Molecular Pathways: Targeting Phosphoinositide 3-Kinase p110-Delta in Chronic Lymphocytic Leukemia

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Abstract

The advent of targeted therapy, specifically to the B-cell receptor (BCR), has changed the convention for the treatment of chronic lymphocytic leukemia (CLL). The phosphoinositide 3-kinase (PI3K) pathway, activated upstream by the BCR, receptor tyrosine kinases, and cytokine receptors, has been a potential target for a multitude of cancers, but until the recent introduction of isoform-specific inhibitors has not been widely used. In this review, we focus on describing the intricate upstream and downstream signaling, leading to cell survival mediated by PI3K in B cells with a specific focus on the impact and importance of the p110δ isoform (which is localized to hematopoietic cells and regulates distinct cellular functions in B cells). In addition, the clinical advances from targeting p110δ are described, with a focus on clinical outcome, toxicities, and rational combination therapies. The experiences with p110δ in CLL have led to a more fundamental understanding of CLL signaling defects and may be predictive of other BCR-directed therapeutics. Clin Cancer Res; 18(15); 4013–8. ©2012 AACR.

Background

The phosphoinositide 3-kinase pathway in chronic lymphocytic leukemia

Cellular signaling involving protein kinases regulates important cellular functions including proliferation, growth, and cell survival; one such pathway is the phosphoinositide 3-kinase (PI3K) pathway (Fig. 1). There are 3 classes of PI3K isoforms; however, only the class I isoforms phosphorylate inositol lipids to form second messenger phosphoinositides and have been associated with tumorigenesis (1, 2). The class I PI3K isoforms can be subcategorized into class IA and class IB PI3Ks (3, 4). Class IA encompasses p110α, p110β, and p110δ (catalytic domains) bound by p85, p50, or p55 (regulatory domains; refs. 3, 5). Class IB is made up solely of the p110δ (catalytic domain) bound by the regulatory domain p101 (3, 5). Activation of PI3K class IA isoforms can occur via a B-cell receptor (BCR)-dependent or -independent (via receptor tyrosine kinases, cytokine receptors) manner. Upon ligation of the BCR, the proteins on the cell surface begin to change. Although activation is dependent on BCR signaling mediated by the antigen receptor, composed of membrane immunoglobulin M, it possesses a short cytoplasmic tail incapable of transmitting signals generated by receptor stimulation. Instead, these signals are transduced by the disulfide-linked helper molecules Igα and Igβ that are noncovalently associated with the antigen receptor. The Igα–Igβ complex contains an immunoreceptor tyrosine-based activation motif (ITAM). Upon receptor cross-linking, Src family kinases (such as Lyn, Fyn, and Blk) and spleen tyrosine kinase are brought into proximity with the receptor to phosphorylate the tyrosine residue on the ITAM on CD19 and/or BCP (6–8). This creates an Src homology 2 (SH2)-binding domain capable of binding SH2 domain proteins. This occurs similarly with receptor tyrosine kinases, where ligand binding leads to autophosphorylation of the tyrosine residue on the ITAM, again creating an SH2-binding domain (9), and with cytokine receptors, where formation of the receptor complex results in activation of the receptor-associated janus-activated kinase (JAK) tyrosine kinases (10). JAK activation is followed by phosphorylation of tyrosine residues in various proteins (including receptors such as gp130 and IRS family members) again providing binding sites for SH2 domain-containing proteins (10). The creation of the SH2-binding domain allows for the binding of the SH2 domain of the p85 subunit (or another regulatory subunit) of PI3K, allowing for activation of class IA PI3K (11). Once this occurs, p85 releases its conformational inhibitory effect on the catalytic subunit of PI3K (11, 12). Specifically, the catalytic class I PI3K enzymes are now able to convert PtdIns(3,4)P₂ into PtdIns(3,4,5)P₃ in the cell membrane (13). These events are directly regulated by 2 phosphates, PTEN and SHIP, both of which hydrolyze PtdIns(3,4,5)P₃ to PtdIns(4,5)P₂ and PtdIns(3,4)P₂, respectively (1, 13, 14).
In addition to class IA PI3Ks, there also exists class IB PI3Ks that function identically but are activated by G-protein-coupled receptors and regulated by a p110 subunit (3, 5). Regardless, once PtdIns(3,4)P$_2$ has been phosphorylated to PtdIns(3,4,5)P$_3$, it recruits via binding to the amino-terminal pleckstrin homology (PH) domain and downstream signaling proteins such as Tec kinases, PDK (phosphoinositide-dependent kinases), Akt, ILK, and Rac GEF. The prototype of these molecules is Akt (also known as PKB), which functions as a serine/threonine kinase (Akt1/2/3) that have a broad range of substrates (15). The role of the PI3K/AKT pathway in cell survival has been well described, with the PI3K/AKT pathway acting to antagonize apoptosis, promoted by a variety of environmental stresses, through interfering with downstream proteins (15–17). Binding of PI3K to PDK leads to phosphorylation of Akt at threonine-308 and serine-473 (15, 17). This activation of Akt leads to increased survival in a dual fashion: first, by inhibiting activation of apoptosis (by interfering with the expression of Fasl, suppression of the extrinsic caspase cascade by inhibition of death gene transcription, and repression of the intrinsic caspase cascade by decreasing the dissociation of the Bad/Bcl-X$_i$ complex, inhibition of cytochrome c release, and phosphorylation and thus inactivation of caspase 9) and also by activating NF-κB (which turns on cell survival signals such as c-FLIP that block caspase-8 activation; refs. 16–18). Thus, activation of the PI3K/AKT pathway increases activation of survival signals, whereas concurrently inhibiting apoptotic signals leads to an overall increase in cell survival.

