the plasma membrane and phosphorylation at Thr³⁰⁸ and Ser⁴⁷³ residues (13, 15). Phosphorylation at Thr³⁰⁸, a necessary and sufficient step at Akt activation, is mediated by 3-phosphoinositide–dependent protein kinase 1, another pleckstrin homology domain–containing kinase on the inner leaflet of the plasma membrane (7, 16). Maximal Akt activation requires phosphorylation at Ser⁴⁷³ residue by a poorly characterized 3-phosphoinositide–dependent protein kinase 1 (ref. 16; Fig. 1).

Akt activation mediates multiple biological activities including increased survival, proliferation, and growth of tumor cells (7, 17, 18). Another speculated Akt effect is related to tumor-induced angiogenesis (19). The effect of Akt on survival of cancer cells is related to its antiapoptotic properties (20). Dominant-negative alleles of Akt block cell survival even in the presence of many mediators of survival such as insulin-like growth factor 1 (IGF-I; ref. 21). This effect of Akt is likely multifactorial because Akt directly phosphorylates several components of the cell death machinery (21). For example, phosphorylation of BAD (a proapoptotic member of the Bcl-2 family) prevents its binding and inactivation of the survival factor Bcl-X₇ (22–24). Similarly, Akt phosphorylates and inhibits the catalytic activity of caspase-9, a known proapoptotic protease (23, 24). A third mechanism of Akt antiapoptotic effect is mediated by phosphorylation of FKHR, a member of the Forkhead family of transcription factors, which inhibits the nuclear translocation and activation of proapoptotic FKHR gene target proteins, such as Bim and Fas ligands (14, 22, 23). In addition to its direct antiapoptotic effect, Akt influences indirectly two central regulators of cell death, nuclear factor-κB and p53 (13, 14, 24). Nuclear factor-κB is a known mediator promoting cell survival in response to many survival stimuli. It is indirectly activated by Akt through direct phosphorylation and activation of IκB kinase, thereby inducing degradation of the nuclear factor-κB inhibitor (IκB; refs. 22, 24). The effect on p53 is mediated by the phosphorylation and activation of the p53-binding protein murine double minute-2, a ubiquitin ligase–containing proteasome, resulting in the degradation of the proapoptotic tumor suppressor p53 (ref. 22; Fig. 1).

In addition to its cell survival effect, Akt also enhances proliferation and growth of tumor cells (25, 26) via its effects on cell cycle regulation by modulating cyclin D1, an important factor in the G₁-S phase transition of the cell. Through phosphorylation and inhibition of cyclin D1, Akt prevents its degradation and leads to its accumulation in the cell (26). Akt kinase also mediates phosphorylation and activation of mammalian target of rapamycin (mTOR; ref. 25). A serine/threonine kinase that serves as a molecular sensor regulating protein synthesis in response to availability of nutrients, mTOR regulates biogenesis by activating p70 ribosomal S6 kinase and enhancing translation (27). Another mTOR effect is inhibition of 4E-binding protein 1 (PHAS-I), a translational repressor. Enhanced mRNA translation results in up-regulation of multiple proteins involved in cell cycle progression from G₁ to S phase (27). The mTOR pathway plays a role in pathogenesis of multiple solid tumors and hematologic malignancies including multiple myeloma (refs. 21, 28–30; Fig. 1).

**Fig. 1.** Schematic of the PI3K pathway and downstream proteins regulating cell cycle and proliferation. Inhibitors of this pathway include direct inhibitors of the PI3K pathway such as perifosine, enzastaurin, and mTOR inhibitors (CCI-779 and RAD001), as well as indirect inhibitors such as Hsp90 inhibitors (KOS-953 and 17-AAG) and IL-6 and IGF-IR inhibitors.

