The success of imatinib in CML has prompted great interest in developing targeted therapies for other types of blood cancers. Unlike CML, most other blood cancers lack a genetically altered common target that is amendable for therapeutic targeting. Instead, pathways that are active in normal cells at select times are often aberrantly continuously activated. An example of this is B-cell receptor (BCR) signaling in normal B cells, which facilitates proliferation, migration, and survival in the setting of activation by an antigen but which is promptly downregulated as exposure to this diminishes. In contrast, BCR signaling is often constitutively active in select B-cell malignancies, including CLL and NHL. The etiology of this pathway activation is multifactorial, as a result of enhanced microenvironment activation, mutation of activating genes, or silencing of specific phosphatases involved in proximal B-cell receptor signaling. Although this pathway is quite complex and offers several therapeutic targets for pharmacologic intervention, one of the more proximal kinases activated is PI3K.

The PI3K Pathway

The PI3K pathway is a key component of cell survival in many cancers, including CLL and low-grade NHL. PI3K is activated by receptors or the small GTPase Ras and includes multiple isoforms. There are three classes of PI3K isoforms; however, only the class I isoforms phosphorylate inositol lipids to form second messenger phosphoinositides. Specifically, class I PI3K enzymes convert PtdIns(3,4)P2 into PtdIns(3,4,5)P3 that recruit downstream signaling proteins such as BTK, PDK, AKT, ILK, and Rac GEF1,2 that can generate survival signals. Class I isoforms are made up of two subsets (IA and IB). Class IA encompasses p110 alpha, p110 beta, and p110 delta (catalytic domains) that are bound by p85, p50, or p55 (regulatory domains). Class IB is made up solely of the p110 gamma (catalytic domain) bound by the regulatory domain p101. The p110 alpha and p110 beta isoforms are ubiquitously expressed, and knock-out mice for both are embryonic lethal.3 It is thought that this widespread functionality of PI3K signaling is at least partially responsible for the significant cellular toxicity associated with pan-PI3K inhibitors such as LY294002.4 However, in recent years it has been shown that these catalytic isoforms have different expression profiles in different cell types.5-8

The expression of PI3K-delta is generally restricted to hematopoietic cells.9 Mice with deleted or kinase dead PI3K-delta exhibit a B-cell defect, with a lack of B1 lymphocytes, decreased mature B-cell numbers, and impaired antibody production.3,5,10 Biochemically, B cells derived from PI3K-delta knock-out mice also demonstrate less AKT phosphorylation when activated, decreased PIP3 levels, and decreased ability to phosphorylate a YXXM phospho-specific peptide.3 These mouse studies suggest that specific targeting of the PI3K-delta isoform may be cytotoxic to B cells with minimal toxicity to other hematopoietic cell types. Forced expression of PI3K-delta was shown to be transforming in cell lines.11 These collective findings provided a rationale to pursue isoform-specific PI3K inhibitors preclinically and clinically in hematologic malignancies, including CLL. The acquisition of a platform of novel PI3K-delta inhibitors by Calistoga, a small biotechnology company with a very collaborative research team, prompted rapid exploitation of this potential target in a variety of hematologic malignancies including CLL, which we will describe in this review.

Preclinical Targeting of the PI3K-delta Pathway in CLL

With the goal of determining the feasibility of targeting the isoform PI3K-delta in CLL, our group collaboratively worked with Calistoga to initially examine 20 patients with CLL for expression of the different PI3K isoforms. In these patient samples, abundant PI3K-delta and PI3K-gamma protein expression was found, but very modest PI3K-alpha and essentially no PI3K-beta.12 Beyond this work, we confirmed a previous observation that CLL cells have increased resting activity of PI3K activity as compared to normal B cells. This finding was concurrently corroborated by Lan-
nutti and colleagues, who demonstrated downstream constitutive phosphorylation of Threonine-308 on AKT, suggesting activated PDK1 and PI3K in CLL cells as compared to normal cells. Collectively, these findings demonstrated that CLL cells may depend on PI3K-delta for constitutive AKT activation and survival. To further confirm this finding, small interfering RNA targeting p110-delta was performed in CLL cells demonstrating knock-down of target protein, diminished AKT/Thr-308 phosphorylation, and apoptosis. These findings provided rationale for further targeting of CLL with a potential isoform-specific inhibitor of PI3K.

