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Inhibitors of B-cell Receptor Signaling for patients with B-cell malignancies

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Abstract

The B-cell receptor (BCR) complex and its associated protein-tyrosine kinases play a critical role in the development, proliferation, and survival of normal or malignant B cells. Regulated activity of the BCR complex promotes the expansion of selected B cells and the deletion of unwanted or self-reactive ones. Compounds that inhibit various components of this pathway, including spleen tyrosine kinase (Syk), Bruton's tyrosine kinase (Btk), and phosphoinositide-3 kinase (PI3K), have been developed. Herein we summarize the rationale for use of agents that can inhibit BCR-signaling to treat patients with either indolent or aggressive B-cell lymphomas, highlight early clinical results, and speculate on the future application of such agents in the treatment of patients with various B-cell lymphomas.

Keywords

B-cell receptor; PI-3 kinase; Bruton's tyrosine kinase; Syk; PCI-32765; CAL-101; AVL-292

THE B-CELL RECEPTOR COMPLEX – STRUCTURE AND FUNCTION

The BCR is composed of antigen-specific membrane bound immunoglobulin (Ig) non-covalently complexed with Ig-accessory molecules CD79a and CD79b, which have cytoplasmic domains that contain Immunoreceptor Tyrosine-based Activation Motifs (ITAM) ^{1, 2}. Upon ligation of the BCR, this complex can recruit intracellular kinases, adapter proteins, and other co-stimulatory molecules, leading to stimulation of signaling pathways that govern the B-cell's fate ^{3, 4}. Antigen binding to surface immunoglobulin induces phosphorylation of the ITAMs of CD79a and CD79b by Lyn kinase or other src-family kinases ⁵. Once activated these ITAMs can recruit the spleen tyrosine kinase (Syk), which in turn also becomes activated. Activated Syk enhances the generation of second messengers, including B-cell linker protein (BLNK), which bridges the BCR associated kinases with several signaling pathways, leading to the phosphorylation of multiple downstream molecules, including Bruton's tyrosine kinase (Btk) ^{6, 7}. Figure 1 depicts a simplified schema of the BCR complex and associated kinases pertinent to a discussion of the emerging therapies.

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Btk is a non-receptor tyrosine kinase of the Tec kinase family. It is primarily expressed in B cells, but not in T cells or plasma cells⁸. Upon activation by Lyn or Syk, Btk phosphorylates phospholipase C γ 2 (PLC- γ 2), triggering calcium (Ca²⁺) mobilization and activation of other pathways, including the mitogen-activated protein kinase (MAPK) pathway and the nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B)^{8,9}. Btk also can be activated in response to stimulation via other receptors, including chemokine receptors CXCR4 and CXCR5¹⁰, Toll-like receptor (TLR) family members (e.g. TLR9)^{11,12}, Wnt receptors¹³, and a receptor for the B-cell activating factor (BAFF) (e.g. BR3)¹⁴.

Signaling through the BCR also activates phosphoinositol-3 kinase (PI3K), which is composed of two subunits: a regulatory subunit (p85) that is required for docking the enzyme to the activated receptor complex and a 110 kD protein (p110), which houses the enzyme's catalytic activity¹⁵. Though PI3K can be activated by many cell surface chemokine and cytokine receptors, BCR-related Lyn-dependent phosphorylation of the ITAM in the cytoplasmic domain of CD19 also can provide a docking site for the p85 regulatory subunit of PI3K, allowing for recruitment of p110 catalytic subunit to the cell membrane^{16,17}. PI3K catalyzes the production of phosphatidylinositol 3,4,5-triphosphate, which recruits and activates Akt, also known as protein kinase B. Akt, in turn, mediates a positive effect on cell survival, proliferation, growth, and metabolism, by downstream signaling through other pathways activated by mammalian target of rapamycin (mTOR), NF- κ B, or other factors^{15,18}. Phosphatidylinositol 3,4,5-triphosphate also activates Btk, highlighting the complex interactions and overlap between these BCR-associated protein tyrosine kinases¹⁹.

PI3K isoforms are defined by the p110 catalytic subunit, of which there are three variants, designated alpha, beta, or delta²⁰. The delta isoform is predominantly expressed in leukocytes and lymphomas, whereas the alpha and beta isoforms are expressed more ubiquitously, but may be upregulated in various solid tumors²¹.

