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# Inhibitors of B-Cell Receptor Signaling for the Treatment of Chronic Lymphocytic Leukemia

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## Introduction

Chronic lymphocytic leukemia (CLL) is a clonal lymphoid disease characterized by the proliferation and accumulation of small CD5/CD19/CD23-positive lymphocytes in the blood, lymph nodes, spleen, liver and bone morrow. It is the most common leukemia in the western world, accounting for approximately 30% of all leukemia's in Europe and North America, with an annual incidence rate of three to five cases per 100,000 [1]. At present, available therapies are only partially effective in patients with CLL and the vast majority of patients cannot be cured with current treatment strategies. Recent treatment of CLL consists of combined cytotoxic chemotherapy with monoclonal antibodies, which has significantly improved the quality and duration of response, as well as survival [2]. However, intensive treatment is often too toxic, particularly in older patients. Hence, there is an obvious need for developing more specific and effective drugs in the treatment of these diseases. Therefore, the development of novel treatment strategies is highly desirable, especially those with targeted actions and lower toxicities.

The management of CLL has greatly evolved in the last decade with the advent of targeted therapies, which have improved response and survival. Several new agents have been explored and have shown promise in CLL treatment including new monoclonal antibodies and BCL-2 inhibitors, such as oblimersen, obatoclax, and ABT-263 [2,3]. In addition, several small molecular kinase inhibitors have been developed to target the proximal B-cell receptor (BCR) signaling pathway including spleen tyrosine kinase inhibitor, Bruton's tyrosine kinase inhibitors and phosphatidylinositol 3-kinase (PI3K) inhibitors [4,5].

BCR plays a critical role in recognition of antigens and activation of B-cells. BCR signaling is essential for normal and malignant B cell development. BCR signaling pathway is a major therapeutic target in CLL [4,5]. Signals propagated through the BCR increase CLL cell survival *in vitro* and there is growing evidence that such signals are continuously delivered to the leukemic cells *in vivo* [6,7]. Transduction of the BCR signal is a complex process that involves multiple kinases representing therapeutic targets in CLL patients. The signal is propagated by SRC-family kinases (Lyn, FYN or BLK) which phosphorylate the immunoreceptor tyrosine-based activation motifs (ITAMs) in the Igα and Igβ chains of the BCR.

## Lyn Inhibitors

Lyn is overexpressed and abnormally distributed in CLL cells as compared to normal B cells and appears to be critical for CLL cell growth [8,9]. Lyn is a potential therapeutic target for antileukemic drugs. Dasatinib ((N-(2chloro-6-methylphenyl)-2-(6-(4-(2-hydroxyethyl) piperazin-1-yl)-2-methylpyrymidin-4-ylamino) thiazole-5-carboxamide; Sprycel®, Bristol-Myers Squibb; BMS-354825) is a BCR/ABL kinase inhibitor that is FDA-approved for the treatment of CML (Figure 1) [10]. However, it is also a potent Lyn kinase inhibitor and Btk inhibitor. This compound induces apoptosis via inhibition of Lyn kinase in primary CLL cells. Dasatinib as a single agent has activity in relapsed and refractory CLL, particularly in the reduction of nodular tumor masses, but seems to have weak efficacy on peripheral blood lymphocytes [11,12].

In a phase II study performed by Amrien et al. [11] 15 patients were treated with dasatinib as a single agent [11]. Five patients were fludarabine-refractory and 11 (73%) had high risk cytogenetics. Six patients showed nodal remission, demonstrating a decrease in less than 50% in lymphocyte count, while two patients actually demonstrated a partial response (PR). However, significant myelosuppression was noted with grade 3 or 4 neutropenia in 10 patients and thrombocytopenia in 6 patients. Dasatinib has been evaluated in CLL also in combination with other drugs, including fludarabine and rituximab. In an open-label phase 2 trial dasatinib was combined with fludarabine in twenty refractory CLL patients [13]. Reductions in lymph node size were observed in most patients. A lymph node reduction of ≥ 20% provided a significantly improved progression free survival (PFS) and overall survival (OS) as compared to non-responders. Dasatinib and fludarabine combination treatment has clinical efficacy in heavily pretreated refractory patients. Kater et al. [14] conducted an open-label phase 2 trial of dasatinib at a dose of 100 mg once daily for 28 days

