

REVIEW

PI3K δ Inhibitors in Cancer: Rationale and Serendipity Merge in the Clinic

David A. Fruman¹ and Christian Rommel²

ABSTRACT

Several phosphoinositide 3-kinase (PI3K) inhibitors are in the clinic and many more are in preclinical development. CAL-101, a selective inhibitor of the PI3K δ isoform, has shown remarkable success in certain hematologic malignancies. Although PI3K δ signaling plays a central role in lymphocyte biology, the degree of single-agent therapeutic activity of CAL-101 during early-phase development has been somewhat unexpected. CAL-101 works in part by blocking signals from the microenvironment that normally sustain leukemia and lymphoma cells in a protective niche. As PI3Ks enter the arena of molecular-targeted therapies, CAL-101 provides proof of principle that isoform-selective compounds can be effective in selected cancer types and patient populations.

SIGNIFICANCE: A key question is whether compounds targeting a single PI3K catalytic isoform can provide meaningful single agent efficacy in cancer cells that express multiple isoforms. Clinical studies of the drug CAL-101 have provided a significant advance by showing that selective targeting of PI3K δ achieves efficacy in chronic lymphocytic leukemia, in part through targeting the tumor microenvironment. *Cancer Discovery*; 1(7); 562-72. ©2011 AACR.

PHOSPHOINOSITIDE 3-KINASE AS A DRUG TARGET

Phosphoinositide 3-kinase (PI3K) was first discovered as an enzymatic activity coprecipitating with oncoproteins and activated growth factor receptors (1, 2). This spurred research in PI3K as an interesting new signaling molecule and a potential drug target. Subsequent cloning efforts identified 8 distinct genes encoding PI3K catalytic subunits (3-5). Four of these PI3K isoforms (PI3K α , PI3K β , PI3K γ , and PI3K δ) are categorized as class I enzymes because they can use phosphatidylinositol-4,5-bisphosphate (PtdIns-4,5-P₂) as a substrate to generate phosphatidylinositol-3,4,5-trisphosphate (PIP₃). Elevated PIP₃ in cellular membranes drives several hallmarks of the cancer phenotype: cell proliferation, survival, metabolic reprogramming, and migration (6, 7).

Diverse mechanisms can contribute to increased PIP₃ levels. Some of these are cell-intrinsic, such as oncogenic tyrosine kinases

(e.g., EGFR, BCR-ABL) or loss of the lipid phosphatase tumor suppressor PTEN. Cell-extrinsic cues, including growth factors, cytokines, chemokines, and adhesion molecules, also promote PI3K activation and PIP₃ production. One reason that PI3K has received such attention as a cancer target is that inhibiting PI3K should block growth and survival signals emanating from both the cell-intrinsic mutations and the microenvironment. Supporting this prediction, first-generation PI3K inhibitors wortmannin and LY294002 cause cell-cycle arrest and death in a broad array of tumor cell lines and stromal coculture models.

These studies led to the concept of PI3K as a convergence point for diverse upstream oncogenic inputs such that PI3K inhibition could provide a general strategy for cancer therapeutics (8). The fact that PI3K enzymes are primarily lipid kinases with distinct structure from classic protein kinase targets afforded the opportunity for drug selectivity. However, it soon became apparent that LY294002 (and, to a lesser extent, wortmannin) are not selective; they inhibit not only class I PI3Ks, but many other lipid and protein kinases in cells, including mTOR, a central regulator of cell growth and proliferation (9-11). Consequently, LY294002 has been instrumental in establishing the importance of class I PI3K and mTOR signaling in tumor cell biology but is a blunt instrument for understanding whether a specific PI3K isoform or subgroup has a required function (Fig. 1).

These considerations, along with the poor pharmacologic properties of wortmannin and LY294002, prompted development of second-generation, more selective "pan-PI3K" inhibitors. Compounds that target all class I PI3Ks are effective in mouse models of cancer with acceptable toxicity, and several pan-PI3K inhibitors have finally entered the stage of clinical

Authors' Affiliations: ¹Department of Molecular Biology and Biochemistry and Institute for Immunology, University of California, Irvine, Irvine, California, and ²Intellikine, La Jolla, California

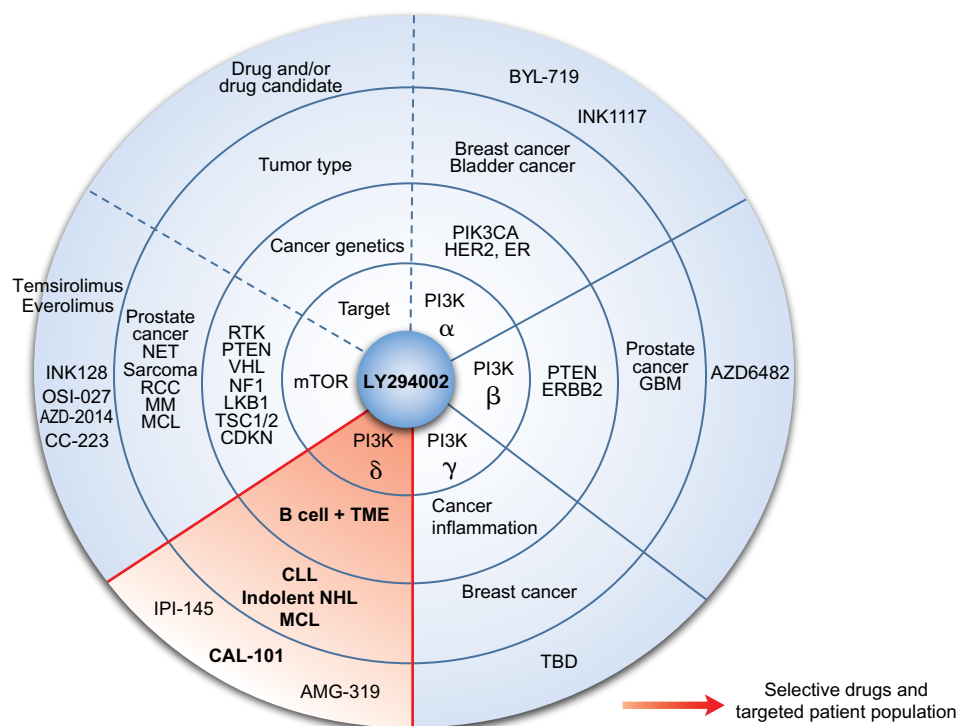
Note: Supplementary data for this article are available at *Cancer Discovery* Online (<http://www.cancerdiscovery.aacrjournals.org>).

Corresponding Author: David A. Fruman, University of California, Irvine, Department of Molecular Biology and Biochemistry, 3242 McGaugh Hall, Irvine, CA 92697-3900. Phone: 949-824-1947; Fax: 949-824-8551; E-mail: dfruman@uci.edu

doi: 10.1158/2159-8290.CD-11-0249

©2011 American Association for Cancer Research.

Figure 1. Targeting PI3K and mTOR has broad applicability in cancer. A first-generation PI3K inhibitor (LY294002, center) inhibits all class I PI3K isoforms and also inhibits mTOR directly. The concentric circles indicate the PI3K isoform-selective inhibitors or mTOR inhibitors and their most likely targets with respect to tumor type and genetics. Bold print refers to topics of focus in this review. GBM, glioblastoma multiformae; MCL, mantle cell lymphoma; NET, neuroendocrine tumor; RCC, renal cell carcinoma; TBD, to be determined; TME, tumor microenvironment.



development in oncology (12–19). On the other hand, targeting the entire group of class I PI3Ks is a “one-size-fits-all” strategy that might not achieve the selectivity for cancer cells that is a primary goal of molecular medicine: to target a selected patient population based on a defined molecular defect such that inhibiting the molecule has a greater impact in cancer cells than normal tissue. It is generally assumed that targeting a single or subgroup of isoforms will achieve the goal of limiting systemic toxicities (20). However, a central question has been whether anticancer efficacy can be achieved by just targeting a single isoform alone. Cell line studies suggest considerable redundancy among class I PI3Ks for sustaining proliferation and survival (21). Another challenge has been for medicinal chemists to design compounds that selectively target PI3K isoforms that are closely related in sequence and structure. Progress in this area has been advanced by x-ray crystal structures of each class I isoform (22–26) and by a landmark survey of diverse PI3K inhibitor chemotypes (11).

PI3K α has received the most attention as a target in oncology. This is because gain-of-function mutations in the gene encoding PI3K α (*PIK3CA*) occur very frequently in human tumors (27–29) (Fig. 1). Genetic experiments using cell lines and mouse models have supported the idea that activating *PIK3CA* mutations are both necessary and sufficient for tumor formation and maintenance (30–32). In addition, pharmacologic interrogations of cancer cell line panels have shown a good correlation between *PIK3CA* mutation status and sensitivity to pan-PI3K inhibitors or to compounds targeting both PI3K and mTOR (33–35). Early results from clinical studies support this correlation between *PIK3CA*

mutation status and response to PI3K/mTOR inhibitors (36). PI3K α is also expressed in lymphocytes and one study found that compounds with some selectivity for PI3K α inhibit chemotaxis and survival of chronic lymphocytic leukemia (CLL) cells *in vitro* (37). Compounds with high selectivity for PI3K α are in early-stage clinical development (INK1117 and BYL-719), and one preclinical compound (A66) showed at least equivalent efficacy to a pan-PI3K/mTOR inhibitor in mouse models of *PIK3CA* mutant tumors (38).

