

Isotype-specific inhibition of the phosphatidylinositol-3-kinase pathway in hematologic malignancies

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Abstract: In the last decade, the advent of biological targeted therapies has revolutionized the management of several types of cancer, especially in the realm of hematologic malignancies. One of these pathways, and the center of this review, is the phosphatidylinositol-3-kinase (PI3K) pathway. The PI3K pathway seems to play an important role in the pathogenesis and survival advantage in hematologic malignancies, such as leukemia, lymphoma, and myeloma. The objectives of the present review, hence, are to describe the current knowledge on the PI3K pathway and its isoforms, and to summarize preclinical and clinical studies using PI3K inhibitors, focusing on the advances made in hematologic malignancies.

Keywords: phosphatidylinositol-3-kinase pathway, inhibitors, leukemia, lymphoma, myeloma

Introduction

In the last decade, the advent of biological targeted therapies has revolutionized the management of several types of cancer, especially in the realm of hematologic malignancies. For example, monoclonal antibody therapy has been shown to increase response and survival rates in patients with non-Hodgkin and Hodgkin lymphoma and chronic lymphocytic leukemia. Furthermore, orally administered small molecules have become the mainstay of treatment for plasma cell myeloma and chronic myeloid leukemia.

The reasons for these advancements are several, but the main driver has been the increased and improved understanding of the multiplicity of intracellular pathways that could be affected during the oncogenic process, providing cancer cells with a wide variety of mechanisms by which to thrive, survive, and multiply. One of these pathways, and the center of this review, is the phosphatidylinositol-3-kinase (PI3K) pathway. The PI3K pathway seems to play an important role in the pathogenesis and survival advantage in hematologic malignancies, such as leukemia, lymphoma, and myeloma. For this purpose, a series of PI3K inhibitors are in ongoing preclinical and clinical development with enthusiasm for the treatment of these conditions.

The objectives of the present review are to describe the current knowledge of the PI3K pathway and its isoforms, and to summarize ongoing preclinical and clinical studies using PI3K inhibitors, focusing on the advances made in hematologic malignancies.

PI3K pathway

PI3K forms part of the PI3K/acute transforming retrovirus (AKT)/mammalian target of rapamycin (mTOR) pathway, which is a complex cascade of signal transduction

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that regulates proliferation and intracellular metabolism, as well as cell survival, differentiation, motility, and migration (Figure 1). The PI3K/AKT/mTOR pathway has been associated not only with normal hematopoiesis but also with the development and maintenance of a number of malignancies. There are three classes of PI3K described, ie, class I, II, and III.

Class I PI3K has been associated with oncogenesis.¹ PI3K is a heterodimer formed by two subunits, one catalytic and one regulatory. The catalytic subunit, p110, has four isotypes, ie, alpha, beta, gamma, and delta, encoded by the genes *PIK3CA*, *PIK3CB*, *PIK3CG*, and *PIK3CD*, respectively.² The p110-alpha isotype has a role in insulin-dependent signaling, p110-beta in platelet aggregation, thrombosis, and insulin signaling, and p110-gamma and delta are expressed mainly in leukocytes and have roles in lymphocyte activation, mast cell degranulation, and chemotaxis.³ The catalytic p110 subunit associates with one of three p85-related regulatory subunits, p85-alpha, p85-beta, and p55-gamma, which are encoded by the genes *PIK3R1*, *PIK3R2*, and *PIK3R3*, respectively.² The p85 regulatory subunit is activated via receptor tyrosine kinase stimulation by binding to phosphotyrosine residues in the receptor tyrosine kinase. Such a process relieves the catalytic p110 subunit from the inhibition of the regulatory

p85 subunit. PI3K then localizes in the cellular membrane where the subunit p110 converts phosphatidylinositol (4,5)-biphosphate into phosphatidylinositol (3,4,5)-triphosphate, which then activates downstream AKT with participation of 3-phosphoinositide-dependent protein kinase-1 (PDK1), PDK2, and mTOR-ricor. AKT, a serine/threonine kinase, in turn modulates the activity of mTORC1, BCL-2 (BAD), FOXO, p53, p27, C-MYC, insulin-like growth factors, and cyclin D1, resulting in dysregulation of metabolism and protein synthesis, and cell growth, proliferation, and survival.⁴