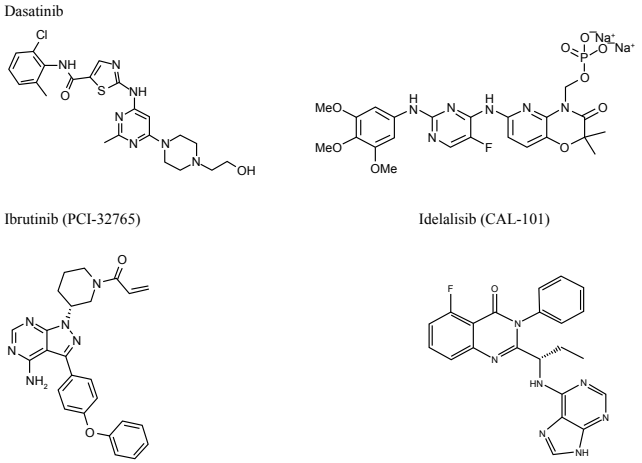


**Figure 1:** Chemical structure of B-Cell receptor signaling inhibitors active in CLL.

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combined with fludarabine in twenty refractory CLL patients [14]. In patients who did not reach at least a partial remission (PR), fludarabine was added at a dose of 40 mg/m$^2$ for 3 consecutive days every 28 days for 2-6 cycles. Most patients showed reduction in lymph node size and 18% of the patients reached a PR.

## Bruton's Tyrosine Kinase Inhibitors

Bruton's tyrosine kinase (Btk) is a member of the src-related BTK/Tec family of cytoplasmic tyrosine kinases. Btk is primarily expressed in hematopoietic cells, and is important in B-lymphocyte development and differentiation [15,16]. It is required for B-cell maturation, and is overexpressed in a number of B-cell malignancies including CLL. Ibrutinib, (PCI-32765, Pharmacyclics) is an orally bioavailable, small-molecule inhibitor of Btk with potential activity in lymphoid malignancies (Figure 1). This agent prevents B-cell activation and inhibits DNA synthesis and CLL cell survival. Moreover, ibrutinib effectively blocks survival signals provided externally to CLL cells from the microenvironment and inhibits migration in response to tissue homing chemokines (CXCL12, CXCL13) [17,18]. Ibrutinib shows encouraging clinical activity in patients with B-cell malignancies, particularly in CLL patients [19-21]. Data from the Phase 1/2 trials demonstrated that high-risk CLL patients responded to ibrutinib equally as well as low-risk patients [22]. In CLL, the response is characterized by a rapid resolution of lymphadenopathy and/or organomegaly accompanied by a transient increase in lymphocyte counts, caused by re-distribution of tissue-resident CLL cells into the peripheral blood. Byrd et al. [21] reported new and updated results from a large phase Ib/II study of 116 patients treated with this agent [21]. Patients previously received two or more therapies including a purine analog or had a high-risk CLL. Overall responses rates were 71% in treatment naïve patients, 67% in relapsed or refractory patients, and 50% in high risk patients with early relapse or 17p deletion. CR rate was 10%, 3% and 0%, respectively. Median PFS and OShave not been met for any of the cohorts including patients with high-risk factors. The majority of adverse events (AEs) have been Gr ≤ 2 in severity, most commonly diarrhea (54%), fatigue (29%), upper respiratory tract infection (29%), rash (28%), nausea (26%) and arthralgias (25%). Serum immunoglobulin evaluation revealed a significant increase in IgA with no decline in IgG or IgM. Ibrutinib in combination with rituximab is also a safe, well tolerated regimen for high-risk CLL patients, which induces very high early response rates. Recently, Burger et al. [23] conducted a Phase 2 single-center clinical trial of ibrutinib combined with rituximab in high-risk CLL patients [23]. Out of 20 patients, 17 patients achieved a PR and three achieved a PR with persistent lymphocytosis.

## Spleen Tyrosine Kinase Inhibitors

Spleen tyrosine kinase (Syk) plays a key role in B cell receptor mediated survival in certain B cell malignancies. Syk is a 72 kDa cytosolic non-receptor tyrosine kinase that is involved in signal transduction in a variety of cell types, including B lymphocytes, mast cells and macrophages [24]. Several studies highlight the critical role of Syk in the molecular mechanisms regulating BCR signaling [25]. Syk as a key component of BCR signaling pathway is critical for normal and malignant B-cell development and proliferation [26,27]. Inhibition of Syk pathway prevents CLL cells from interacting with the microenvironment and inhibition of Syk promotes proapoptotic signals [28,29].

