Molecular Pathways: Targeting the Phosphoinositide 3-Kinase (PI3-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 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 utilized. 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.
Background

*The phosphoinositide 3-kinase (PI3-Kinase) pathway in CLL:*

Cellular signaling involving protein kinases regulates important cellular functions including proliferation, growth and cell survival; one such pathway is the phosphoinositide 3-kinase (PI3-Kinase) pathway (Figure 1). There are three classes of PI3-Kinase 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 PI3-Kinase isoforms can be sub-categorized into class IA and class IB PI3-Kinases (3, 4). Class IA encompasses p110α, p110β and p110δ (catalytic domains), bound by p85, p50 or p55 (regulatory domains) (3, 5). Class IB is made up solely of the p110γ (catalytic domain) bound by the regulatory domain p101 (3, 5). Activation of PI3-Kinase class 1A isoforms can occur via a B-cell receptor (BCR) dependent or independent (via receptor tyrosine kinases, cytokine receptors, etc.) manner. Upon ligation of the BCR the proteins on the cell surface begin to change. Although activation is dependent on B-cell receptor signaling mediated by the antigen receptor, comprised of membrane IgM, 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 and phosphorylate the tyrosine residue on the ITAM on CD19 and/or BCAP (6-8). This creates a 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 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 a 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 PI3-Kinase allowing for activation of class IA PI3-Kinases (11). Once this occurs, p85 releases its conformational inhibitory effect on the catalytic subunit of PI3-Kinase (11, 12). Specifically, the catalytic class I PI3-Kinase enzymes are now able to convert PtdIns(3,4)P$_2$ into PtdIns(3,4,5)P$_3$, in the cell membrane (13). These events are directly regulated by two phosphates PTEN and SHIP; both which hydrolyze PtdIns(3,4,5)P$_3$ to PtdIns(4,5)P$_2$ and PtdIns(3,4)P$_2$, respectively (1, 13, 14). In addition to class IA PI3-Kinases, there also exists class IB PI3-Kinases that function identically but are activated by G-protein coupled receptors and regulated by a p101 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, downstream signaling proteins such as Tec kinases, PDK, Akt, ILK and Rac GEF. The prototype of these molecules is Akt (also known as PKB), which functions as three serine/threonine kinases (Akt1/2/3) that have a broad range of substrates (15). The role of the PI3-Kinase/AKT pathway in cell survival has been well described, with the PI3-Kinase/AKT pathway acting to antagonize apoptosis, promoted by a variety of environmental stresses, through interfering with downstream proteins (15-17). Binding of PI3-Kinase to PDK (phosphoinositide-dependent kinases) leads to phosphorylation of Akt at Thr$^{308}$ and Ser$^{473}$ (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$_L$ complex, inhibition of cytochrome c release, and phosphorylation; and thus inactivation, of caspase 9) and also by activating NF-$\kappa$B (which turns on cell survival signals; such as c-FLIP, that blocks caspase 8 activation) (13 2001, 16-18). Thus, activation of the PI3-Kinase/AKT pathway increases
activation of survival signals while concurrently inhibiting apoptotic signals leading to an overall increase in cell survival.

**Validation of p110δ as a therapeutic target for CLL:**

With the PI3-Kinase/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 PI3-Kinase/AKT pathway has proven quite complex; as the pathway is involved in the maintenance of a multitude of cell types; as it is critical to fundamental cell processes; such as metabolism, growth, proliferation and survival (1, 17). It is thought that this widespread functionality of PI3-Kinase signaling is at least partially responsible for the significant toxicity associated with pan-PI3-Kinase inhibitors as these inhibitors (such as LY294002 and wortmannin) typically inhibit all of the class I PI3-Kinase isoforms (14, 19). The most notable of these is the induction of hyperglycemia, caused by inhibition of PI3K (specifically p110α) in the β islet cells of the pancreas (14). In recent years it has been shown that the different class I isoforms have non-redundant roles and different expression profiles in different cell types (3, 20-22). The p110α and p110β isoforms are ubiquitously expressed, and knock-out mice for both are embryonic lethal (4). In the past decade it has been determined that the p110δ and p110γ isoforms of PI3-Kinase 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 PI3-Kinase in B-cells. Further, 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 knock-out mice also demonstrate less AKT phosphorylation when activated and have decreased PtdIns(3,4,5)P3 levels and phosphopeptide activity (4). In contrast, p110γ isoform knock-out mice, while not embryonic lethal, have predominately 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, PI3-Kinase signaling has been found to be constitutively activated and at least in IGHV unmutated CLL it has been found to be overexpressed at the gene level (26). Moreover, in vitro studies have demonstrated increased general activity of PI3-Kinase 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 non-specific PI3-Kinase inhibitors, by reducing the off target effects in non-hematopoietic tissues, while maintaining the ability to inhibit the PI3-Kinase 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 (formally 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). In vitro, the selectivity of GS-1101 to p110δ has been reported to be IC₅₀ of 2.5nM compared to 820, 565 and 89nM for p110α, β, and γ, respectively (34). In addition GS-1101 was found to be 400 fold more selective for class I PI3-Kinases than other related kinases (34). GS-1101 is thus a selective p110δ
inhibitor that has the potential to be a relevant therapeutic for the treatment of CLL and other related disorders.