The role of PI3K β in cancer is less clear. Overexpression of wild-type PI3K β is sufficient to transform chicken embryo fibroblasts (39), and a constitutively active mutant can cause prostate cancer in mice (40). However, activating mutations in the *PIK3CB* gene are not observed in human tumors. A recent crystal structure of PI3K β (26) provided a potential explanation by showing that the catalytic subunit (p110 β) is constrained by an additional inhibitory contact with the regulatory subunit, an interaction that is not present in PI3K α . Despite the multiple inhibitory contacts between p110 β and the regulatory subunit, PI3K β seems to have basal activity in cells (11). Indeed, evidence has emerged that PI3K β is required for PIP₃ accumulation in epithelial tumor cells lacking PTEN phosphatase activity (Fig. 1). In a mouse model of prostate cancer driven by PTEN loss, deletion of PI3K β but not PI3K α reduced tumorigenesis (41). PI3K β activation downstream of tyrosine kinases is difficult to detect (42); nevertheless, inactivating PI3K β impaired breast cancer development in a model driven by an activated tyrosine kinase, ERBB2 (43) (Fig. 1). PI3K β activity might also provide a resistance mechanism in tumors treated with selective PI3K α

inhibitors (44). These findings have raised interest in clinical testing of PI3K β inhibitors or combined PI3K α /PI3K β inhibitors in cancer.

PI3K γ is expressed at highest levels in leukocytes but is also detectable in some other cell types. There have been sporadic reports of PI3K γ activity in certain tumor types, yet overall the role of PI3K γ as a cell-intrinsic oncoprotein seems limited. To date, much more effort has been devoted to PI3K γ as a target in inflammatory diseases driven by leukocytes (16, 20). Intriguingly, PI3K γ might have a very important role in the development of solid tumors through its role in innate immune cells. Inflammation driven by tumor-associated myeloid cells is now considered a hallmark of cancer, contributing to both cancer cell expansion and angiogenesis (7). A recent study showed that PI3K γ is essential for myeloid cell adhesion to endothelium and extravasation into tumor sites in mice (45) (Fig. 1). Blockade of PI3K γ specifically in myeloid cells is sufficient to diminish tumor growth with a concomitant reduction in vascular endothelial growth factor production and angiogenesis.

This discussion highlights the prominent role of PI3K α and the potential contributions of PI3K β and PI3K γ in cancer. What about PI3K δ , the focus of this review? Like PI3K γ , the PI3K δ isoform is mainly expressed in leukocytes and has been widely viewed as a target for immune-related diseases (46). A large amount of effort has been devoted to understanding the biology of PI3K δ in the immune system and to develop and characterize PI3K δ -selective inhibitors. PI3K δ is expressed in most lymphoid tumors but so are other class I isoforms, and activating mutations have not been found for PI3K δ . Selective inhibitors of PI3K δ are not strongly cytotoxic *in vitro* (47–49). It has therefore been surprising and exciting that the PI3K δ -targeted compound CAL-101 has achieved remarkable clinical responses in certain B-cell malignancies (Fig. 1). As discussed in this review, the efficacy and tolerability of CAL-101 make sense in light of the somewhat restricted role for PI3K δ in leukocyte biology and the specific diseases where CAL-101 has been effective. From the perspective of pharmaceutical development, the success of CAL-101 emphasizes the importance of (i) knowing your target; (ii) selecting the right disease/patient population; and (iii) treating the disease with an isoform-selective PI3K drug.

Target Validation of PI3K δ

The B-cell receptor (BCR) for antigen and the coreceptor CD19 strongly activate PI3K, as first reported in the mid-1990s (50–52). Subsequent work showed that inhibitory receptors limit B-cell activation by reducing PIP₃ (53, 54). The first genetic evidence for PI3K function in B cells came from studies of mice lacking the class I regulatory isoform p85 α (55, 56). Deletion of p85 α diminished the numbers of mature B cells and caused profound defects in B-cell proliferation and antibody production. In 2002, three groups reported similar B-cell defects in mice with targeted inactivation of *Pik3cd*, the gene encoding the PI3K δ catalytic subunit (often referred to as p110 δ) (57–59). One of these studies, from Okkenhaug and colleagues (59), used a knock-in approach in which a point mutation (D910A) produced a p110 δ protein lacking lipid kinase activity but expressed at normal levels. The development of a kinase-dead

knock-in model was a historic event in the PI3K field, because this approach prevents compensatory upregulation of other isoforms and retains potential noncatalytic functions of the protein (60). Hence, the kinase-dead knock-in strains are more faithful models of pharmacologic kinase inhibitors. Indeed, the p110 δ ^{D910A} strain has been the mouse model of choice for most subsequent studies of PI3K δ function. As a case in point, p110 δ ^{D910A} B cells were used as a reference in a study of IC87114, the first PI3K δ -selective inhibitor to be published (47). Comparison of IC87114-treated and p110 δ ^{D910A} B cells revealed similar impairments in BCR-mediated signaling and proliferation and equivalent defects in survival mediated by the cytokine interleukin-4 (IL-4).

IC87114 was originally discovered by ICOS Pharmaceuticals and subsequently developed further by Calistoga Pharmaceuticals. The high degree of selectivity of IC87114 towards PI3K δ was achieved well before the p110 δ crystal

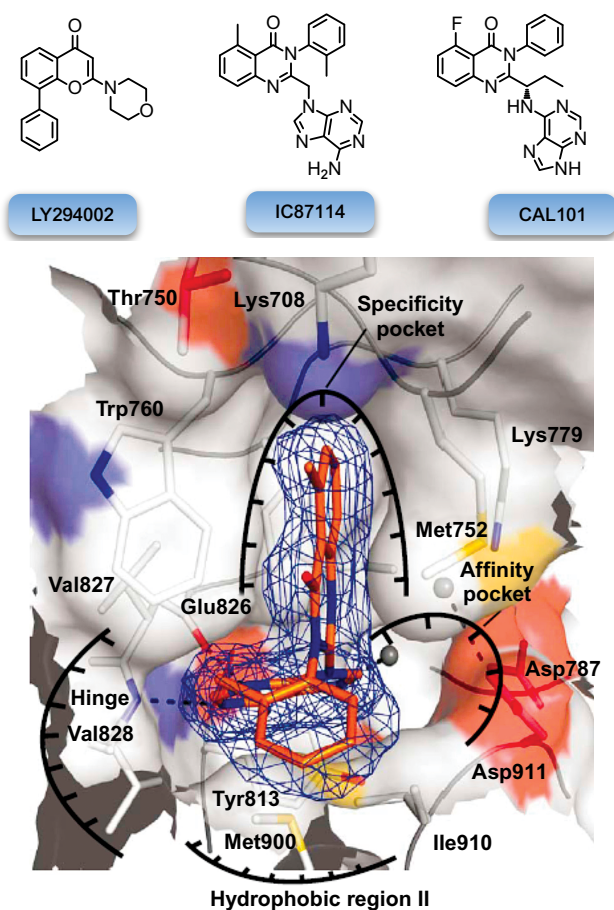


Figure 2. The evolution of a selective PI3K δ inhibitor. The nonselective PI3K/mTOR inhibitor LY294002 along with wortmannin were standard pharmacologic tools for PI3K studies from 1994 through the mid-2000s. The ICOS compound IC87114 was the first highly selective PI3K δ inhibitor to be described. The mechanism of selectivity was revealed by the crystal structure of p110 δ /IC87114 (bottom) (22). Calistoga developed CAL-101 (now GS-1101) based on the IC87114 scaffold.

structure was reported, yet IC87114 remains one of the most selective PI3K δ inhibitors known. We now understand that IC87114 binding is favored by the conformational flexibility of the catalytic domain of p110 δ and by the ability of IC87114 to interact with amino acid residues outside the ATP-binding pocket (Fig. 2) (22). The high selectivity of IC87114, together with the clear-cut B-cell deficits and overall benign phenotype of p110 δ ^{D910A} mice, validated this compound class as potential therapeutics. Medicinal chemistry efforts using the IC87114 lead compound yielded the clinical candidate CAL-101 (Fig. 2). Before discussing CAL-101 efficacy and mechanism, we first review the known functions of PI3K δ in B cells.