Fostamatinib (N4-(2, 2-dimethyl-3-oxo-4-pyrid1, 4oxazin-6-yl)-5-fluoro-N2-(3, 4, 5-trimethyoxyphenyl)-2,4-pyrimidinediamine, R788, FosD, AstraZeneca/Rigel Pharmaceuticals) is the first oral Syk inhibitor in development as a novel therapeutic approach for lymphoid malignancies. It is converted to an active drug, R406 with activity against Syk [25]. R788 effectively inhibits BCR signaling in vivo, resulting in reduced proliferation and survival of the malignant B cells and significantly prolonged survival of the Eμ-TCL1 mice with CLL model of leukemia. These data suggest that CLL should be an appropriate disease for clinical trials with R788 [30]. Fostamatinib disodium is under clinical development for the treatment of B-cell lymphoid malignancies. In a phase 1/2 clinical trial, Friedberg et al. [31] investigated fostamatinib disodium in the treatment of patients with relapsed and refractory B-cell NHL and CLL. In the phase 1 part of the trial 2 cohorts of 6 patients each received 1of 2 oral dose levels, 200 mg or 250 mg twice daily [31]. In the phase 2 study the patients were treated with fostamatinib disodium at doses of 200 mg twice daily. The highest response rate (55%) was observed in patients with SLL/CLL. Median progression-free survival was 4.2 months. Common toxicities observed in this study included diarrhea, fatigue, and cytopenias. These results indicate that fostamatinib disodium is a promising rational targeted therapy for CLL and B-cell lymphomas.

## PI3K Inhibitors

Phosphatidylinositol 3-kinases (PI3Ks) are a family of lipid kinases that mediate signals from cell surface receptors [32]. PI3K is essential to BCR signaling. PI3K regulates cellular functions, including survival and migration, by integrating and transmitting signals from diverse surface molecules including the BCR. Activation of PI3K requires Lyn-dependent phosphorylation of CD19 surface molecule expressed during all stages of B-cell ontogeny. There are 3 classes of PI-3K (I, II and III) categorized on the basis of the structure and substrate specificity. The class IA PI3K isoforms (PIK3Cα, PIK3Cβ, and PIK3Cδ) are heterodimeric proteins that contain a p110 catalytic subunit and a p85 regulatory subunit. Expression of the PI3K p110 δ isoform (PI3K-δ) is largely restricted to lymphocytes where it plays a key role in B cell proliferation and survival. The PI3K pathway is a central pro-survival mechanism in CLL. Identification of the PI3K-δ isoform unlocks a new therapeutic potential for this leukemia. Herman et al. [33] demonstrated that PI3K has increased enzymatic activity and that its isoform PI3Kδ is expressed in CLL-cells and PI3K activity is higher in these cells than in B cells from healthy volunteers [33]. Inhibition of PI3K activity in vitro induces CLL cell apoptosis and death. A number of potential therapeutics targeting this signaling pathway have been recently generated and some of them seem to be active in lymphoid malignancies [34,35].

Idelalisib, (CAL-101 GS-1101, Calistoga Pharmaceuticals), is an oral PI3K p110δ- selective inhibitor which has shown preclinical and clinical activity against CLL and other lymphoid malignancies. In the first phase I clinical trial in patients with heavily pretreated CLL, it showed acceptable toxicity, positive pharmacodynamic effects and favorable clinical activity in patients with refractory disease, bulky lymphadenopathy and poor-prognosis cytogenetics [36]. Moreover, GS-1101-based combination therapies with rituximab and/or bendamustine induced major and rapid reductions in lymphadenopathy and durable tumor control in previously treated CLL patients [37,38].

In conclusion, the BCR pathways play a seminal role in the development of CLL. Ibrutinib and idelalisib (CAL-101) are highly selective Btk and PI3K p110δ inhibitors currently undergoing clinical development. These drugs should significantly improve the prognosis of this frequently fatal disease in the near future.

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