There are three isoforms of class II PI3Ks, ie, PI3K-C2-alpha, PI3K-C2-beta, and PI3K-C2-gamma. However, no regulatory subunits have been described for these enzymes. PI3K-C2-alpha seems to have a role in clathrin-mediated vesicle trafficking, neurosecretory granular exocytosis, smooth muscle contraction, and insulin signaling, and PI3K-C2-beta in cell migration and neuronal cell survival.³ The function of PI3K-C2 has not yet been determined. Class III PI3K, also known as vacuolar protein sorting 34, is encoded by the *PI3KC3* gene, and associates with the putative serine/threonine protein tyrosine kinase p150 regulatory subunit. Vacuolar protein sorting 34 has a role in vesicular trafficking of membrane proteins to the lysosome, and has also been implicated in activation of the mTOR pathway.³

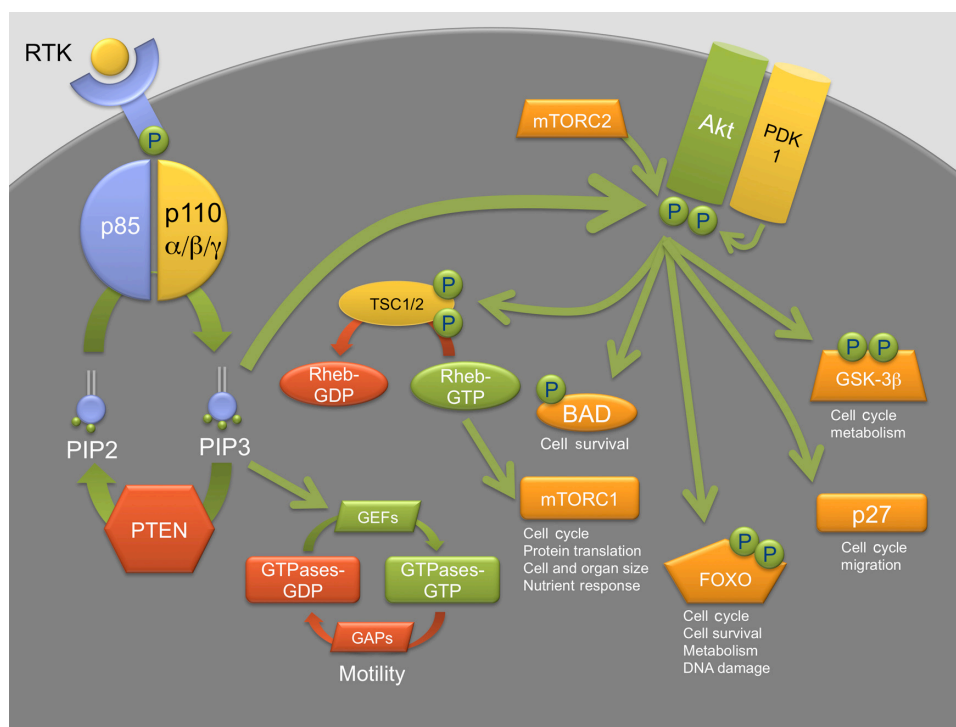


Figure 1 PI3K/AKT/mTOR pathway.

Abbreviations: PI3K, phosphatidylinositol-3-kinase; AKT, protein kinase B; mTOR, mammalian target of rapamycin.

PI3K pathway targeting in hematologic malignancies

The PI3K p110-delta isotype plays a critical role in the signaling pathways of various types of leukemia. Hence, it has become an attractive target for pharmacotherapy. While other isoforms of class I PI3K molecules (ie, p110-alpha and p110-beta) are expressed ubiquitously in mammalian tissue, the p110-delta subunit appears to be more selectively expressed in leukocytes.⁵ Preclinical data on acute myeloid leukemia and chronic lymphocytic leukemia has identified the p110-delta subunit as a predominant isoform in these diseases.^{6,7} Sujobert et al showed consistently increased activity of p110-delta, in contrast with p110-alpha and p110-beta, in leukemic blasts from patients with a diagnosis of acute myeloid leukemia who participated in clinical trials by the “Groupe Ouest-Est des Leucémies et des Autres Maladies du Sang”.⁷ Similarly, Herman et al showed increased activity of p110-delta in cells from patients with chronic lymphocytic leukemia. Additionally, constitutive activation of p110-delta was significantly higher in malignant cells when compared with normal B-cells from the same patients.⁶