**Validation of p110δ as a therapeutic target for chronic lymphocytic leukemia**

With the PI3K/AKT pathway playing such a diverse role in the regulation of cell survival/apoptosis, it is a prime target for the treatment of B-cell malignancies such as chronic lymphocytic leukemia (CLL), characterized by prolonged malignant cell survival. However, inhibiting the PI3K/AKT pathway has proved quite complex, as the pathway is involved in the maintenance of a multitude of cell types and it is critical to fundamental cell processes such as metabolism, growth, proliferation, and survival (1, 17). It is thought that this widespread functionality of PI3K signaling is at least partially responsible for the significant toxicity associated with pan-PI3K inhibitors, as these inhibitors (such as LY294002 and wortmannin) typically inhibit all of the class I PI3K isoforms (14, 19). The most notable of these is the induction of hyperglycemia caused by inhibition of PI3K (specifically p110δ) in the β islet cells of the pancreas (14). In recent years, it has been shown that the different class I isoforms have nonredundant roles and different expression profiles in different cell types (3, 20–22). The p110α and p110β isoforms are ubiquitously expressed, and knockout mice for both are embryonic lethal (4). In the past decade, it has been determined that the p110δ and p110γ isoforms of PI3K are expressed primarily in cells of hematopoietic lineage, such as B and T cells (12, 23). This suggests an important role for these isoforms of PI3K in B cells. Furthermore, mice with deleted or mutated p110δ exhibit a B-cell defect with a lack of B1 lymphocytes (as well as marginal zone B cells), decreased mature B cell numbers, and impaired antibody production (4, 20, 24). Biochemically, B cells derived from p110δ knockout mice also show less Akt phosphorylation when activated and have decreased PtdIns(3,4,5)P$_3$ levels and phosphopeptide activity (4). In contrast, p110γ isoform knockout mice, while not embryonic lethal, have predominantly a T-cell defect with no B-cell developmental or functional abnormalities (4). These mouse studies suggest that isoform-specific targeting of the p110δ isoform may be cytotoxic to B cells with minimal toxicity to other hematopoietic cell types. To further understand the role of p110δ in B cells, forced expression of p110δ was evaluated and found to be transforming in cell lines (25). In CLL cells, PI3K signaling has been found to be constitutively activated and at least in IGHV-unmutated CLL has been found to be overexpressed at the gene level (26). Moreover, in vitro studies have shown increased general activity of PI3K in the pathogenesis of CLL and other B-cell diseases with convergence of CD40L, BAFF, fibronectin, and BCR signaling through this pathway (27–31). The specificity of the p110δ makes it a promising drug target for B-cell lymphoproliferative disorders, including CLL, as it reduces the cellular toxicity associated with nonspecific PI3K inhibitors by reducing the off-target effects in nonhematopoietic tissues, while maintaining the ability to inhibit the PI3K pathway in a way that alters B-cell survival.

**Clinical–Translational Advances**

**GS-1101 (CAL-101)—a selective p110δ inhibitor**

The identification of the hematopoietic-selective isoform p110δ unlocks a new therapeutic potential for B-cell malignancies as it led to the clinical development of isoform-
specific kinase inhibitors. One such agent is GS-1101 (formerly known as CAL-101), the first p110δ inhibitor in clinical use, initially developed by Calistoga Pharmaceuticals and now Gilead. GS-1101 is an orally bioavailable, potent, and selective inhibitor of the p110δ isoform that is currently under clinical evaluation in B-cell malignancies (32, 33). \textit{In vitro}, the selectivity of GS-1101 to p110δ has been reported to be IC$\text{}_{50}$ of 2.5 nmol/L compared with 820, 565, and 89 nmol/L for p110α, β, and γ, respectively (34). In addition, GS-1101 was found to be 400-fold more selective for class I PI3K than other related kinases (34). GS-1101 is thus a selective p110δ inhibitor that has the potential to be a relevant therapeutic for CLL and other related disorders.