![fig1.png](attachment:fig1.png)
PI3K Pathway and Human Cancer

Studies have shown that multiple genes encoding proteins involved in the PI3K pathway are mutated in human cancers (31, 32). The earliest and most compelling evidence of the involvement of the PI3K pathway in human cancer came from studies involving deletion of phosphatase and tensin homologue (PTEN) tumor suppressor gene in multiple cancer types (33, 34). PTEN protein product is a phosphatase that has a dual activity toward both phosphotyrosine and phospholipid substrates. Dephosphorylation of PI3K products, mainly PIP_3, by PTEN leads to the decreased level of this phospholipid and a concomitant reduction in Akt activity (34). Conversely, the loss of PTEN expression has been detected in many cancers (34, 35). A high incidence of hematopoietic tumors and lymphoid hyperplasia was observed in PTEN-knockout mice, suggesting a role of PTEN in regulating hematopoietic cell proliferation, cell death, and malignant transformation (33, 34).

Multiple Myeloma and the Role of PI3K Pathway

Myeloma is a B-cell malignancy characterized by excess monoclonal plasma cells in the bone marrow. Multiple studies have shown that the PI3K pathway is involved in the development of multiple myeloma (25). Specifically, previous studies showed that interleukin-6 (IL-6) stimulates growth and survival of multiple myeloma cells, at least in part, via the PI3K/Akt pathway (36). Similarly, IGF-I is a growth factor for multiple myeloma, which also activates the PI3K pathway (25, 37, 38). mTOR, a major survival checkpoint regulator downstream of Akt, has shown significant antiapoptotic activity in multiple myeloma (25, 29). It is a serine/threonine kinase that enhances the phosphorylation and subsequent activation of p70 ribosomal S6 kinase and 4E-binding protein 1, associated with multiple myeloma cell proliferation and survival (29).

Clinical Translational Advances

Agents that target the PI3K pathway in multiple myeloma. Improved understanding of the pathways involved in multiple myeloma cell transformation has led to the development of several new treatment approaches. PI3K/Akt pathway inhibitors are already being studied in phase II trials, whereas others are still in phase I trials or preclinical studies showing antitumor activity. PI3K/Akt pathway inhibitors can be divided into direct versus indirect inhibitors of specific members of the pathway.

Direct inhibitors of the PI3K/Akt pathway. These include the Akt inhibitor perifosine, the PKC inhibitor enzastaurin, and the mTOR inhibitors RAD001 and CCI-779.

Akt inhibitors. Due to its central role in the proliferation, survival, migration, and drug resistance of multiple myeloma cells in vitro, Akt represents a promising target for novel therapies. Perifosine [I(1,1-dimethyl-4-(octadecyloxy)hydroxy-phosphinyloxy)-piperidinium inner salt, NSC 639966; Keryx Biopharmaceuticals] is a novel oral Akt inhibitor that belongs to a class of lipid-related compounds called alkylphospholipids. Perifosine was found to inhibit phosphorylation of Akt (at both Ser^473 and Thr^308 positions) and its downstream molecules, FKHR1 and glycogen synthase kinase 3α/β, in a time- and dose-dependent fashion (39). It triggered significant cytotoxicity against multiple myeloma cells, both in vitro and in vivo, even in the presence of stromal cells that induce drug resistance (39). This cytotoxic effect was mediated via caspase-8, caspase-9, and poly[ADP-ribose] polymerase cleavage and was associated with c-jun NH2-terminal kinase activation (39). In addition, perifosine enhances the antitumor activity of multiple other agents such as bortezomib and dexamethasone (39). A phase II clinical trial to test the efficacy of perifosine treatment in patients with relapsed multiple myeloma is ongoing. To date, it has been well tolerated with a favorable side effect profile, which includes mainly nausea, vomiting, and fatigue, but no myelosuppression or neuropathy (40). The multiple myeloma trial is ongoing and has already shown early activity, specifically in combination with dexamethasone (40). A follow-up study of the combination of perifosine and bortezomib based on synergistic antitumor cytotoxicity in vitro is now being initiated.