In 2000, Borlado et al developed a mouse model in which increased activity of PI3K was associated with development of infiltrating malignant lymphomatous lesions.⁸ Since then, a number of studies have focused on evaluating the pathophysiologic role played by the PI3K pathway in lymphoma. Uddin et al demonstrated that the PI3K pathway was constitutively active in diffuse large B-cell lymphoma (DLBCL) cell lines and primary cells from DLBCL patients.⁹ PI3K activity, reflected by AKT phosphorylation, was identified in 52% of primary DLBCL cells. Interestingly, AKT-activated DLBCL patients had a worse 5-year overall survival rate. In the same experiment, inhibition of the PI3K pathway induced apoptosis in DLBCL cells. A retrospective study has also shown that AKT-activated DLBCL patients have worse outcomes even when treated with a rituximab-containing regimen.¹⁰ Rudelius et al investigated the activity of the PI3K pathway in mantle cell lymphoma, and showed constitutive activation in mantle cell lymphoma cell lines and also in primary cells derived from patients with blastoid (100% of cases) and typical mantle cell lymphoma (30% of cases), measured by activated AKT.¹¹ Blastoid mantle cell lymphoma, although rare, is a hard-to-treat lymphoma with extremely poor outcomes. PI3K pathway activation has also been demonstrated in follicular lymphoma and Hodgkin lymphoma cell lines.^{12,13}

The PI3K pathway also seems of importance in plasma cell myeloma. Ikeda et al showed that p110-delta, the catalytic portion of PI3K, was overactive not only in plasma

cell myeloma cell lines but also in the malignant cells of 24 patients with a diagnosis of plasma cell myeloma.¹⁴

Based on the results of these experiments, the clinical use of p110-delta isotype-specific inhibitors of the PI3K pathway is of interest and specific molecules targeted at p110-delta could become an additional weapon in our armamentarium against specific types of leukemia, lymphoma, and myeloma. The isotype-specific effects of selected PI3K inhibitors are shown in Table 1.

Idelalisib (GS-1101, CAL-101)

Idelalisib is a highly specific inhibitor of the PI3K p110-delta subunit that has oral bioavailability. Idelalisib is a PI3K inhibitor that is more advanced in its clinical development, and has been studied in leukemia, lymphoma, and myeloma.

Preclinical data

In vitro studies using idelalisib in chronic lymphocytic leukemia and acute myeloid leukemia have shown promising activity in terms of inhibiting constitutively active oncogenic stimuli, inducing apoptosis, and blocking survival signaling from the microenvironment while minimally impacting normal hematopoietic cells.^{6,15} Additional in vitro studies in chronic lymphocytic leukemia cell lines have shown that idelalisib-treated cells have inhibited chemotaxis, down-regulation of chemokines, and impaired susceptibility to cell survival signals.¹⁶ Furthermore, it has been shown that treatment with idelalisib can sensitize chronic lymphocytic leukemia cells to chemotherapy. Data from Herman et al have shown a beneficial effect of combination therapy with idelalisib and the immunomodulator lenalidomide.¹⁷ Lenalidomide has been associated with a cytokine release syndrome when given in chronic lymphocytic leukemia, which is thought to be

Table 1 Biochemical profile of selected PI3K inhibitors against class I, II, and III PI3Ks (IC₅₀ levels in nM)

Inhibitor	Class I				Class II	Class III
	p110-alpha	p110-beta	p110-gamma	p110-delta		
Idelalisib	820	565	89	2.5	>10 ³	978
AMG 319	–	–	–	<10	–	–
BAG956	56	446	117	35	–	–
BEZ235	4	75	5	7	–	–
BGT226	4	63	–	38	–	–
BKM120	52	166	262	116	–	2,410
IC87114	1,820	>1,000	1,240	70	–	–
IPI145	1,602	85	27	2.5	–	–
PI103	2	3	15	3	–	–

Note: A dash means data are not available.