Negative regulators modulate the intensity and duration of BCR-signaling. The Fc receptor for Ig (Fc γ RIIB), for instance, contains an immunoreceptor tyrosine-based inhibitory motif (ITIM) that can bind phosphatases, such as the src homology-2-containing inositol phosphatase (SHIP)²². These phosphatases can dephosphorylate activated signaling molecules and ITAMs of the accessory molecules, thereby suppressing the signal that can be transmitted via the BCR. Activation of Fc γ RIIB also recruits the phosphatase and tensin homolog (PTEN), which can catalyze the removal of the 3' phosphate of phosphoinositides to suppress activation of the PI3K/AKT pathway²³.

Stimulation via the BCR dictates the fate of developing B-cells. The tightly regulated activity of the BCR complex governs the expansion of selected B-cells and the deletion of unwanted or self-reactive ones. When immature B cells expressing surface IgM are first exposed to self-antigens in the marrow, clones that are highly reactive with self-antigens are deleted, with the exception of those that successfully undergo reiterative Ig rearrangements to produce a new Ig with a requisite specificity, a process termed receptor editing²⁴. There is evidence that receptor editing can also occur in mature B-cells in the periphery and contribute to the generation of Ig expressed by the neoplastic B cells of some patients with chronic lymphocytic leukemia (CLL)²⁵.

Following this negative selection step, BCR signaling continues to regulate the development of non-autoreactive mature B-cells into naïve B-cells. In the absence of antigen-binding, basal or tonic BCR signaling through the extra-cellular signal-regulated kinase (ERK)/mitogen activated protein (MAP) kinase pathway is required for continued B-cell maturation, in concert with chemokines and cytokines signals, such as BAFF, which are

elaborated by the supportive stroma in the tumor microenvironment²⁶. Interruption of these signals below threshold levels can result in apoptosis or developmental regression with re-expression of pro-B cell and pre-B cell genes and reactivation of V(D)J recombination²⁷⁻²⁹.

THE B-CELL RECEPTOR IN B-CELL LYMPHOMAS

Neoplastic B cells of most lymphomas generally retain expression of surface immunoglobulin and have intact BCR complexes. Interaction of the BCR with self or environmental antigens could lead to constitutive BCR-signaling, which in turn could provide the neoplastic B cells with growth and/or survival signals that play a role in the pathogenesis of such cancers. Such stimulation might obviate an oncogenic requirement for activating somatic mutations in tyrosine kinases or other molecules involved in BCR-signaling, though some mutations have been identified. Activation via BCR-signaling has been proposed to play a pathogenic role in various B-cell malignancies, and is summarized below.

Marginal Zone Lymphoma

Marginal zone lymphoma presents direct evidence of the role of B-cell receptor signaling in B-cell lymphoma. Chronic antigenic B-cell receptor stimulation by *H. pylori*, *Chlamydia psittaci*, *Campylobacter jejuni*, or self-antigens (as in Sjögren's syndrome or Hashimoto's thyroiditis) can directly drive lymphomagenesis³⁰. Furthermore, eradication of such microorganisms also can lead to regression of the lymphoma³¹.

Gastric MALT lymphomas generally express somatically mutated surface immunoglobulins, stimulated to proliferate upon exposure to target antigens. Neoplastic B-cell proliferation may be augmented by tumor-infiltrating T cells³². Genetic analysis also supports the constitutive activation of BCR signaling in splenic marginal zone lymphoma, finding gain-of-functions mutations in CARD11, a scaffolding molecule that links the BCR to the NF- κ B pathway; and inactivating mutations of A20, a ubiquitin modification enzyme that negatively regulates BCR signaling by inactivating secondary messengers³³. Although eradication of the antigenic stimulus may be the optimal manner for treatment of patients with such lymphomas, small molecule inhibitors of BCR-signaling still might play a therapeutic role. Such inhibitors could interrupt BCR signaling that is critical for the survival and/or growth of marginal zone lymphoma and prove useful in treatment of patients who have lymphoma that is refractory to clearance of the responsible microorganism or in patients who have lymphomas with BCR that are stimulated by ubiquitous self-antigen(s).