**Preclinical antitumor activity of GS-1011**

GS-1101 has been shown to affect cell survival and microenvironmental signaling \textit{in vitro} and \textit{in vivo}. \textit{In vitro}, we have shown that patients with CLL express p110δ (at both the gene and protein level), the target for GS-1101 (35). Subsequently, we found that GS-1101 can induce apoptosis in CLL cells, although responses varied. Lannutti and colleagues further showed an observed significant sensitivity (defined as an EC$\text{}_{50}$ < 1 μmol/L) to CAL-101 in 26% of the CLL samples (11 of 42) evaluated (34). We found that the induction of apoptosis was selective for CLL cells as compared with normal B cells or other hematopoietic cells and occurred independently of traditional prognostic markers (such as cytogenetic abnormality or IGVH mutational status; ref. 35). Furthermore, the induction of apoptosis via GS-1101 was found to be via a caspase-dependent mechanism (35). In addition to a direct induction of apoptosis, we showed that GS-1101 can inhibit induced survival mechanisms by blocking the protective effect of multiple microenvironmental stimuli (such as CD40L) by preventing activation of downstream signaling (such as phosphorylation of AKT; ref. 35). Similarly, Fiorari and colleagues recently presented data suggesting that GS-1101 can overcome both BMSC- and EC-mediated CLL cell protection, indicating that GS-1101 inhibits BMSC- and EC-derived prosurvival signals (36). More recently, Hoellenriegel and colleagues showed that GS-1101 can inhibit both the chemotaxis toward CXCL12 and CXCL13 and the migration beneath stroma cell layers, suggesting a potential mobilization effect (37). In addition, they showed that GS-1101 inhibits chemokine (such as CCL3 and CCL4) and cytokine (such as interleukin-6 and TNF) secretion mediated by BCR stimulation or nurse-like cells (37). Concurrent with this, chemosensitization was observed to other cytotoxic drugs (such as fludarabine and bendamustine; ref. 37). Along these lines, Davids and colleagues recently presented data suggesting that GS-1101 can sensitize stroma-exposed CLL cells to other agents (such as fludarabine) by inhibition of stroma–CLL contact, leading to an increase in mitochondrial apoptotic priming of the CLL cells (38). These data suggest that GS-1101 will be beneficial in the treatment of CLL by acting to directly induce apoptosis and to inhibit microenvironmental interactions, which would be leading to increased cellular survival, proliferation, and migration.

**Clinical experience with GS-1101**

Clinical results from either the single-agent CAL-101 (GS-1101) trial or dual therapy trials involving GS-1101 have not yet been published in manuscript form and to date have only been presented in part at international meetings; however, even early clinical trial data suggest promising results. At the American Society of Hematology (ASH) 2010 scientific meeting, Furman and colleagues reported the results from the first 37 patients treated with GS-1101. These patients showed a significant decrease in lymphadenopathy (occurring in 100% of the patients with bulky disease) with 91% of patients having at least a 50% reduction in lymph node size (39). As expected, significant lymphocytosis [absolute lymphocyte count (ALC) rising by more than 50% compared with baseline] occurred in 60% of patients with maxima during the first 2 cycles of treatment (39). Despite the lymphocytosis, when traditional response criteria were applied the overall progressive response rate was 33% (39). Pharmacodynamic studies on peripheral blood mononuclear cells showed a decrease in phosphorylated AKT after just 1 week of treatment (39). Corroborating these results, Hoellenriegel and colleagues showed that patients with CLL receiving GS-1101 showed decreases in CLL3, CLL4, and CXCL13 as well as reduced phosphorylation of AKT (threonine-308) by the end of the first cycle (37). Coutre and colleagues updated the patient responses in mid-2011 with a total of 34 patients enrolled. The ALC trends still upheld leaving the overall intention-to-treat response rate by International Workshop on Chronic Lymphocytic Leukemia 2008 criteria at 26%; despite this, however, 46% of patients remained on treatment (32). In addition to GS-1101 as a single agent, it is currently being investigated in combination with rituximab, bendamustine, ofatumumab, and fludarabine. The first reports of GS-1101 in combination therapy in CLL were presented briefly at the ASH 2010 meeting followed by an update at the ASCO meeting in 2011. In 2011, Flinn and colleagues reported that compared with baseline, on-treatment peripheral lymphocyte counts were stable or decreased in 8 of 8 patients with CLL (40). Following up on this study, Sharman and colleagues reported that although lymphocyte mobilization occurred in some patients, it was not as prominent as what had been reported for the single-agent studies (33). A distinct reduction in lymphadenopathy (>50% decrease in lymph node area) was observed in more than 75% of patients (33). Furthermore, Sharman and colleagues reported that the GS-1101 combination trial with rituximab and bendamustine showed a 80% clinical response rate concurrent with a decrease in CLL-associated chemokines and cytokines such as CCL3, CCL4, CXCL13, and TNF-α (33). Together, this suggests that although GS-1101 is showing promising results as a single agent, it is showing even greater potential in combination studies.
Conclusions

Inhibition of p110δ signaling in CLL appears to be a promising new therapeutic approach in treating CLL. Although GS-1101 is the only well-described p110δ molecule for the treatment of lymphoid malignancies, multiple other compounds are currently entering clinical development, further showing the legitimacy of p110δ as a target. Although preliminary clinical trial data have shown promising results, combination studies (such as those currently under way with anti-CD20 antibodies and bendamustine) are of even greater interest, given the distinct differences in the mechanisms of action of such kinase inhibitors and traditional chemotherapeutics or antibody therapy and the promising preliminary results currently available. Ongoing studies to discover the influence of PI3K in the micro-environment are important and will help our understanding of the differential effects on cells in the peri-

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