Abbreviations: IC₅₀, half maximal inhibitory concentration; PI3K, phosphatidylinositol-3-kinase.

due to upregulation of p110-delta and subsequent activation of AKT.¹⁸ When lenalidomide is given in conjunction with idelalisib, there is less upregulation of cellular prosurvival mediators like phosphorylated AKT, suggesting that concomitant administration of these drugs may produce a dampened immune response while still providing cytotoxicity.

Idelalisib has shown preclinical activity against a number of lymphoma cell lines. Idelalisib downregulated AKT phosphorylation in DLBCL, mantle cell lymphoma, and follicular lymphoma cell lines, and induced a several-fold increase in levels of apoptotic markers, such as caspase 3 and poly-(ADP-ribose) polymerase cleavage.¹⁵ Meadows et al evaluated the effect of idelalisib in Hodgkin lymphoma cell lines.¹⁹ High levels of p110-delta and phosphorylated AKT were found in five of five (100%) Hodgkin lymphoma cell lines investigated. Exposure to idelalisib not only decreased levels of p110-delta and phosphorylated AKT but also disrupted tumor microenvironment-mediated survival signals mediated by CCL5, CCL17, and CCL22 in cocultures of Hodgkin lymphoma cells and bone marrow stem cells. Idelalisib also induced dose-dependent apoptosis of Hodgkin lymphoma cells at 48 hours. Reduced cell viability and increased apoptosis were observed when combining idelalisib with the mTOR inhibitor everolimus.

When idelalisib was tested against plasma cell myeloma cell lines and primary cells, the drug exhibited potency against cells that expressed p110-delta.¹⁴ Peripheral blood mononuclear cells from healthy donors were not significantly affected by idelalisib. In order to mimic the bone marrow microenvironment, which is thought to be protective for malignant cells in plasma cell myeloma, bone marrow stem cells, interleukin-6, and insulin-like growth factor-1 were added to plasma cell myeloma cells in culture but these did not provide protection against idelalisib-induced apoptosis and cell death. The authors also hypothesized that idelalisib would have antiangiogenesis activity; the drug was tested on human umbilical vein endothelial cells to assess for inhibition of capillary-like tube formation. Idelalisib inhibited capillary-like tube formation in a dose-independent and time-dependent manner.¹⁴ Additionally, idelalisib showed a synergistic effect when combined with the proteasome inhibitor bortezomib and tested against two of the cell lines that had previously been shown to express p110-delta.¹⁴

Clinical data

A series of Phase I/II studies are currently available in abstract form. Not surprisingly, these studies are focused mainly on the treatment of patients with relapsed or refractory

hematologic malignancies, although a few studies are already looking at combination therapy in untreated patients. Selected Phase I/II studies using idelalisib either alone or in combination are shown in Table 2. Single-agent idelalisib studies in patients with chronic lymphocytic leukemia, indolent non-Hodgkin lymphoma, and mantle cell lymphoma have shown overall response and complete remission rates in the range of 40%–50% and 2%–5%, respectively. The response rates for idelalisib in combination with chemotherapy are expectedly higher, with overall response and complete remission rates in the range of 80%–100% and 30%–50%, respectively. The most common grade 3 or higher adverse events associated with idelalisib have been pneumonia, diarrhea, and liver enzyme elevation. The design of the four Phase III studies has been reported in abstract form at the 2013 American Society of Clinical Oncology annual scientific meeting (Table 3).

Other PI3K p110-delta inhibitors AMG 319

AMG 319 is a highly selective inhibitor of PI3K p110-delta and has oral bioavailability. In a preclinical study evaluating more than 20 B-cell malignancy cell lines, AMG 319 was found to be a potent inhibitor of AKT activation and effective at reducing the number of viable cells.²⁰ However, different cell lines had variable responses to this drug. Further analysis showed that when AMG 319 was given concomitantly with vincristine in vitro and in vivo using a xenograft model of DLBCL, a synergistic effect was noted and there was increased cytotoxicity.²¹ A recent study evaluated AMG 319 alone or in combination with a series of monoclonal antibodies, targeted agents, and chemotherapy drugs in a panel of rituximab-sensitive and rituximab-resistant lymphoma cell lines as well as primary cells from patients with B-cell and T-cell lymphomas. AMG 319 showed dose-dependent activity against all cell lines. Additionally, AMG 319 induced cell death in primary cells at lower doses than in cell lines. Also, pre-exposure to AMG 319 increased the biological activity of rituximab and ofatumumab in terms of antibody-dependent and complement-dependent cytotoxicity. The apoptotic mechanism of AMG 319 seems to be mediated via the mitochondrial pathway. Ongoing clinical studies are shown in Table 4.