Protein kinase C inhibitors. Protein kinases C (PKC) family proteins are composed of 11 members of serine threonine kinases that mediate proliferation, survival, migration, and angiogenesis in many malignancies (41). These proteins directly activate the PI3K pathway through Akt protein. Enzastaurin [4-(1-[1-[(2-pyridinylmethyl)-4-piperidinyl]-1H-pyrrole-2,5-dione,3-(1-methyl-1H-indol-3-yl)-4-(1-[-1(2-pyridinylmethyl)-4-piperidinyl]-1H-indol-3-yl), LY317615; Eli Lilly and Company] is an acyclic bisindolylmaleimide, an oral PKCβ selective inhibitor. It suppresses not only PKC signaling but also the PI3K/Akt pathway, thereby inhibiting tumor-induced angiogenesis as well as tumor cell survival and proliferation (42). Enzastaurin also suppresses vascular endothelial growth factor–induced angiogenesis in animal models, indicating that it suppresses tumor growth through multiple mechanisms (42, 43). In multiple myeloma, PKC overexpression has been associated with t(4;14) translocation (44). Importantly, PKC signaling pathways have been implicated in multiple myeloma cell proliferation, apoptosis, and migration (43, 45). Studies conducted by our group and others have shown that enzastaurin specifically inhibits membrane, cytosolic, and nuclear phosphorylation of homologous PKC isoform residues, as well as associated kinase activity, induced by the major PKC activator 12-O-tetradecanoylphorbol-13-acetate (tumor-promoting phorbol ester; refs. 46, 47). Consequently, it also abrogates 12-O-tetradecanoylphorbol-13-acetate–induced phosphorylation of signaling molecules downstream of PKC, including myristolated alanine-rich C kinase substrate (47). Importantly, in multiple myeloma cells, enzastaurin also inhibits PKC activation triggered by growth factors and cytokines secreted by bone marrow stromal cells; costimulation with the extracellular matrix protein fibronectin, vascular endothelial growth factor, or IL-6; and multiple myeloma patient serum. In addition, enzastaurin inhibited multiple myeloma cell adhesion as well as vascular endothelial growth factor– and IGF-I–triggered multiple myeloma cell migration (47). Finally and most importantly, tumor growth, survival, and angiogenesis were abrogated by enzastaurin in an in vivo xenograft model of human multiple myeloma (47). Enzastaurin has been tested in clinical trials in diffuse large-cell lymphoma and glioblastoma and showed promising activity in lymphoma with minimal side effects, including fatigue, peripheral edema, and nausea (48). A phase II trial of enzastaurin in patients with relapsed/refractory multiple myeloma is planned to start in 2007.
mTOR inhibitors. Rapamycin has been in clinical use for more than two decades as an immunosuppressive agent to prevent allograft rejection. The delineation of the role of mTOR in the pathogenesis of many malignancies led to the use of rapamycin and several other analogues as anticancer agents (49–51). Specifically, two rapamycin analogues have been tested in preclinical and clinical studies in multiple myeloma: CCI-779 and RAD001 (52, 53). CCI-779 (temsirolimus, Wyeth) has shown in vitro and in vivo activity against multiple myeloma cell lines, specifically those with PTEN mutations (54, 55). Similarly, RAD001 (everolimus, Novartis) inhibits proliferation at nanomolar concentrations against a broad range of multiple myeloma cells in vitro (52). It also has antitumor activity in a severe combined immunodeficient/nonobese diabetic mouse model of human multiple myeloma (52). Importantly, the combination of rapamycin with other novel agents used in multiple myeloma, such as lenalidomide and 17-allylamino-17-demethoxygeldanamycin (17-AAG), has shown synergistic activity in vitro (56, 57). In addition, rapamycin has shown strong antiangiogenic activity even at low concentrations (53, 58). Interestingly, rapamycin also showed inhibitory activity on osteoclasts in vitro, indicating that it may inhibit in vivo bone lytic lesion formation in multiple myeloma (57). A phase II study of i.v. CCI-779 once a week in patients with relapsed multiple myeloma has shown exciting responses (59). In addition, a phase II trial of oral RAD001 is currently being conducted in patients with relapsed and refractory multiple myeloma. Based on preclinical studies, a phase II trial of RAD001 in combination with lenalidomide and another phase I/II clinical trial of CCI-779 in combination with bortezomib are currently under way in patients with relapsed/refractory multiple myeloma.

Indirect inhibitors of the PI3K pathway. These include IL-6 and IGF-1 inhibitors as well as heat shock protein-90 (Hsp90) inhibitors.