PI3K δ in B Cells

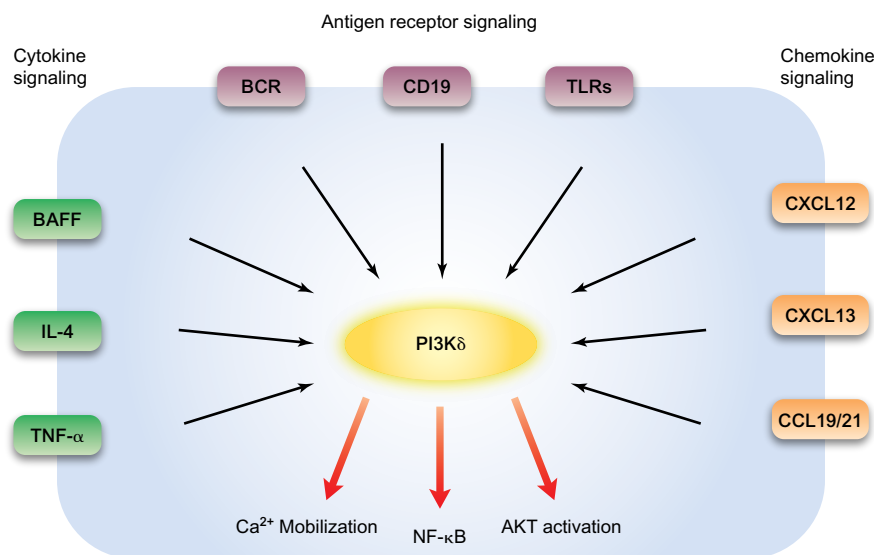
In mice, the naïve mature B-cell pool is divided into three major subsets: follicular (FO) B cells that recirculate, marginal zone (MZ) B cells that reside in the spleen, and B-1 cells that are abundant in body cavities. Genetic inactivation of PI3K δ function greatly reduces development of MZ and B-1 cells (57–59). Mice treated with IC87114 also display an altered MZ B-cell compartment (48); indeed, loss of MZ B cells is a useful biomarker of PI3K δ inhibition in mice. Although selective loss of PI3K δ has little impact on numbers of mature FO B cells, the function of these cells is severely compromised (57–59). *In vitro*, B cells fail to enter the cell cycle after BCR stimulation and display reduced proliferation in response to Toll-like receptor ligands (e.g., LPS) (Fig. 3). Survival mediated by the cytokines IL-4 or BAFF is also diminished (Fig. 3). These defects are not a consequence of altered development as wild-type B cells treated with IC87114 show equivalent changes (47). *In vivo*, the antibody response to T-cell-independent antigens is reduced at least 100-fold. PI3K δ -deficient mice also display aberrant responses to T-cell-dependent antigens. This might be partly the result of defective antigen presentation by B cells (61) as well as defects in the follicular helper T-cell subset (62). PI3K δ does not play a positive role in all B-cell responses. For example, PI3K δ inhibition promotes immunoglobulin class

switching *in vitro* (63) and specifically augments the production of IgE both *in vitro* and *in vivo* (64, 65).

The mechanism for diminished BCR responses in PI3K δ -deficient B cells has been studied extensively [Fig. 3; for more detail, see review articles (46, 66)]. In response to BCR crosslinking, PI3K δ -deficient B cells display a reduced Ca²⁺ mobilization response and an almost complete loss of AKT phosphorylation, a common readout of PI3K activation in cells (57–59). Again, similar results were observed in wild-type cells treated with IC87114 (47). The reduced Ca²⁺ response is likely to explain much of the functional deficits, because the Ca²⁺ signalosome controls early events in antigen recognition and capture as well as later events such as transcription factor activation and gene expression. NF- κ B activation and FOXO inactivation are both impaired in PI3K δ -deficient B cells (47, 57).

The survival of peripheral B cells in the absence of antigen is sustained by the cytokine BAFF (Fig. 3) as well as tonic (basal) signaling through the BCR (67, 68). Chronic BCR signaling also sustains survival in a subset of diffuse large B-cell lymphoma (DLBCL) (69) and is implicated in survival of CLL cells (70). PI3K δ -deficient mice show only a modest decrease in the FO B-cell pool (57–59), suggesting that other mechanisms can compensate to maintain survival. Srinivasan and colleagues (71), have shown that constitutively active PI3K α is sufficient to maintain FO B-cell survival after BCR inactivation. However, the converse experiment has shown that PI3K α loss-of-function does not affect B-cell survival (72). Investigation of compound mutant mice showed that loss of both PI3K α and PI3K δ , but not PI3K α or PI3K δ alone, is required to eliminate peripheral FO B cells (72). One implication of this result is that selective pharmacologic PI3K δ inhibition should not lead to disappearance of mature FO cells, a conclusion supported by studies of mice treated with IC87114 (48). Assuming that BCR-positive B-cell tumors rely on similar mechanisms for survival, these results also predict that cell-intrinsic survival mechanisms might be resistant to selective PI3K δ inhibition. Preclinical data with CAL-101 support this prediction, as discussed subsequently.

Figure 3. Diverse stimuli converge on PI3K δ in B cells. The B-cell receptor (BCR) for antigen, the B-cell coreceptor (CD19, part of a complex with CD21 and CD81), and Toll-like receptors for pathogen-associated molecular patterns all activate PI3K δ . Cytokines derived from lymphoid stromal cells (BAFF) and T cells (IL-4, TNF- α) also activate PI3K δ . Chemokines, which signal through GPCRs to PI3K β and PI3K γ in most other cell types, activate PI3K δ in B cells. The key outputs of PI3K δ activity for B-cell proliferation and survival are shown below the red arrows. PI3K, phosphoinositide 3-kinase; IL-4, interleukin-4; TNF- α , tumor necrosis factor- α ; GPCR, G-protein-coupled receptors.



An unexpected and important phenotype of PI3K δ -deficient B cells is their reduced response to chemokines (Fig. 3). Like other immune cells, B cells rely on chemokine signals to control their positioning within lymphoid tissue both in the absence and presence of antigen (73, 74). Resting FO B cells express high levels of the receptor CXCR5, which binds to CXCL13 localized in B-cell follicles. Activated FO B cells upregulate CCR7, whose ligands CCL19 and CCL21 are abundant in T-cell zones, thus allowing activated B cells to meet up with cognate T cells. B cells at most developmental stages also express CXCR4, whose ligand CXCL12 is important during bone marrow development and peripheral B-cell recirculation. CXCR4, CXCR5, and CCR7 are all G-protein-coupled receptors, which in most cells activate the PI3K γ isoform (and sometimes PI3K β) rather than PI3K α or PI3K δ (5). Nevertheless, chemokine-mediated signaling and migration are markedly impaired in PI3K δ -deficient B cells (48, 75). The mechanism for chemokine receptor coupling to PI3K δ in B cells remains mysterious, but likely has central relevance to the efficacy of CAL-101 as discussed subsequently.

PI3K δ is also a signaling hub in T cells, mast cells, and other immune cells (Supplementary Fig. S1) (46, 76). However, PI3K δ inactivation generally has more modest effects on these other cell types. This distinction is partly the result of functional overlap of PI3K δ with other PI3K isoforms. For example, thymocyte development is severely compromised by combined loss of PI3K γ and PI3K δ but not by inactivation of PI3K δ alone (77–79). PI3K δ does play a significant role in clonal expansion and differentiation of CD4 T cells (80–82). With respect to chemokine responses in T cells, the role of class I PI3K as a whole is rather limited, and PI3K γ seems to be the relevant isoform (83). Curiously, in some myeloid subsets, the PI3K γ isoform couples not only to chemokine receptors, but also to tyrosine kinase-linked receptors that conventionally signal through the other class I isoforms (45). Overall, mouse models of PI3K δ deficiency support the prediction that acute PI3K δ inhibition should affect the function of B cells more dramatically than most other immune cell types but with selective effects on trafficking and antigen responses.

THE CAL-101 STORY

CAL-101 is a quinazoline—(S)-2-{1-[(9H-purin-6-yl)amino]propyl}-5-fluoro-3-phenylquinazolin-4(3H)-1—that inhibits PI3K δ with IC₅₀ value of 2.5nM *in vitro* (84). The compound inhibits other class I PI3Ks only at much higher concentrations (40- to 300-fold higher IC₅₀) and has essentially no activity against other lipid kinases and protein kinases including the PIKK-family kinases mTOR and DNA-PK (84, 85). In cell-based assays, CAL-101 blocks PI3K δ -dependent responses at greater than 240-fold lower concentrations than responses dependent on other isoforms (85).

For the first CAL-101 study in human disease, Calistoga selected allergic rhinitis (Clinical Trials identifier NCT00836914). This choice reflects the initial positioning of PI3K δ inhibitors in the inflammation drug discovery pipelines (86). Although PI3K δ remains undoubtedly an attractive target for immune disease therapy, the subsequent studies of CAL-101 in B-cell malignancies have established drugs of this class as an exciting new therapeutic strategy for

the treatment of hematologic cancers. Therapeutic application in both inflammation and B-cell malignancies is reminiscent of the anti-CD20 antibody rituximab.

The first study of CAL-101 in cancer (NCT00710528) recruited patients with relapsed or refractory B-cell malignancies. These diseases included CLL, non-Hodgkin's lymphoma (NHL), acute myeloid leukemia (AML), and multiple myeloma (MM). Although most of the data have not yet been published, some of the results have been presented at conferences over the past year (87, 88). The most impressive clinical responses were in CLL and certain subtypes of NHL. In this phase I dose-escalation study, primary goals were to study CAL-101 safety, pharmacokinetics (PK), and pharmacodynamics. Therefore, it was surprising and encouraging that CAL-101 caused lymph node shrinkage in all evaluable patients with CLL and across all dose levels. A significant percentage of subjects showed durable responses (approximately 25% overall response rate) and remained on study for many cycles of treatment. CAL-101 showed a favorable tolerability profile, even after sustained treatment. Mouse studies had suggested that PI3K δ inhibition would greatly elevate levels of IgE (64, 65), but this has not yet been reported in the human patients. It will be important to determine whether reported dose-limiting toxicities are mechanism-based and predictive of PI3K δ inhibitors as a class (not only for oncology, but also to support development in other disease settings). It is worth pointing out that the CLL subjects were mostly older adults with comorbid conditions, who had been heavily pretreated and had failed previous therapies. Hence, achieving meaningful single-agent clinical responses with manageable side effects was a significant feat. Applying a PI3K δ inhibitor in front-line therapy might produce even better results.