IC87114

IC87114 is another selective inhibitor of PI3K p110-delta. IC87114 was shown to inhibit proliferation of acute myeloid leukemia blast cells without significant toxicity to normal hematopoietic cells in vitro.^{7,22} In acute myeloid leukemia

Table 2 Selected clinical trials using idelalisib with data reported at the 2013 American Society of Clinical Oncology annual scientific meeting

Author	Phase	Disease	n	Alone/combination	Outcome	Toxicity (>Grade 3)
Brown et al ⁴⁴	I	Relapsed or refractory CLL	54	Alone	ORR 56% CR 4% Median PFS 17 months	Pneumonia 19% Diarrhea 6% Fever 4%
Barrientos et al ⁴⁵	I	Relapsed or refractory CLL	52	In combination with rituximab and/or bendamustine	ORR 81% CR 2% 2-year PFS 62%	Diarrhea 14% Pneumonia 12% ALT/AST elevation 10%
Benson et al ⁴⁶	I	Relapsed or refractory indolent NHL	64	Alone	ORR 48% CR 2% Median PFS 8 months	ALT/AST elevation 25% Pneumonia 16% Diarrhea 8%
Leonard et al ⁴⁷	I	Relapsed or refractory indolent NHL	78	In combination with rituximab and/or bendamustine	ORR 81% CR 28% 20-month PFS 66%	ALT/AST elevation 17% Pneumonia 15% Diarrhea 8%
Spurgeon et al ⁴⁸	I	Relapsed MCL	40	Alone	ORR 40% CR 5% Median PFS 4 months	ALT/AST elevation 20% Diarrhea 18% Pneumonia 13%
Wagner-Johnston et al ⁴⁹	I/II	Relapsed MCL	22	In combination with everolimus (Id + E), bortezomib (Id + V), or bendamustine/rituximab (Id + BR)	ORR 25% (Id + E), 50% (Id + V), 100% (Id + BR) CR 0% (Id + E, Id + V), 50% (Id + BR) Median PFS not reached	Thrombocytopenia 27% Neutropenia 14% Rash 14% ALT/AST elevation 5%
O'Brien et al ⁵⁰	II	Untreated CLL >65 years	50	In combination with rituximab	ORR 96% CR not reported 2-year PFS 91%	AST/ALT elevation 22% Pneumonia 20% Diarrhea 16%

Abbreviations: CLL, chronic lymphocytic leukemia; CR, complete response; MCL, mantle cell lymphoma; NHL, non-Hodgkin lymphoma; ORR, overall response rate; PFS, progression-free survival; Id, idelalisib; E, everolimus; V, bortezomib; BR, bendamustine/rituximab; ALT, alanine transaminase; AST, aspartate transaminase.

blasts, IC87114 decreases AKT phosphorylation in a dose-dependent manner, with maximal PI3K inhibition at 10 μ M.⁷ When compared against a pan-PI3K inhibitor (LY294002), similar reductions in AKT phosphorylation were observed, indicating that p110- δ is the main contributor to PI3K activity in acute myeloid leukemia blasts. IC87114 was also able to inhibit AKT phosphorylation after FLT3-ligand stimulation. Finally, acute myeloid leukemia blast proliferation was almost

completely blocked by IC87114. Furthermore, IC87114 in combination with etoposide has shown synergism in acute myeloid leukemia cell lines by reducing viable cell numbers, which was associated with a reduction in nuclear factor kappa B activity. IC87114 did not appear to enhance the activity of etoposide in terms of proliferation and survival of normal hematopoietic progenitors.²² Of note, this molecule has also shown activity against acute promyelocytic leukemia cells in

Table 3 Selected Phase III studies using idelalisib with data reported at the 2013 American Society of Clinical Oncology annual scientific meeting