CAL-101 was an example of a PI3K-delta-specific inhibitor that is 100-fold more selective for PI3K-delta compared to other PI3K isoforms. Whole blood studies in basophils demonstrated appropriate inhibition of PI3K-delta at 50 nM concentrations, whereas PI3K-gamma-related targets were not inhibited until μM concentrations were attained. Furthermore, a kinase screen demonstrated significant selectivity for PI3K-delta. The selectivity for PI3K-delta and other favorable drug properties prompted the choice of CAL-101 as a lead candidate for clinical development in hematologic malignancies and initiation of preclinical studies with this agent. Here we found that CAL-101 exhibits dose-dependent induction of apoptosis in CLL tumor cells that is independent of patient genomic features, including immunoglobulin gene variable heavy gene (IVGH) mutational status and del(17p13.1). In contrast, CAL-101 did not promote cytotoxicity toward T cells or natural killer cells. We next confirmed the effect of microenvironment stimuli on CLL cells, including that soluble factors such as CD40L, BAFF, and TNF-α and contact factors (fibronectin and stromal cell coculture) all can activate PI3K and downstream AKT, thereby promoting CLL survival. However, treatment with CAL-101 in the context of any of these microenvironment protective features was shown to abrogate the AKT phosphorylation and protection from cell death. CAL-101 modulation of microenvironment protection was confirmed later by the Burger laboratory that also demonstrated disrupted CLL cell migration, adhesion, and diminished CLL production of chemokines that recruit immune cells to tumor sites. Similar findings in mantle cell lymphoma and other types of B-cell lymphoma, as well as multiple myeloma, were also shown. Collectively, these in vitro studies provided a strong rationale for moving forward to the clinic with CAL-101 with particular focus on targeting CLL and other types of B-cell lymphomas as the lead study diseases. Additionally, these preclinical studies provided for biomarkers of target inhibition such as AKT/Thr-308 phosphorylation loss by flow cytometry, cytokine production by tumor and normal immune cells, and also chemokine production by tumor cells.

CAL-101: Early Clinical Development

The selectivity of CAL-101 and also a very favorable toxicity profile observed in preclinical toxicology at doses where PI3K-delta was inhibited allowed an initial study in healthy volunteers. This phase I study demonstrated favorable pharmacokinetics during a week of CAL-101 oral administration with absent lymphocyte count changes or notable toxicity. A phase I study of CAL-101 in select lymphoid malignancies was initiated in July 2008 beginning at a 50-mg once daily dosage. Unlike many phase I studies that struggle through enrollment with only modest efficacy, dramatic reduction in tumor size was noted in the first patient at the 1-month response evaluation. Accrual to this phase I trial was quite rapid, with efficient data communication and extensive investigator involvement typical of early studies performed with small biopharmaceutical companies. Dose escalation proceeded through several dose levels with a loss of linear pharmacokinetics at higher doses examined. The most predominate toxicity observed with CAL-101 was reversible transaminitis early during therapy, which usually resolved with temporary discontinuation of the drug. Re-initiation of CAL-101 at lower doses generally was possible without recurrence of transaminitis. Perplexing, however, was the finding that this toxicity occurred much more frequently in patients with lymphoma rather than with CLL. This liver toxicity and the nonlinear pharmacokinetics observed with CAL-101 required exploration of multiple dose levels that ultimately delayed development of this compound proceeding to phase II formal testing. Results for the CLL and low-grade lymphoma cohort were most promising, prompting focused expansion in each of these groups. In contrast, single-agent activity in aggressive large cell lymphoma, refractory acute myeloid leukemia, and multiple myeloma was not observed.