A key observation in the patients with CLL was that lymph node responses were accompanied by a transient elevation in circulating lymphocyte counts, known as lymphocytosis. This phenomenon is likely the result of lymphocytes being released from lymphoid tissue microenvironments and/or failing to home from the blood into lymph nodes. The observation that lymphocytosis occurs, but is transient, suggests that CAL-101 acts by dislodging CLL cells from the protective niche of the lymph node, rendering them susceptible to gradual apoptosis from loss of survival signals. It is also possible that CAL-101 has some direct proapoptotic effect on CLL cells both in lymphoid tissue and after release into the blood.

There is growing consensus that PI3K inhibitors, like most targeted therapies, will be most effective when used in combination (7, 89). One current study (NCT01088048) is testing combinations of CAL-101 with two commonly used agents in CLL and indolent NHL (iNHL): rituximab (anti-CD20) and bendamustine. The results that have been presented, albeit preliminary, indicate high response rates with all combinations in both diseases. Interestingly, patients with CLL treated with CAL-101 plus rituximab or bendamustine did not display lymphocytosis. One interpretation is that the combination treatment is directly toxic to malignant cells even in their microenvironmental niche. Another possibility is that CAL-101 releases the cells from these niches to increase vulnerability to toxic effects of the companion agent. Regardless of the

mechanism, these results provide encouragement for testing PI3K δ inhibitor combinations in other disease settings, including blood cancers that do not respond to single-agent PI3K δ inhibition. Combining CAL-101 with lenalidomide in CLL also shows promise based on preclinical studies (90). An important objective for such combination studies is to establish appropriate long-term dose levels and safety margins.

The initial clinical data from CAL-101 trials has created tremendous excitement in the field of PI3K drug development. Thousands of biologists and chemists have been working for the past decade to validate targets and bring compounds forward, and CAL-101 provides proof-of-concept that these efforts will pay off with significant advances in clinical management of cancer. At the same time, there is considerable surprise that the biggest success to date has come in indolent blood cancers (CLL, iNHL) and that so far the most effective drug is selective for PI3K δ . Leukemia and lymphomas generally express all class I PI3K isoforms, rarely carry mutations in PI3K genes (91), and have lower incidence of PTEN loss compared with epithelial tumors (92, 93). CLL and iNHL do not generally express activated receptor tyrosine kinases or oncogenic Ras, yet these patients responded better than those with AML, a disease with high frequency of receptor tyrosine kinase activation (Flt3, Kit) or activated Ras and associated with elevated p110 δ expression (94, 95).

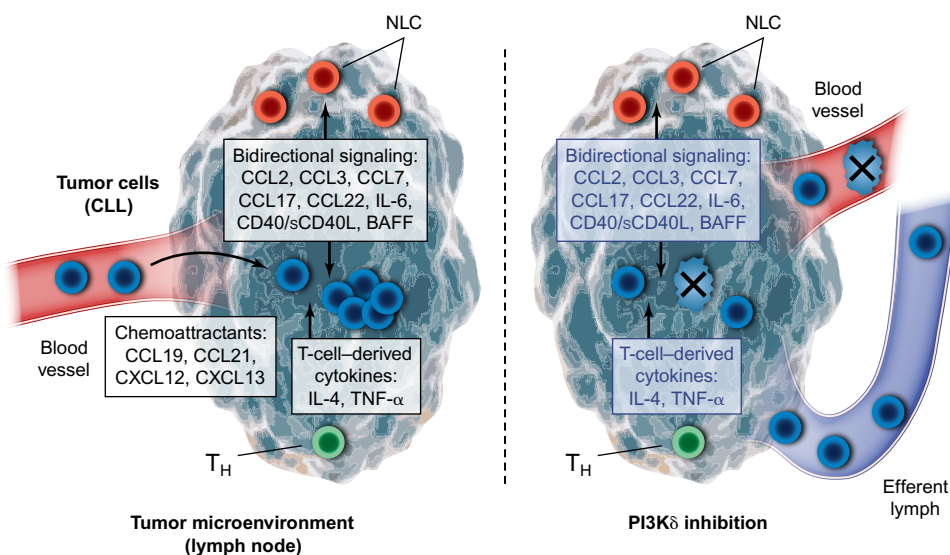
In addition, the first set of preclinical studies of CAL-101 did not clearly predict the dramatic efficacy of the drug in human trials (84, 85, 96). Cytotoxic effects on human CLL samples in tissue culture are inconsistent and generally achieved only at CAL-101 concentrations approaching 10 μ M. This is approximately 1,000 times the IC₅₀ value for CAL-101 in PI3K δ -selective cell-based assays and is well within the range where PI3K β and PI3K γ isoform inhibition is observed. These results suggest that selective PI3K δ inhibition has minimal impact on cell-intrinsic survival signals in

B-lineage cancer cells. It should be noted that clinical exposures to CAL-101 are greater than 2 μ M even at the lowest doses tested, suggesting that partial inhibition of non-PI3K δ isoforms may contribute to cytotoxic effects in patients.

Further clues to the efficacy of CAL-101 emerge when examining data from CLL cells cultured in the presence of cytokines or stromal cells. The cytokines BAFF and TNF- α boost survival of primary CLL cells *in vitro* but this effect is attenuated by CAL-101 at the relatively low concentration of 0.1 μ M (96). Similarly, the survival advantage conferred by growth on fibronectin or stromal cell layers is reversed by 0.1 μ M CAL-101 (96). At a concentration of 0.5 μ M, CAL-101 completely blocks survival signaling from BCR engagement and significantly reduces survival of CLL cells cultured on specialized “nurse-like cells” (NLC) derived from peripheral blood monocytes (97). Together these observations suggest that PI3K δ is essential for CLL cell survival pathways initiated by extrinsic factors in the microenvironment. This mechanism could also contribute to the impressive efficacy of CAL-101 combination therapies. Indeed, CAL-101 potentiates the prodeath effects of bendamustine, fludarabine, and dexamethasone in CLL cells grown on stromal cells (97).

A recent publication from Calistoga, in collaboration with MD Anderson Cancer Center, provided additional key insights into the mechanism of action of CAL-101 (97). One important finding was that CAL-101 blocks CLL cell chemotaxis in response to CXCL12 and CXCL13. In accord, CAL-101 inhibits chemokine-dependent increases in AKT phosphorylation. These results are consistent with studies of PI3K δ -deficient mouse B cells mentioned previously and provide a likely explanation for the release of CLL cells from the lymph node environment into the circulation. CAL-101 also suppresses the secretion of cytokines by both the CLL cells (CCL2, CCL3) and stromal cells (CXCL13) in coculture.

Figure 4. Inhibiting PI3K δ targets both malignant B cells and the tumor microenvironment. (Left) CLL cells are drawn from the vasculature into lymphoid organs by chemotactic signals from chemokines. In the lymph node microenvironment, CLL cells signal bidirectionally with nurse-like cells (NLC) to generate a suite of cytokines and chemokines to promote survival. T cells in the lymphoid tissue can also secrete factors to promote CLL survival. Not shown: adhesion receptors promote maintenance of CLL cells in the protective niche of the lymph node and stabilize interactions with NLC, T cells, other stromal cells, and extracellular matrix. (Right) On treatment with the PI3K δ inhibitor, loss of survival cell-intrinsic survival signals and disruption of the protective niche causes some CLL cells to die in the lymph node. Most of the CLL cells are released into the efferent lymph and eventually into the blood, causing lymphocytosis. This eventually resolves as circulating CLL cells die from niche exclusion.



The coculture of CLL and NLC triggers release of additional factors (CCL7, CCL17, CCL22, soluble CD40L, IL-6, and TNF- α) whose secretion is reduced by CAL-101. An important element of this study was the analysis of cytokine levels in peripheral blood of patients with CLL treated with CAL-101. The data show a uniform decrease in CCL2, CCL3, and CXCL13 in patient plasma after 28 days of treatment compared with pretreatment measurements (97). Assessment of AKT phosphorylation in circulating CLL cells was used as a pharmacodynamics marker and showed a strong correlation with reduced chemokine concentrations. This indicates that at pharmacologically active doses, CAL-101 disrupts the CLL microenvironment, resulting in reduced production of chemokines.

Putting all the data together, one can now propose a model to explain the efficacy of CAL-101 in patients with CLL (Fig. 4). PI3K δ inhibition reduces both the production of chemokines and the response of CLL cells to these factors. This leads to release of CLL cells from the protective niche in lymphoid tissue. Concurrently, those cells remaining in the niche receive weakened survival signals from microenvironmental factors such as BAFF, CD40L, adhesion molecules, and extracellular matrix. Some of the tumor cells may die rapidly but the majority of cells seem to exit lymphoid tissue and circulate in the blood. Gradually, CLL cells excluded from the lymphoid tissue niche undergo apoptosis.

When rituximab or bendamustine is included in the treatment regimen, the destruction of these circulating B cells is accelerated.

OUTLOOK

It seems that PI3K inhibitors are now destined to enter clinical practice in CLL and possibly other blood cancer settings. Calistoga became part of a larger pharma company (Gilead), and plans are in place for accelerated clinical trials and rapid registration of CAL-101 (now named GS-1101). Several other companies have PI3K inhibitor programs (Table 1) and some are no doubt diverting resources from inflammation to oncology. At the same time, the CAL-101 data raise several interesting and important questions for further consideration.