Author	n	Disease	Alone/combo	Endpoints	ClinicalTrials.gov ID
Flinn et al ⁵¹	210	Previously treated CLL	Arm A idelalisib + ofatumumab Arm B ofatumumab Randomization 2:1	Primary, PFS Secondary, ORR, CR, OS, lymph node response rate	NCT01659021
Eradat et al ⁵²	390	Previously treated CLL	Arm A idelalisib + BR Arm B idelalisib Randomization 1:1	Primary, PFS Secondary, ORR, CR, OS, lymph node response rate	NCT01569295
Leonard et al ⁵³	375	Previously treated indolent NHL	Arm A idelalisib + rituximab Arm B idelalisib Randomization 2:1	Primary, PFS Secondary, ORR, CR, OS, lymph node response rate	NCT01732913
De Vos et al ⁵⁴	450	Previously treated indolent NHL	Arm A idelalisib + BR Arm B BR Randomization 2:1	Primary, PFS Secondary, ORR, CR, OS, lymph node response rate	NCT01732926

Abbreviations: BR, bendamustine and rituximab; CLL, chronic lymphocytic leukemia; CR, complete response; NHL, non-Hodgkin lymphoma; ORR, overall response rate; OS, overall survival; PFS, progression-free survival.

Table 4 Selected ongoing clinical trials with PI3K inhibitors in hematologic malignancies

ClinicalTrials.Gov ID	Phase	Drug	n	Disease	Alone/combination
NCT01393106	II	Idelalisib	25	Hodgkin lymphoma	Alone
NCT01796470	II	Idelalisib	200	CLL, DLBCL, NHL, MCL	With GS-9973
NCT01693614	II	BKM120	66	DLBCL, FL, MCL	Alone
NCT01882803	II	IPI-145	120	Refractory indolent NHL	Alone
NCT01306643	I/II	Idelalisib	15	Low-grade lymphomas	Alone
NCT01644799	I/II	Idelalisib	30	FL	With lenalidomide and rituximab
NCT01838434	I/II	Idelalisib	99	MCL	With lenalidomide and rituximab
NCT01300026	I	AMG319	50	CLL, lymphoma	Alone
NCT01756118	I	BEZ235	23	ALL, AML, CML	Alone
NCT01396499	I	BKM120	16	Advanced leukemia	Alone
NCT01719250	I	BKM120	18	Relapsed/refractory NHL	Alone
NCT01871675	I	IPI-145	70	CLL, lymphoma	With bendamustine, rituximab or bendamustine/rituximab
NCT01476657	I	IPI-145	250	Advanced hematologic malignancies	Alone

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; MCL, mantle cell lymphoma; NHL, non-Hodgkin lymphoma; PCM, plasma cell myeloma; PI3K, phosphatidylinositol-3-kinase.

a preclinical study.²³ In acute promyelocytic leukemia, inhibition of p110-beta and p110-delta triggered apoptosis in the presence or absence of all-trans retinoic acid. IC87114 has also been shown to reverse acute promyelocytic leukemia cells protective mechanisms against doxorubicin or arsenic trioxide, which correlates with downregulation of MCL-1.

PI3K dual inhibitors

BEZ235

BEZ235 is a dual inhibitor of PI3K and mTOR with oral bioavailability.²⁴ In a preclinical study, this molecule has been shown to induce apoptosis and reduce survival of acute myeloid leukemia cells without significant toxicity to normal CD34+ cells.²⁵ BEZ235 has also demonstrated efficacy in plasma cell myeloma cell lines as well as in a murine xenograft model. The IC₅₀ varied depending on which cell lines were tested, but most of the cell lines had an IC₅₀ of <50 nmol/L. Additionally, three of five patient samples had IC₅₀ <200 nmol/L. The compound was also tested in peripheral blood mononuclear cells and immortalized hepatocyte cells, and stromal cell survival was not affected at drug levels of 800 nmol/L. When interleukin-6 and insulin-like growth factor-1 were added to the compound, no survival benefit was conferred to the plasma cell myeloma cell lines. Using a cell viability assay, BEZ235 has also demonstrated an additive effect when combined with dexamethasone, doxorubicin, and bortezomib.²⁶ In a recent preclinical study, BEZ235 has been shown to be effective against follicular lymphoma cell lines.²⁷ BEZ235 successfully inhibited AKT, mTOR, and S6K phosphorylation in K422, SUDHL16, FL-18, and SUDHL4 cell lines. Inhibition of cell proliferation