**Preclinical antitumor activity of GS-1011:**

GS-1101 has been shown *in vitro* and *in vivo* to affect cell survival and microenvironmental signaling. *In vitro*, we have shown that CLL patients 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 et al. further demonstrated an observed significant sensitivity (defined as an EC_{50} < 1μM) to CAL-101 in 26% of CLL samples (11 of 42) evaluated (34). We found that the induction of apoptosis was selective for CLL cells as compared to normal B-cells or other hematopoietic cells and occurred independently of traditional prognostic markers (such as cytogenetic abnormality or IGVH mutational status) (35). Further, 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 demonstrated 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) (35). Similarly, Fiorcari et al., 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 pro-survival signals (36). More recently, Hoellenrigel et al., showed that GS-1101 can inhibit both the chemotaxis towards 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 IL-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) (37). Along these lines Davids et al. 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 p110δ:**

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 et al., reported the results from the first 37 patients treated with GS-1101. These patients demonstrated 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) raising more than 50% compared to baseline) occurred in 60% of patients with maxima during the first two cycles of treatment (39). Despite the lymphocytosis, when traditional response criteria was applied the overall PR rate was 33% (39). Pharmacodynamic studies on peripheral blood mononuclear cells showed a decrease in phosphorylated AKT after just one week of treatment (39). Corroborating these results Hoellenrigel et al., demonstrated that CLL patients receiving GS-1101 demonstrated decreases in CLL3, CLL4 and CXCL13 as well as reduced phosphorylation of AKT (Thr308) by the end of the first cycle (37). Coutre et al updated the patient responses in mid-2011 with a total of 54 patients enrolled. The ALC trends still uphold leaving the overall intention-to-treat response rate by IWCLL 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 et al., reported that compared to baseline, on-treatment peripheral lymphocyte counts were stable or decreased in 8/8 patients with CLL (40). Following up on this study, Sharman et al., reported that although lymphocyte mobilization occurred in some patients it was not as prominent as what has been reported for the single agent studies (33). A distinct reduction in lymphadenopathy (more than a 50% decrease in lymph node area) was observed in more than 75% of patients (33). Further they 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, CLL4, CXCL13 and TNF-alpha (33). Together this suggests that although GS-1101 is demonstrating promising results as a single agent it is showing even greater potential in combination studies.

Conclusions

Inhibition of p110δ signaling in CLL looks to be a promising new therapeutic approach to 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 has shown promising results, combination studies (such as those currently underway 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 PI3-Kinase in the microenvironment are important and will help our understanding of the differential effects on cells in the periphery and lymph node niches and the ongoing lymphocytosis.
Contributions

SH wrote the first draft, revised subsequent versions, and approved the final paper. AJ organized the structure of the manuscript, reviewed, edited, and revised all versions and approved the final paper.

Figure 1 Legend

Phosphoinositide 3-kinase (PI3K) signaling can be activated via a variety of methods including stimulation from the microenvironment leading to engagement of the B-cell receptor (BCR), cytokine receptors or receptor tyrosine kinases. Upon receptor activation, accessory molecules lead to the activation of PI3K. All three class 1A isoforms of PI3K (p110α, p110β and p110δ), facilitate the phosphorylation of phosphatidylinositol (3,4)-biphosphate (PIP2) into phosphatidylinositol (3,4,5)-trisphosphate (PIP3), leading to the phosphorylation of downstream signaling molecules. GS-1101, a selective p110δ isoform inhibitor, thereby prevents the activity of PI3K and thus inhibits the phosphorylation of downstream kinases; the hallmark of which is AKT. By preventing the phosphorylation of AKT, GS-1101 alters cell homeostasis by: 1) preventing the phosphorylation of GSK3, thereby preventing the transcription of survival genes induced by intact -catenin, 2) preventing the phosphorylation of caspase 9 leaving its protease activity intact and allowing for activation of the intrinsic caspase cascade, 3) preventing the phosphorylation of Frhl1 which blocks the binding of 14-3-3 allowing Frkh1 to translocate to the nucleus and transcribe genes related to cell death (such as FASL), and 4) preventing the phosphorylation of the IKK complex which inhibits its ability to phosphorylate IKBα preventing the release of the NF-κB complex and translocation to the nucleus; thereby again preventing survival gene transcription. Thus, inhibition of p110δ by GS-1101 tips the signaling balance from cell survival to cell death.
References


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