One question is whether targeting additional PI3K isoforms would provide even greater benefit. The pan-PI3K inhibitors and PI3K/mTOR inhibitors currently in clinical trials have been tested mainly in patients with solid tumors. Would a broad-spectrum PI3K inhibitor provide more rapid elimination of CLL cells, and/or durable responses in patients refractory to CAL-101? This possibility is supported by data showing greater inhibition of CXCR4-mediated CLL chemotaxis by pan-PI3K inhibitors relative to PI3K-selective inhibitors (37). It is possible that pan-PI3K inhibitors will

Table 1. Phosphoinositide 3-kinase inhibitors in development

Discovery	Preclinical	Phase I	Phase II
Eli Lilly ^a	XL-499 (Exelixis) ^f	IPI-145 (Intellikine/Infinity) ^j	CAL-101/GS-
Merck Serono S.A. ^b	KAR-4141 (Karus) ^g	CAL-263 (Calistoga/Gilead) ^k	1101 (Calistoga/Gilead)
Incyte Corporation ^c	UCB Pharma ^h	AMG-319 (Amgen) ^l	
Respivert Limited ^d	Incozen Therapeutics ⁱ	GlaxoSmithKline ^m	
Cellzome ^e			
Pathway Therapeutics			
Genentech			
Intellikine/Infinity			

^aEli Lilly patent applications: WO2009064802; WO2008064018.

^bMerck Serono patent application: WO2011058149.

^cIncyte patent applications: WO2011075630; WO2011008487.

^dRespivert patent applications: WO2011048111.

^eThe company has phosphoinositide 3-kinase program. Status and patent information are not available.

^fThe structure of XL-499 (Exelixis) is not disclosed. The patent information is not available.

^gThe structure of KAR-4141 (Karus) is not disclosed. The patent information is not available.

^hThe development candidate ID and structure are not disclosed. UCB Pharma Patent applications: WO2011058113; WO2011058112; WO2011058111; WO2011058110; WO2011058109; WO2011058108.

ⁱThe development candidate ID and structure are not disclosed. Incozen Patent application: WO2011055215.

^jIPI-145 is a dual PI3K δ / γ inhibitor. The structure is not disclosed. Intellikine/Infinity patent applications: WO2011008302; WO2010129816; WO2010036380; WO2009088986; WO2009088990.

^kThe structure of CAL-263 is not disclosed. Calistoga/Gilead patent application: WO2010123931.

^lThe structure of AMG-319 is not disclosed. Amgen patent applications: WO2011075628; WO2010151740; WO2010151737; WO2010151735; WO2010151791; WO2008118468; WO2008118455; WO2008118454.

^mThe clinical candidate ID and structure are not disclosed. The patent information is not available.

be more effective than selective PI3K inhibitors in leukemias arising from immature B cells (B-ALL), germinal center B cells (DLBCL), plasma cells (MM), or myeloid progenitors (AML). If pan-PI3K inhibitors cause unmanageable side effects, an alternative strategy would be to design compounds that target PI3K along with one other class I isoform. A dual PI3K δ /PI3K α inhibitor would be predicted to suppress survival more completely in malignancies derived from mature B cells. Alternatively, a dual PI3K δ /PI3K γ inhibitor might block chemokine signaling across a broader range of lymphoid malignancies. Dual PI3K δ /PI3K γ inhibitors were found to suppress inflammatory markers more effectively than selective PI3K inhibitors using *in vitro* assays of human lymphocytes (49). A molecule that inhibits PI3K and mTOR might also be useful. Targeting mTOR with an allosteric (Rapalog) or ATP-site kinase inhibitor (TORC1/2) could further suppress survival signals and metabolic responses to environmental cues including growth factors and nutrients.

The mechanism of action of CAL-101 also raises the question of whether blocking microenvironmental signals is a general key to success in targeted cancer therapies. Many drugs that kill cancer cells *in vitro* fail to suppress tumor growth *in vivo* because of cell-extrinsic survival signals. Conversely, the CAL-101 story teaches us that a drug with limited activity on isolated tumor cells can be highly effective *in vivo* by disrupting the microenvironment. Altering the *in vitro* conditions to include stromal support, thereby modeling the microenvironment more faithfully, provided a sensitized system that revealed potent antileukemic activity. Such systems are therefore valuable even at early stages of drug screening. An intriguing possibility is that PI3K inhibition could also provide benefit in solid tumors in which B cells contribute to the supportive microenvironment. For example, B cells are part of the inflammatory infiltrate supporting survival of prostate cancer cells (98).

It is also important to understand more about the microenvironment in malignancies that do not respond to isoform-selective compounds. For example, CAL-101 has shown lesser efficacy in MM versus CLL. Both are relatively slow-growing malignancies arising in B-lineage cells, and patient samples cultured *in vitro* showed similar degrees of growth arrest and apoptosis in the presence of CAL-101 (84, 85, 96). However, the MM patient population did not respond as favorably to CAL-101 treatment in clinical trials. Whereas the protective niche for CLL cells is in lymph nodes and the spleen, the primary niche for MM cells is in the bone marrow. In this case, *in vitro* models were not predictive of *in vivo* efficacy because CAL-101 was able to block the prosurvival effects of IL-6 or bone marrow stromal cells in MM cell lines in culture (84).

Selectively targeting PI3K isoforms to promote rather than disrupt immune responses to tumors is an important goal. CAL-101 is not cytotoxic to normal lymphocytes even at 10 μ M. However, it is important to keep in mind that PI3K inhibitors are likely to impact significantly the differentiation and migration of T cells, thereby influencing the immune response to tumor cells. IC87114 suppresses proliferation of both murine and human T cells (82). CAL-101 strongly suppresses cytokine production by human T cells, even when the drug is present at a low concentration (0.1M) (96). PI3K inhibition also promotes the induction of a regulatory T-cell fate but can also inhibit

the function of these cells (66, 76). PI3K inhibition affects the activation of natural killer cells (99) with possible detrimental effects on immunosurveillance and antibody-dependent cellular cytotoxicity (ADCC) (100). Because rituximab and other monoclonal antibody therapies require ADCC for maximal efficacy, interfering with ADCC will limit the efficacy of combination approaches. PI3K isoforms (including PI3K δ) regulate maturation and migration of macrophages and dendritic cells (101–103), which play important roles in the microenvironment of many tumors. In summary, understanding how PI3K inhibitors influence the function of T cells, natural killer cells, and myeloid cells will be key to choosing the optimal pharmacologic profile.

Discussions of PI3K inhibitor efficacy in cancer often touch on the issue of cancer stem cells. Most normal stem cells in tissues exhibit low PI3K activity, and artificial elevation of PI3K or AKT, or inactivation of FOXO proteins, promotes exit from quiescence and stem cell exhaustion (104–107). Consequently, there is concern that cancer stem cells will be resistant to PI3K inhibition, analogous to the imatinib-resistance of leukemic stem cells (LSC) in CML. Although some studies suggest that LSCs have elevated PI3K/AKT activity relative to normal hematopoietic stem cells (105), a recent article showed that LSCs in AML have elevated FOXO function that is necessary to maintain this compartment (108). Therefore, PI3K inhibitors such as CAL-101 might be less effective in AML because such agents would enforce FOXO activity in the LSC compartment. It will be interesting to examine the differentiation state of resistant cells in patients who exhibit incomplete responses to PI3K inhibitors. This can be done using multiparameter flow cytometry or through genomewide studies of transcriptional or epigenetic signatures.

A broader question is whether the success of CAL-101 should alter the development path for other PI3K inhibitors. Current dogma is that candidate therapeutics should be validated extensively in cell-based assays and then rodent tumor models before initiating human trials. However, cell lines and xenograft models have significant limitations for predicting drug response of human tumors growing in their natural host (14). As discussed previously, coculture systems to mimic the microenvironment are helpful but do not always provide predictive value. Genetically engineered mice have advantages for modeling cancer development and maintenance but have a high cost and do not recapitulate the genetic complexity of *bona fide* human tumors. In the case of CAL-101, no data have been published from mouse tumor models (probably as a result of poor PK of this drug in mice) and the data from isolated cell lines did not predict a strong cytotoxic effect. On the other hand, the clinical data from human patients demonstrated clear efficacy even in a relatively small (and therefore relatively inexpensive) phase I study. The clinical trial was initiated before much of the mechanistic data on chemokines and microenvironmental signals was generated. These considerations support the merit of moving compounds quickly into the clinic as soon as favorable tolerability can be shown in preclinical toxicity studies. Contrary to early predictions, most PI3K inhibitors (including pan-PI3K and PI3K/mTOR) appear to be reasonably well tolerated and there is strong justification for evaluating their efficacy in patients with cancer, ideally selected (enriched) cancer types.

Regardless of the path to the clinic, it is clear that trials should incorporate extensive analysis of biomarkers of treatment response. These can be biomarkers of intracellular signaling (phospho-AKT, phospho-S6) as well as immune activation (cytokines, chemokines). Correlation of biomarker changes with treatment response will help reveal the mechanism of drug action *in vivo*. In the case of the CAL-101 trial in CLL, the dramatic reductions in circulating cytokines and chemokines provided important clues to explain drug efficacy (97). Measuring chemokines and cytokines in plasma might help establish a blood-based clinical biomarker for correlation with drug dose/exposure and therapeutic efficacy.