was due to increased apoptosis characterized by a two-fold increase in caspase 3 activation. In a mouse xenograft model, BEZ235 was associated with minimal toxicity and reduced phosphorylated levels of AKT and S6k, downstream effectors of PI3K and mTOR. Levels of phosphorylated AKT and mTOR were detected in tissue samples from patients with mantle cell lymphoma, facilitating the generation of a bortezomib-resistant mantle cell lymphoma cell line. In this experiment, individual inhibition of PI3K and mTOR showed limited antiproliferative effects; however, dual inhibition with BEZ235 inhibited cell growth.²⁸ Ongoing clinical studies are shown in Table 4.

BAG956

BAG956 is a cell-permeable dual inhibitor of PI3K and PDK1. The IC₅₀ value for the p110-delta subunit is 35 nM.²⁹ BAG956 is effective against FLT3-mutant acute myeloid leukemia cell lines as well as chronic myeloid leukemia cell lines expressing BCR-ABL.³⁰ In both cell lines, BAG956 has been shown to decrease the levels of phosphorylated AKT in a time-dependent manner while no changes were detected in levels of total AKT. Similarly, levels of cyclin D2 decreased with increasing exposure to BAG956. The compound also showed synergy against FLT3-mutant acute myeloid leukemia cells and BCR-ABL chronic myeloid leukemia cells when combined with imatinib, nilotinib, and rapamycin. Given the observed synergy, studies in mice were undertaken. The tumor burden was the lowest in mice treated with the combination of BAG956 and nilotinib or rapamycin than in mice treated with vehicle or either agent alone.

BGT226

BGT226 is a dual PI3K and mTOR inhibitor that has been tested in multiple myeloma cell lines as well as samples from patients. It was shown to have an IC_{50} ranging from 20 to 100 nmol/L and inhibited cell growth in a time-dependent and dose-dependent manner. In three of four cell lines tested, the compound actually caused apoptosis as opposed to just inhibiting cellular proliferation. BGT226-induced apoptosis is mediated by caspase 3 and characterized by upregulation of BIM, MCL-1, and BAD, and downregulation of BCL-2, BAX, and BCL-XL.³¹ BGT226 induced cell cycle arrest in the G1 phase due to downregulation of cyclin D1 and cyclin D2. The compound was also shown to abrogate the stimulatory effects of interleukin-6, insulin-like growth factor-1, and stromal cell media in multiple myeloma cells.³¹ Similarly, in acute leukemia cells, BGT226 demonstrated strong inhibition of serine and threonine AKT phosphorylation and increased cleavage of caspase 3.³² Additionally, cells treated with BGT226 were able to override halting of cell growth and induction of autophagy to induce apoptosis in a cell cycle-independent manner. In this experiment, the apoptotic properties of BGT226 were more pronounced as compared with BEZ235.

PI-103

PI-103 is a dual inhibitor of PI3K and mTOR. Although this molecule is a more selective inhibitor of the p110- α subunit, it still has some inhibitory effect on the p110- δ subunit, with an IC_{50} of 8 nM and 48 nM, respectively.³³ In acute myeloid leukemia cells, PI-103 resulted in cell cycle arrest in the G1 phase. PI-103 also induced leukemic cell proliferation and induced mitochondrial apoptosis in blast cells, but had little effect on the clonogenic and proliferative properties of normal CD34+ cells.³⁴ In chronic lymphocytic leukemia cells, PI-103 has been shown to inhibit chemotaxis and also reduce the spontaneous migration of chronic lymphocytic leukemia cells beneath marrow stromal cells, a process called pseudoemperipoiesis.³⁵ Additionally, PI-103 appears to induce apoptosis in chronic lymphocytic leukemia cells as well as normal peripheral blood mononuclear cells, although the cytotoxic effect on peripheral blood mononuclear cells is less pronounced than in chronic lymphocytic leukemia cells. This effect seems to be mediated by caspase 7 and poly-(ADP-ribose) polymerase. Finally, PI-103 seemed to sensitize chronic lymphocytic leukemia cells to fludarabine and partially reversed the protective effect of stromal cells on fludarabine-induced apoptosis. In an experiment on primary multiple myeloma cells, PI-103 showed higher activity against cells harboring t(4;14) and t(14;16)

characterized by increased cyclin D2 activity; however, cells with t(11;14) showing increased cyclin D1 activity showed resistance against PI-103.³⁶