Chronic Lymphocytic Leukemia (CLL)

BCR signaling has been extensively investigated in CLL, and evidence of its importance was recently reviewed^{22,34}. Indirect evidence of the role of the BCR in CLL development and clinical outcomes is inferred from the usage of restricted and stereotypic immunoglobulin variable gene sequences³⁵⁻³⁷ as well as data showing that CLL cells collected from the lymph node microenvironment have gene-expression signatures similar to that of B cells stimulated via BCR signaling, as opposed to cells collected from the peripheral blood³⁸. There also is evidence of constitutive activation and high expression of BCR-associated kinases in a subset of patients, including the MAP kinase, ERK, Akt, and the nuclear factor of activated T-cells (NFAT)³⁹. In vitro engagement of surface IgM by anti-IgM results in intracellular calcium influx and the activation of downstream signaling molecules, leading to induction of anti-apoptotic, Bcl-2-family-member protein, the myeloid leukemia cell differentiation protein (Mcl-1), and other pro-survival factors^{40, 41}. The relative capacity of the BCR to stimulate such growth and/or survival factors appears associated with expression levels of surface immunoglobulin, absence of somatic mutation

of the expressed heavy-chain immunoglobulin variable region genes (IGHV), and/or the expression of the zeta-associated protein of 70 kD (ZAP-70), an intracellular tyrosine kinase that can enhance the recruitment and activity of Syk⁴². It is thought for this reason that expression of ZAP-70 correlates with clinical outcomes and aggressiveness, further evidence of the relevance of the BCR-signaling pathway⁴³.

Mantle cell lymphoma (MCL)

In addition to its pathognomonic translocation of t(11;14) resulting in aberrant expression of cyclin D1, overactive BCR signaling also appears to contribute to the pathogenesis of MCL. Phosphoproteomic analysis of MCL cell lines found that the most abundant phosphorylated peptides belonged were ones generated upon stimulation of the BCR signaling, including Syk, Lyn, Btk, and PKC-delta⁴⁴. Primary tumor cells also appeared sensitive to piceatannol, a small molecule that can inhibit Syk⁴⁴. Another group of investigators also performed proteomic analysis of B-cell plasma membranes isolated from patients with MCL and identified high-level expression of a voltage-gated protein channel, HVCN1, which can amplify BCR signaling by inactivating the phosphatase SHP-1⁴⁵.

Diffuse Large B-cell Lymphoma (DLBCL)

Chronic BCR signaling is implicated in the pathogenesis of activated B-cell (ABC) subtype of diffuse large B-cell lymphoma (DLBCL). Historically, this subtype was distinguished from the germinal center subtype by gene expression profiling, and generally characterized by constitutive NF- κ B signaling⁴⁶. Activating mutations of CARD11 accounts for this NF- κ B activity in a subset of cases, by the same mechanism as described above in splenic marginal zone lymphoma⁴⁷. In the remaining cases, constitutive NF- κ B activity appears to be due to BCR signaling as well, based on the cell death induced by shRNA silencing of Btk, CD79A, CD79B, IgM or Ig-kappa⁴⁸. Activating mutations of CD79b that result in immobile clusters of BCR complexes and blunt Lyn-mediated negative feedback have been described in a subset of cases^{48,49}. Augmentation of BCR signaling also has been attributed to increased expression of Bcl6, which increases Syk activity by repressing expression of inhibitory tyrosine phosphatases⁵⁰. Indeed, in vitro DLBCL cell lines and primary tumors appear sensitive to R406, a small molecule that can inhibit Syk⁵¹.

THE INHIBITORS

Several inhibitors have been developed that can inhibit various enzymes involved in BCR signaling. Many of these are called inhibitors of the specific enzyme that they are found to target. However, it should be emphasized that these small molecules generally can inhibit more than one enzyme. Moreover, even though a drug can inhibit a particular enzyme, this does not guarantee that any effect caused by use of the drug will be due to its ability to inhibit that particular enzyme. As such, it is highly unfortunate that each of these drugs are referred to as being an inhibitor of a specific kinase, such as an inhibitor of Syk or an inhibitor of Btk, as this understates the drug's potential "off-target" effects. Indeed each inhibitor will have off-target effects that actually might account for some of its noted biologic and/or clinical activity, as well as its toxicity.