In summary, CAL-101 has opened up many new avenues of research for both laboratory and clinical scientists. As more PI3K pathway inhibitors are tested in human patients, we anticipate further exciting advances in disease management along with new biologic insights.

Disclosure of Potential Conflicts of Interest

D.A. Fruman is a scientific advisor to Intellikine and receives research support from Intellikine. C. Rommel is an employee of Intellikine.

Acknowledgments

We thank Kevan Shokat, Klaus Okkenhaug, Vito Palombello, Yi Liu, and Pingda Ren for helpful comments on the manuscript. We also thank Pingda Ren and Liansheng Li for assistance preparing figures and tables regarding inhibitor compounds.

Grant Support

D.A. Fruman was supported by a Discovery Grant from the University of California Industry-University Cooperative Research Program.

Received September 26, 2011; revised October 21, 2011; accepted October 21, 2011; published OnlineFirst November 30, 2011.

REFERENCES

- Cantley LC, Auger KR, Carpenter C, Duckworth B, Graziani A, Kapeller R, et al. Oncogenes and signal transduction. *Cell* 1991;64:281-302.
- Whitman M, Kaplan DR, Schaffhausen B, Cantley L, Roberts TM. Association of phosphatidylinositol kinase activity with polyoma middle-T competent for transformation. *Nature* 1985;315:239-42.
- Fruman DA, Meyers RE, Cantley LC. Phosphoinositide kinases. *Annu Rev Biochem* 1998;67:481-507.
- Katso R, Okkenhaug K, Ahmadi K, White S, Timms J, Waterfield MD. Cellular function of phosphoinositide 3-kinases: implications for development, homeostasis, and cancer. *Annu Rev Cell Dev Biol* 2001;17:615-75.
- Vanhaesebroeck B, Guillermet-Guibert J, Graupera M, Bilanges B. The emerging mechanisms of isoform-specific PI3K signalling. *Nat Rev Mol Cell Biol* 2010;11:329-41.
- Engelman JA, Luo J, Cantley LC. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. *Nat Rev Genet* 2006;7:606-19.
- Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011;144:646-74.
- Vivanco I, Sawyers CL. The phosphatidylinositol 3-Kinase AKT pathway in human cancer. *Nat Rev Cancer* 2002;2:489-501.
- Brunn GJ, Williams J, Sabers C, Wiederrecht G, Lawrence JC Jr, Abraham RT. Direct inhibition of the signaling functions of the mammalian target of rapamycin by the phosphoinositide 3-kinase inhibitors, wortmannin and LY294002. *Embo J* 1996;15:5256-67.
- Gharbi SI, Zvelebil MJ, Shuttleworth SJ, Hancox T, Saghir N, Timms JF, et al. Exploring the specificity of the PI3K family inhibitor LY294002. *Biochem J* 2007;404:15-21.
- Knight ZA, Gonzalez B, Feldman ME, Zunder ER, Goldenberg DD, Williams O, et al. A pharmacological map of the PI3-K family defines a role for p110alpha in insulin signaling. *Cell* 2006;125:733-47.
- Agarwal R, Carey M, Hennessy B, Mills GB. PI3K pathway-directed therapeutic strategies in cancer. *Curr Opin Invest Drugs* 2010;11:615-28.
- Garcia-Echeverria C, Sellers WR. Drug discovery approaches targeting the PI3K/Akt pathway in cancer. *Oncogene* 2008;27:5511-26.
- Khawaja A. PI3K as a target for therapy in haematological malignancies. *Curr Top Microbiol Immunol* 2010;347:169-88.
- Liu P, Cheng H, Roberts TM, Zhao JJ. Targeting the phosphoinositide 3-kinase pathway in cancer. *Nat Rev Drug Discov* 2009;8:627-44.
- Marone R, Cmiljanovic V, Giese B, Wymann MP. Targeting phosphoinositide 3-kinase: moving towards therapy. *Biochim Biophys Acta* 2008;1784:159-85.
- Wong KK, Engelman JA, Cantley LC. Targeting the PI3K signaling pathway in cancer. *Curr Opin Genet Dev* 2010;20:87-90.
- Workman P, Clarke PA, Raynaud FI, van Montfort RL. Drugging the PI3 Kinome: From Chemical Tools to Drugs in the Clinic. *Cancer Res* 2010;70:2146-57.
- Yap TA, Garrett MD, Walton MI, Raynaud F, de Bono JS, Workman P. Targeting the PI3K-AKT-mTOR pathway: progress, pitfalls, and promises. *Curr Opin Pharmacol* 2008;8:393-412.
- Ruckle T, Schwarz MK, Rommel C. PI3Kgamma inhibition: towards an 'aspirin of the 21st century'? *Nat Rev Drug Discov* 2006;5:903-18.
- Foukas LC, Berenjano IM, Gray A, Khawaja A, Vanhaesebroeck B. Activity of any class IA PI3K isoform can sustain cell proliferation and survival. *Proc Natl Acad Sci U S A* 2010;107:11381-6.
- Berndt A, Miller S, Williams O, Le DD, Houseman BT, Pacold JI, et al. The p110delta structure: mechanisms for selectivity and potency of new PI(3)K inhibitors. *Nat Chem Biol* 2010;6:244.
- Huang CH, Mandelker D, Schmidt-Kittler O, Samuels Y, Velculescu VE, Kinzler KW, et al. The structure of a human p110alpha/p85alpha complex elucidates the effects of oncogenic PI3Kalpha mutations. *Science* 2007;318:1744-8.
- Miled N, Yan Y, Hon WC, Perisic O, Zvelebil M, Inbar Y, et al. Mechanism of two classes of cancer mutations in the phosphoinositide 3-kinase catalytic subunit. *Science* 2007;317:239-42.
- Walker EH, Perisic O, Ried C, Stephens L, Williams RL. Structural insights into phosphoinositide 3-kinase catalysis and signalling. *Nature* 1999;402:313-20.
- Zhang X, Vadas O, Perisic O, Anderson KE, Clark J, Hawkins PT, et al. Structure of lipid kinase p110beta/p85beta elucidates an unusual SH2-domain-mediated inhibitory mechanism. *Mol Cell* 2011;41:567-78.
- Campbell IG, Russell SE, Choong DY, Montgomery KG, Ciavarella ML, Hooi CS, et al. Mutation of the PIK3CA gene in ovarian and breast cancer. *Cancer Res* 2004;64:7678-81.
- Ligresti G, Militello L, Steelman LS, Cavallaro A, Basile F, Nicoletti F, et al. PIK3CA mutations in human solid tumors: role in sensitivity to various therapeutic approaches. *Cell Cycle* 2009;8:1352-8.
- Samuels Y, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, et al. High frequency of mutations of the PIK3CA gene in human cancers. *Science* 2004;304:554.
- Engelman JA, Chen L, Tan X, Crosby K, Guimaraes AR, Upadhyay R, et al. Effective use of PI3K and MEK inhibitors to treat mutant Kras G12D and PIK3CA H1047R murine lung cancers. *Nat Med* 2008;14:1351-6.
- Liu P, Cheng H, Santiago S, Raeder M, Zhang F, Isabella A, et al. Oncogenic PIK3CA-driven mammary tumors frequently recur via PI3K pathway-dependent and PI3K pathway-independent mechanisms. *Nat Med* 2011;17:1116-20.
- Samuels Y, Diaz LA Jr, Schmidt-Kittler O, Cummins JM, DeLong L, Cheong I, et al. Mutant PIK3CA promotes cell growth and invasion of human cancer cells. *Cancer Cell* 2005;7:561-73.