Disease-Specific Activity and Toxicity of CAL-101 in CLL

With respect to CLL and its exploration in the initial phase I study, a dose of 150 mg every 12 hours was identified to be ideal for the phase II dose in CLL based on tolerability and minimal increase in plasma Cmax at increasing doses. Of 54 patients with CLL enrolled in this trial, 84% achieved a decrease in lymph node and spleen size of 50% or greater. An increase in peripheral lymphocyte count of more than 50% was seen in 58% of patients, consistent with other BCR antagonists. Lymphocytosis peaked at 2 months and resolved over time in a subset of patients. As a consequence of the lymphocyte count not falling to 50% of baseline, response across all patients enrolled was 24%. This response was independent of high-risk genetic features, bulky adenopathy, prior therapy, or presence of cytopenias. Median progression-free survival was 15 months, with 46% of patients continuing on therapy at the time of presentation. Side effects with this agent have been mild and have included rare cytopenias and pneumonia. Approximately
6% of patients developed grade 3 or 4 transient liver function abnormalities during the early phase, which was reversible with holding therapy and generally did not recur with resumption at a lower dose level. Several of the pharmacodynamic studies optimized with the preclinical CAL-101 work confirmed target inhibition of PI3K-delta in vivo in patients receiving this agent, including serial loss of AKT-Thr308 phosphorylation, diminished chemokine, and also cytokine levels in plasma. These data collectively provide support that CAL-101 has significant clinical activity in CLL and can be administered as continuous therapy for an extended period of time with very modest toxicity.

Based on the favorable toxicity observed with CAL-101 monotherapy, target inhibition of PI3K-delta, and early lymphocytosis observed that was believed to be representative of the ability of CAL-101 to mobilize CLL cells from protected stromal cell niches, combination studies with other therapies were clearly justified. CAL-101 studies with either monoclonal antibodies (rituximab or ofatumumab) or chemotherapy agents (bendamustine, bendamustine and rituximab) in the phase I setting have been pursued. In these studies, CAL-101 was dosed at 100 mg twice daily or 150 mg twice daily with other drugs administered as standard. Therapy was well-tolerated, with no toxicities in addition to those expected with the single agents. Response data have been presented for the CAL-101 studies combined with rituximab, bendamustine, and bendamustine and rituximab. For patients receiving CAL-101 with rituximab or bendamustine, 90% or more of patients achieved a reduction in lymph node size of 50% or more. For three patients treated with bendamustine, rituximab, and CAL-101, all patients achieved a lymph node response. Using traditional IWCLL 2008 CLL response criteria, more than 80% of patients receiving each of these three regimens met criteria for response. Collectively, these data provide evidence that CAL-101 can be safely combined with other therapies used in CLL and also can contribute to durable remissions with these agents.

Future Directions for CAL-101 and Other PI3K Inhibitors in CLL

The documented success of CAL-101 has prompted transition of the compound from Calistoga, a very small biotechnology company, to Gilead, a much larger pharmaceutical company. This transition resulted in both a renaming of CAL-101 to GS1101 and also the initiation of more definitive registration studies for eventual marketing approval in CLL and low-grade NHL. As an expected consequence of the success of CAL-101, other PI3K-delta specific inhibitors are now being explored in CLL and related B-cell malignancies. Additionally, trials with more broad PI3K inhibitors have also been initiated with documented clinical activity. In particular, PI3K inhibitors targeting the PI3K-alpha isoform may prove worthwhile in CLL. Burger and colleagues have previously demonstrated that PI3K-alpha antagonists abrogate stromal microenvironment protection against chemotherapy-mediated death. Additionally, the success of CAL-101 has brought forth inhibitors of other kinases downstream in the BCR signaling pathway including BTK, which have also demonstrated dramatic clinical responses. At this point, the field of CLL has been mesmerized by the durable activity of these BCR antagonizing agents and also the true potential of these therapeutics to favorably affect the natural history of CLL.

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*No relevant relationships to disclose.

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5. Jou ST, Carpino N, Takahashi Y, et al. Oncogenic transformation of CAL-101 has brought forth inhibitors of other kinases downstream in the BCR signaling pathway including BTK, which have also demonstrated dramatic clinical responses. At this point, the field of CLL has been mesmerized by the durable activity of these BCR antagonizing agents and also the true potential of these therapeutics to favorably affect the natural history of CLL.