IL-6 inhibitors. IL-6 is one of the major cytokines that induce proliferation and survival in multiple myeloma. It activates multiple signaling pathways mediating proliferation, including the PI3K, Ras/mitogen-activated protein kinase/extracellular signal–regulated kinase/keratin/extracellular signal–regulated kinase, and Janus-activated kinase/signal transducers and activators of transcription cascades (36). Monoclonal antibodies targeting IL-6 and the IL-6 receptor have shown preclinical antitumor cytostatic activity in multiple myeloma in vitro and transient antitumor activity in vivo (60). However, clinical trials using anti–IL-6 monoclonal antibody failed to achieve clinical activity in multiple myeloma (60). More recently, superantagonists to the IL-6 receptor have shown strong antitumor activity in vivo, specifically Sant7 (61). Sant7 showed activity even in the demethasone-resistant multiple myeloma cell lines. In addition, it showed synergistic activity in vitro when combined with demethasone and significantly potentiated the activity of demethasone in vivo in a humanized severe combined immunodeficient multiple myeloma model (61). Taken together, these studies provide the framework for studying Sant7 in clinical trials in multiple myeloma.

IGF-I inhibitors. IGF-I is another major regulator of cell survival and activation of PI3K and mitogen-activated protein kinase in multiple myeloma. The IGF-I receptor (IGF-IR) is highly expressed on various hematologic cell lines as well as patient multiple myeloma cells (62). IGF-I triggers multiple myeloma cell proliferation and drug resistance; conversely, a small-molecule IGF-IR tyrosine kinase inhibitor, NVP-ADW742, has shown significant cytotoxicity in vitro and in vivo in multiple myeloma (62). In addition, IGF-IR inhibition decreased phosphorylation and activation of multiple key kinases and kinase targets involved in the PI3K/Akt pathway, including Akt, IκB kinase, FKHR1, p70S6 kinase, and glycogen synthase kinase 3α/β. In addition, IGF-IR inhibition of multiple myeloma cells sensitized the cells to other anticancer agents including doxorubicin and melphalan (62). These results provide the rationale for undergoing clinical trials of IGF-IR inhibitors for the treatment of multiple myeloma patients.

Hsp90 inhibitors. Hsp90 is a ubiquitous molecular chaperone involved in the proper folding of a variety of intracellular proteins involved in proliferation and survival of tumor cells, including bcr/abl chimeric kinases, cell-surface receptors like HER2/neu, and receptors of androgens and estrogens (63). Studies in multiple myeloma have shown that heat shock proteins are involved in multiple intracellular tumor proliferation/survival pathways including the PI3K/Akt pathway (64). More specifically, small-molecule Hsp90 inhibitors (such as geldanamycin, a benzoquinone ansamycin antibiotic produced by the bacterium Streptomyces hygroscopicus, and its analogue 17-AAG) suppressed multiple myeloma cell proliferation and survival both in vitro and in vivo (64). Growth inhibition was associated with suppression of signaling events triggered by IGF-I and IL-6 at multiple levels, including suppression of cell-surface receptor expression (IGF-IR) and inhibition of downstream PI3K signaling mediators such as Akt and IκB kinase (65). Multiple anti-Hsp90 compounds (17-AAG, KOS-953, and IPI-504) are currently being tested in phase I and II clinical trials in patients with multiple myeloma. KOS-953 is a novel form of 17-AAG, which, based on preclinical studies, has been combined with bortezomib in clinical trials and has already shown promising activity even in patients whose multiple myeloma is resistant to bortezomib (66).

Conclusion

In summary, the PI3K pathway is a critical regulator of cell survival and proliferation in multiple myeloma. Several PI3K cascade inhibitors show significant preclinical activity, and clinical trials show early promising activity. These novel agents include direct inhibitors of the pathway such as perifosine, enzastaurin, CCI-779, and RAD001. In addition, indirect inhibitors of the pathway such as IL-6 and IGF-IR inhibitors and Hsp90 inhibitors also inhibit signaling through the PI3K/Akt pathway. The combination of inhibitors of this pathway along with other novel therapeutic agents has great promise to improve patient outcome in multiple myeloma.

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