33. O'Brien C, Wallin JJ, Sampath D, GuhaThakurta D, Savage H, Punnoose EA, et al. Predictive biomarkers of sensitivity to the phosphatidylinositol 3' kinase inhibitor GDC-0941 in breast cancer preclinical models. *Clin Cancer Res* 2010;16:3670-83.
34. Weigelt B, Warne PH, Downward J. PIK3CA mutation, but not PTEN loss of function, determines the sensitivity of breast cancer cells to mTOR inhibitory drugs. *Oncogene* 2011;30:3222-33.
35. Brachmann SM, Hofmann I, Schnell C, Fritsch C, Wee S, Lane H, et al. Specific apoptosis induction by the dual PI3K/mTOR inhibitor NVP-BEZ235 in HER2 amplified and PIK3CA mutant breast cancer cells. *Proc Natl Acad Sci U S A* 2009;106:22299-304.
36. Janku F, Tsimberidou AM, Garrido-Laguna I, Wang X, Luthra R, Hong DS, et al. PIK3CA mutations in patients with advanced cancers treated with PI3K/AKT/mTOR axis inhibitors. *Mol Cancer Ther* 2011;10:558-65.
37. Niedermeier M, Hennessy BT, Knight ZA, Henneberg M, Hu J, Kurtova AV, et al. Isoform-selective phosphoinositide 3'-kinase inhibitors inhibit CXCR4 signaling and overcome stromal cell-mediated drug resistance in chronic lymphocytic leukemia: a novel therapeutic approach. *Blood* 2009;113:5549-57.
38. Jamieson S, Flanagan JU, Kolekar S, Buchanan C, Kendall JD, Lee WJ, et al. A drug targeting only p110alpha can block phosphoinositide 3-kinase signalling and tumour growth in certain cell types. *Biochem J* 2011;438:53-62.
39. Kang S, Denley A, Vanhaesebroeck B, Vogt PK. Oncogenic transformation induced by the p110beta, -gamma, and -delta isoforms of class I phosphoinositide 3-kinase. *Proc Natl Acad Sci U S A* 2006;103:1289-94.
40. Lee SH, Pouligiannis G, Pyne S, Jia S, Zou L, Signoretti S, et al. A constitutively activated form of the p110beta isoform of PI3-kinase induces prostatic intraepithelial neoplasia in mice. *Proc Natl Acad Sci U S A* 2010;107:11002-7.
41. Jia S, Liu Z, Zhang S, Liu P, Zhang L, Lee SH, et al. Essential roles of PI(3)K-p110beta in cell growth, metabolism and tumorigenesis. *Nature* 2008;454:776-9.
42. Guillermet-Guibert J, Bjorklof K, Salpekar A, Gonella C, Ramadani F, Bilancio A, et al. The p110beta isoform of phosphoinositide 3-kinase signals downstream of G protein-coupled receptors and is functionally redundant with p110gamma. *Proc Natl Acad Sci U S A* 2008;105:8292-7.
43. Cirao E, Iezzi M, Marone R, Marengo S, Curcio C, Costa C, et al. Phosphoinositide 3-kinase p110[beta] activity: key role in metabolism and mammary gland cancer but not development. *Sci Signal* 2008;1:ra3.
44. Crowder RJ, Phommaly C, Tao Y, Hoog J, Luo J, Perou CM, et al. PIK3CA and PIK3CB inhibition produce synthetic lethality when combined with estrogen deprivation in estrogen receptor-positive breast cancer. *Cancer Res* 2009;69:3955-62.
45. Schmid MC, Avraamides CJ, Dippold HC, Franco I, Foubert P, Ellies LG, et al. Receptor tyrosine kinases and TLR/IL1Rs unexpectedly activate myeloid cell PI3kgamma, a single convergent point promoting tumor inflammation and progression. *Cancer Cell* 2011;19:715-27.
46. Okkenhaug K, Ali K, Vanhaesebroeck B. Antigen receptor signalling: a distinctive role for the p110delta isoform of PI3K. *Trends Immunol* 2007;28:80-7.
47. Bilancio A, Okkenhaug K, Camps M, Emery JL, Ruckle T, Rommel C, et al. Key role of the p110delta isoform of PI3K in antigen and IL-4 receptor signaling: comparative analysis of genetic and pharmacologic interference with p110delta function in B cells. *Blood* 2006;107:642-50.
48. Durand CA, Hartvigsen K, Fogelstrand L, Kim S, Iritani S, Vanhaesebroeck B, et al. Phosphoinositide 3-kinase p110delta regulates natural antibody production, marginal zone and B-1 function, and autoantibody responses. *J Immunol* 2009;183:5673-84.
49. Williams O, Houseman BT, Kunkel EJ, Aizenstein B, Hoffman R, Knight ZA, et al. Discovery of dual inhibitors of the immune cell PI3Ks p110delta and p110gamma: a prototype for new anti-inflammatory drugs. *Chem Biol* 2010;17:123-34.
50. Gold MR, Abersold R. Both phosphatidylinositol 3-kinase and phosphatidylinositol 4-kinase products are increased by antigen receptor signaling in B cells. *J Immunol* 1994;152:42-50.
51. Gold MR, Duronio V, Saxena SP, Schrader JW, Abersold R. Multiple cytokines activate phosphatidylinositol 3-kinase in hemopoietic cells. Association of the enzyme with various tyrosine-phosphorylated proteins. *J Biol Chem* 1994;269:5403-12.
52. Tuveson DA, Carter RH, Soltoff SP, Fearon DT. CD19 of B cells as a surrogate kinase insert region to bind phosphatidylinositol 3-kinase. *Science* 1993;260:986-9.
53. Ono M, Bolland S, Tempst P, Ravetch JV. Role of the inositol phosphatase SHIP in negative regulation of the immune system by the receptor Fc(gamma)RIIB. *Nature* 1996;383:263-6.
54. Scharenberg AM, El-Hillal O, Fruman DA, Beitz LO, Li Z, Lin S, et al. Phosphatidylinositol-3,4,5-trisphosphate (PtdIns-3,4,5-P3)/Tec kinase-dependent calcium signaling pathway: a target for SHIP-mediated inhibitory signals. *EMBO J* 1998;17:1961-72.
55. Fruman DA, Snapper SB, Yballe CM, Davidson L, Yu JY, Alt FW, et al. Impaired development and proliferation in absence of phosphoinositide 3-kinase p85alpha. *Science* 1999;283:393-7.
56. Suzuki H, Terauchi Y, Fujiwara M, Aizawa S, Yazaki Y, Kadowaki T, et al. Xid-like immunodeficiency in mice with disruption of the p85alpha subunit of phosphoinositide 3-kinase. *Science* 1999;283:390-2.
57. Clayton E, Bardi G, Bell SE, Chantry D, Downes CP, Gray A, et al. A crucial role for the p110delta subunit of phosphatidylinositol 3-kinase in development and activation. *J Exp Med* 2002;196:753-63.
58. Jou ST, Carpino N, Takahashi Y, Piekorz R, Chao JR, Wang D, et al. Essential, nonredundant role for the phosphoinositide 3-kinase p110delta in signaling by the receptor complex. *Mol Cell Biol* 2002;22:8580-91.
59. Okkenhaug K, Bilancio A, Farjot G, Priddle H, Sancho S, Peskett E, et al. Impaired B and T-cell antigen receptor signaling in p110delta PI 3-kinase mutant mice. *Science* 2002;297:1031-4.
60. Vanhaesebroeck B, Ali K, Bilancio A, Geering B, Foukas LC. Signalling by PI3K isoforms: insights from gene-targeted mice. *Trends Biochem Sci* 2005;30:194-204.
61. Al-Alwan MM, Okkenhaug K, Vanhaesebroeck B, Hayflick JS, Marshall AJ. Requirement for phosphoinositide 3-kinase p110delta signaling in antigen receptor-mediated antigen presentation. *J Immunol* 2007;178:2328-35.
62. Rolf J, Bell SE, Kovcsdi D, Janas ML, Soond DR, Webb LM, et al. Phosphoinositide 3-kinase activity in T cells regulates the magnitude of the germinal center reaction. *J Immunol* 2010;185:4042-52.
63. Omori SA, Cato MH, Anzelon-Mills A, Puri KD, Shapiro-Shelef M, Calame K, et al. Regulation of class-switch recombination and plasma cell differentiation by phosphatidylinositol 3-kinase signaling. *Immunity* 2006;25:545-57.
64. Doi T, Obayashi K, Kadowaki T, Fujii H, Koyasu S. PI3K is a negative regulator of IgE production. *Int Immunol* 2008;20:499-508.
65. Zhang TT, Okkenhaug K, Nashed BF, Puri KD, Knight ZA, Shokat KM, et al. Genetic or pharmaceutical blockade of p110delta phosphoinositide 3-kinase enhances IgE production. *J Allergy Clin Immunol* 2008;122:811-9.e2.
66. Fruman DA, Bismuth G. Fine tuning the immune response with PI3K. *Immunol Rev* 2009;228:253-72.
67. Kraus M, Alimzhanov MB, Rajewsky N, Rajewsky K. Survival of resting mature B lymphocytes depends on BCR signaling via the Igalphabeta heterodimer. *Cell* 2004;117:787-800.
68. Schiemann B, Gommerman JL, Vora K, Cachero TG, Shulga-Morskaya S, Dobles M, et al. An essential role for BAFF in the normal development of B cells through a BCMA-independent pathway. *Science* 2001;293:2111-4.
69. Davis RE, Ngo VN, Lenz G, Tolar P, Young RM, Romesser PB, et al. Chronic active B-cell-receptor signalling in diffuse large lymphoma. *Nature* 2010;463:88-92.
70. Efremov DG, Gobessi S, Longo PG. Signaling pathways activated by antigen-receptor engagement in chronic lymphocytic leukemia B-cells. *Autoimmun Rev* 2007;7:102-8.
71. Srinivasan L, Sasaki Y, Calado DP, Zhang B, Paik JH, DePinho RA, et al. PI3 kinase signals BCR-dependent mature survival. *Cell* 2009;139:573-86.