IPI-145

IPI-145 is a potent oral inhibitor of the PI3K- δ and PI3K- γ isoforms. IPI-145 was screened against a panel of over 400 diverse kinases using KINOMEscan® technology (DiscoveRx Corporation, Fremont, CA, USA), and was found to selectively bind to PI3K class I isoforms with no significant activity against other protein or lipid kinases, including class II PI3Ks.³⁷ In PI3K- γ -specific and δ -specific assays, IPI-145 inhibited AKT phosphorylation at the nanomolar level, and was shown to be approximately 14 times more potent than idelalisib in terms of δ blockade when compared directly. At the cellular level, IPI-145 inhibited the proliferation of CD19+ B-cells stimulated with anti-IgM and anti-CD40 antibodies. IPI-145 also inhibited the proliferation of CD3+ T-cells stimulated with Concanavalin A.

In a recent Phase I study, 55 patients with relapsed or refractory lymphoma were treated with IPI-145.³⁸ Doses >15 mg twice daily induced complete inhibition of PI3K- δ and increasing suppression of PI3K- γ with increasing dose. In 36 patients who received >15 mg twice daily, the most common adverse events were neutropenia and increased alanine aminotransferase. Early clinical activity was seen in T-cell lymphomas, with one complete remission and one partial remission, and B-cell lymphomas, with two complete remissions and nine partial remissions; 90% of the responses were seen by 3 months of therapy. Ongoing clinical trials are shown in Table 4.

Pan-PI3K inhibitors

BKM120 inhibits all class I PI3K isoforms with at least 50-fold selectivity for mTOR and other protein kinases, such as HER1, JAK2, and PDK1. BKM120 has been shown to decrease levels of pAKT in cell lines and xenograft tumor models. In vitro studies have shown that BKM120 exhibits preferential inhibition of tumor cells bearing PI3KCA mutations.³⁹ Additional preclinical studies suggest that BKM120 has activity against DLBCL and chronic lymphocytic leukemia lines. In DLBCL, BKM120 induces an increase in the proapoptotic PUMA and BIM and downregulation of the antiapoptotic BCL-XL and MCL-1.⁴⁰ Similarly, in chronic lymphocytic leukemia primary cells, BKM120 decreased phosphorylation of AKT and FOXO3a, leading to downregulation of MCL-1 and induction of BIM.^{41,42} BKM120 also inhibited BCR and stroma-dependent AKT

pathway activation and inhibited cell chemotaxis, sensitizing chronic lymphocytic leukemia cells to bendamustine and fludarabine.⁴² BKM120 has also shown activity against plasma cell myeloma cell lines and primary cells, with limited cytotoxicity in normal lymphocytes.⁴³ Additionally, BKM120 prolonged survival of plasma cell myeloma-bearing mice with synergistic effects in combination with dexamethasone. BKM120 causes cell cycle arrest by upregulating p27 and downregulating cyclin D1, and induces apoptosis by downregulating XIAP and inducing BIM. Ongoing clinical trials are shown in Table 4.

Conclusion

Improvements in the understanding of the PI3K pathway have led to the rational development and use of PI3K inhibitors, mainly in patients with hematologic malignancies. Idelalisib, a p110-delta inhibitor, appears to be the PI3K inhibitor under more advanced development. However, pan-PI3K and dual inhibitors are undergoing development and are already showing promising preclinical and clinical efficacy. The oral bioavailability of such drugs makes them interesting for the management of patients with chronic indolent conditions in whom quality of life might be the main goal of therapy. Our job as clinical researchers is, based on solid preclinical experience, to carefully design and carry out meaningful prospective studies using these compounds alone or in combination to improve responses, quality of life, and ultimately survival.

Disclosure

The authors report no conflicts of interest in this work.

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