72. Ramadani F, Bolland DJ, Garcon F, Emery JL, Vanhaesebroeck B, Corcoran AE, et al. The PI3K isoforms p110alpha and p110delta are essential for pre-receptor signaling and development. *Sci Signal* 2010;3:ra60.
73. Cyster JG. Chemokines, sphingosine-1-phosphate, and cell migration in secondary lymphoid organs. *Annu Rev Immunol* 2005;23:127-59.
74. Okada T, Cyster JG. migration and interactions in the early phase of antibody responses. *Curr Opin Immunol* 2006;18:278-85.
75. Reif K, Okkenhaug K, Sasaki T, Penninger JM, Vanhaesebroeck B, Cyster JG. Cutting edge: differential roles for phosphoinositide 3-kinases, p110gamma and p110delta, in lymphocyte chemotaxis and homing. *J Immunol* 2004;173:2236-40.
76. Okkenhaug K, Fruman DA. PI3Ks in lymphocyte signaling and development. *Curr Top Microbiol Immunol* 2010;346:57-85.
77. Janas ML, Varano G, Gudmundsson K, Noda M, Nagasawa T, Turner M. Thymic development beyond beta-selection requires phosphatidylinositol 3-kinase activation by CXCR4. *J Exp Med* 2010;207:247-61, S1-2.
78. Swat W, Montgrain V, Doggett TA, Douangpanya J, Puri K, Vermi W, et al. Essential role of PI3Kdelta and PI3Kgamma in thymocyte survival. *Blood* 2006;107:2415-22.
79. Webb LM, Vigorito E, Wymann MP, Hirsch E, Turner M. Cutting edge: T-cell development requires the combined activities of the p110gamma and p110delta catalytic isoforms of phosphatidylinositol 3-kinase. *J Immunol* 2005;175:2783-7.
80. Okkenhaug K, Patton DT, Bilancio A, Garcon F, Rowan WC, Vanhaesebroeck B. The p110delta isoform of phosphoinositide 3-kinase controls clonal expansion and differentiation of Th cells. *J Immunol* 2006;177:5122-8.
81. Patton DT, Garden OA, Pearce WP, Clough LE, Monk CR, Leung E, et al. Cutting edge: the phosphoinositide 3-kinase p110 delta is critical for the function of CD4+CD25+Foxp3+ regulatory T cells. *J Immunol* 2006;177:6598-602.
82. Soond DR, Bjorgo E, Moltu K, Dale VQ, Patton DT, Torgersen KM, et al. PI3K p110delta regulates T-cell cytokine production during primary and secondary immune responses in mice and humans. *Blood* 2010;115:2203-13.
83. Nombela-Arrieta C, Lacalle RA, Montoya MC, Kunisaki Y, Megias D, Marques M, et al. Differential requirements for DOCK2 and phosphoinositide-3-kinase gamma during T and B lymphocyte homing. *Immunity* 2004;21:429-41.
84. Ikeda H, Hideshima T, Fulciniti M, Perrone G, Miura N, Yasui H, et al. PI3K/p110[delta] is a novel therapeutic target in multiple myeloma. *Blood* 2010;116:1460-8.
85. Lannutti BJ, Meadows SA, Herman SE, Kashishian A, Steiner B, Johnson AJ, et al. CAL-101, a p110delta selective phosphatidylinositol-3-kinase inhibitor for the treatment of malignancies, inhibits PI3K signaling and cellular viability. *Blood* 2011;117:591-4.
86. Rommel C, Camps M, Ji H. PI3K delta and PI3K gamma: partners in crime in inflammation in rheumatoid arthritis and beyond? *Nat Rev Drug Disc* 2007;7:191-201.
87. Furman RR, Byrd JC, Brown JR, Coutre SE, Benson DM, Wagner-Johnston ND, et al. CAL-101, An Isoform-Selective Inhibitor of Phosphatidylinositol 3-Kinase P110[delta], Demonstrates Clinical Activity and Pharmacodynamic Effects In Patients with Relapsed or Refractory Chronic Lymphocytic Leukemia. *ASH Annual Meeting Abstracts* 2010;116:55.
88. Furman RR, Byrd JC, Flinn IW, Coutre SE, M.BD, Brown JR, et al. Interim results from a phase I study of CAL-101, a selective oral inhibitor of phosphatidylinositol 3-kinase p110d isoform, in patients with relapsed or refractory hematologic malignancies. *J Clin Oncol* 2010;28:15s (abstr 3032).
89. Courtney KD, Corcoran RB, Engelman JA. The PI3K pathway as drug target in human cancer. *J Clin Oncol* 2010;28:1075-83.
90. Herman SE, Lapalombella R, Gordon AL, Ramanunni A, Blum KA, Jones J, et al. The role of phosphatidylinositol 3-kinase-delta in the immunomodulatory effects of lenalidomide in chronic lymphocytic leukemia. *Blood* 2011;117:4323-7.
91. Bousquet M, Recher C, Queleen C, Demur C, Payrastra B, Brousset P. Assessment of somatic mutations in phosphatidylinositol 3-kinase gene in human lymphoma and acute leukaemia. *Br J Haematol* 2005;131:411-3.
92. Sakai A, Thieblemont C, Wellmann A, Jaffe ES, Raffeld M. PTEN gene alterations in lymphoid neoplasms. *Blood* 1998;92:3410-5.
93. Palomero T, Sulis ML, Cortina M, Real PJ, Barnes K, Ciofani M, et al. Mutational loss of PTEN induces resistance to NOTCH1 inhibition in T-cell leukemia. *Nat Med* 2007;13:1203-10.
94. Billotet C, Grandage VL, Gale RE, Quattropiani A, Rommel C, Vanhaesebroeck B, et al. A selective inhibitor of the p110delta isoform of PI 3-kinase inhibits AML cell proliferation and survival and increases the cytotoxic effects of VP16. *Oncogene* 2006;25:6648-59.
95. Sujobert P, Bardet V, Cornillet-Lefebvre P, Hayflick JS, Prie N, Verdier F, et al. Essential role for the p110delta isoform in phosphoinositide 3-kinase activation and cell proliferation in acute myeloid leukemia. *Blood* 2005;106:1063-6.
96. Herman SE, Gordon AL, Wagner AJ, Heerema NA, Zhao W, Flynn JM, et al. Phosphatidylinositol 3-kinase-delta inhibitor CAL-101 shows promising preclinical activity in chronic lymphocytic leukemia by antagonizing intrinsic and extrinsic cellular survival signals. *Blood* 2010;116:2078-88.
97. Hoellenriegel J, Meadows SA, Sivina M, Wierda WG, Kantarjian H, Keating MJ, et al. The phosphoinositide 3'-kinase delta inhibitor, CAL-101, inhibits receptor signaling and chemokine networks in chronic lymphocytic leukemia. *Blood* 2011;118:3603-12.
98. Ammirante M, Luo JL, Grivennikov S, Nedospasov S, Karin M. B-cell-derived lymphotoxin promotes castration-resistant prostate cancer. *Nature* 2010;464:302-5.
99. Kerr WG, Colucci F. Inositol phospholipid signaling and the biology of natural killer cells. *J Innate Immun* 2011;3:249-57.
100. Zebedin E, Simma O, Schuster C, Putz EM, Fajmann S, Warsch W, et al. Leukemic challenge unmasks a requirement for PI3Kdelta in NK cell-mediated tumor surveillance. *Blood* 2008;112:4655-64.
101. Del Prete A, Vermi W, Dander E, Otero K, Barberis L, Luini W, et al. Defective dendritic cell migration and activation of adaptive immunity in PI3Kgamma-deficient mice. *EMBO J* 2004;23:3505-15.
102. Krishnamoorthy N, Oriss TB, Paglia M, Fei M, Yarlagadda M, Vanhaesebroeck B, et al. Activation of c-Kit in dendritic cells regulates T helper cell differentiation and allergic asthma. *Nat Med* 2008;14:565-73.
103. Papakonstanti EA, Zwaenepoel O, Bilancio A, Burns E, Nock GE, Houseman B, et al. Distinct roles of class IA PI3K isoforms in primary and immortalised macrophages. *J Cell Sci* 2008;121:4124-33.
104. Kharas MG, Okabe R, Ganis JJ, Gozo M, Khandan T, Paktinat M, et al. Constitutively active AKT depletes hematopoietic stem cells and induces leukemia in mice. *Blood* 2010;115:1406-15.
105. Yilmaz OH, Valdez R, Theisen BK, Guo W, Ferguson DO, Wu H, et al. Pten dependence distinguishes haematopoietic stem cells from leukaemia-initiating cells. *Nature* 2006;441:475-82.
106. Zhang J, Grindley JC, Yin T, Jayasinghe S, He XC, Ross JT, et al. PTEN maintains haematopoietic stem cells and acts in lineage choice and leukaemia prevention. *Nature* 2006;441:518-22.
107. Torhova Z, Kollipara R, Huntly BJ, Lee BH, Castrillon DH, Cullen DE, et al. FoxOs are critical mediators of hematopoietic stem cell resistance to physiologic oxidative stress. *Cell* 2007;128:325-39.
108. Sykes SM, Lane SW, Bullinger L, Kalaitzidis D, Yusuf R, Saez B, et al. AKT/FOXO Signaling Enforces Reversible Differentiation Blockade in Myeloid Leukemias. *Cell* 2011;146:697-708.

CANCER DISCOVERY

PI3K δ Inhibitors in Cancer: Rationale and Serendipity Merge in the Clinic

David A. Fruman and Christian Rommel

Cancer Discovery 2011;1:562-572. Published OnlineFirst November 29, 2011.

Updated version

Access the most recent version of this article at:
doi:[10.1158/2159-8290.CD-11-0249](https://doi.org/10.1158/2159-8290.CD-11-0249)

Supplementary Material

Access the most recent supplemental material at:
<http://cancerdiscovery.aacrjournals.org/content/suppl/2011/10/27/2159-8290.CD-11-0249.DC1>

Cited articles

This article cites 108 articles, 50 of which you can access for free at:
<http://cancerdiscovery.aacrjournals.org/content/1/7/562.full#ref-list-1>

Citing articles

This article has been cited by 10 HighWire-hosted articles. Access the articles at:
<http://cancerdiscovery.aacrjournals.org/content/1/7/562.full#related-urls>

E-mail alerts

[Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions

To request permission to re-use all or part of this article, use this link
<http://cancerdiscovery.aacrjournals.org/content/1/7/562>.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.