Nevertheless there is excitement about the use of these inhibitors as they have apparent biologic and clinical activity in the treatment of patients with NHL. Patients treated with these inhibitors typically experience a decrease in lymph node size and an increase in lymphocytosis. This is felt to represent a disruption of the nodal microenvironment and trafficking of leukemic cells from the lymphoid compartment into the blood. Due to this phenomenon, traditional response criteria may underestimate the true clinical benefit of such

agents, as only a portion of patients qualified as achieving an objective response according to standard International-Working-Group criteria ⁵².

Fostamatinib

One of the first compounds designed and developed to inhibit the BCR pathway was fostamatinib, a prodrug of R406, which can inhibit Syk. Preclinical testing utilizing Eμ-TCL1 transgenic mouse model of CLL demonstrated that fostamatinib could inhibit BCR-signaling and reduce the growth and/or survival of malignant B-cells in vivo. ⁵³ However, fostamatinib had only modest cytotoxic activity on leukemic B cells in vitro, suggesting that this drug acted on survival-stimulating pathways that might be activated primarily in vivo ⁵³. A phase I/II clinical trial involving patients with relapsed or refractory B-cell lymphomas, including DLBCL (23 patients at the phase II dose), FL (21 patients), CLL (11 patients), and MCL (9 patients) observed objective response rates ranging from 10% for patients with FL or MCL, 22% for patients with DLBCL, and 55% for patients with CLL. ⁵⁴ Treatment generally was associated with relatively few adverse events, which included low-grade diarrhea, fatigue, myelosuppression, hypertension, and nausea ⁵⁴. Fostamatinib also appears to have clinical activity in the treatment of patients with rheumatoid arthritis, which has been a major target disease for development of this drug ⁵⁵. Nevertheless, other compounds have been identified as inhibitors of Syk and have promising preclinical activity ⁵⁶.

GS-1101 (formerly CAL-101)

GS-1101 is the first-in-class oral small molecule that selectively can inhibit the delta isoform PI3K. In vitro kinase profiling assays demonstrated potent inhibition of purified PI3Kδ, with an IC₅₀ of 2.5nM, which is 40- to 300-fold more than its activity against the other PI3K isoforms ⁵⁷. In vitro treatment of lymphoma cell lines and primary CLL cells decreased pAKT levels and induced cellular apoptosis⁵⁸. Normal peripheral blood mononuclear cells, normal T-cells, and acute myeloid leukemia cells exhibited far less sensitivity to GS-1101 in vitro, further supporting its isoform specificity ⁵⁸. In addition to its direct effect on the PI3K/AKT/MTOR pathway the leukemic cell, GS-1101 impairs the cross talk between leukemic cells and the microenvironment. In vitro models of the CLL microenvironment demonstrated that GS-1101 inhibits CXCL12 and CXCL13 mediated chemotaxis, down-regulates stromal mediated chemokine secretion, and mitigates stromal-cell mediated resistance to cytotoxic drugs ⁵⁹.

Based on the in vitro data, Phase I studies were conducted in patients with relapsed or refractory hematologic malignancies, including 55 patients with CLL, 63 with indolent non-Hodgkin lymphoma (iNHL), 40 with MCL, and 9 with DLBCL ⁶⁰. Interim results showed objective response rates of 52% in patients with MCL and 59% in iNHL in those patients receiving doses greater than 100mg twice daily. ⁶¹ Eighty-four percent of patients with CLL experienced reductions in lymph node size upon therapy, including patients with CLL cells that harbored deletions in the short-arm of chromosome 17 (del(17p)) ⁶². Pharmacodynamic analysis demonstrated that the CLL cells of treated patients had a 90% reduction in the levels of phospho-AKT levels after dosing. Treatment also caused reductions in plasma chemokine levels ⁶². As is observed with the other inhibitors, patients with CLL typically experienced an increase in the absolute lymphocyte count upon therapy, consistent with an egress of leukemia cells from the lymphoid tissues into the blood. Nevertheless, responses have been durable for patients who continued to take GS-1101, with the median progression-free survival not yet reached after a median of 11 months on treatment ⁶². Twenty-four percent of treated patients with CLL experienced neutropenia and 24% developed pneumonia while undergoing therapy. Transaminase elevations were observed in 16% of patients with lymphoma who were treated with GS-1101, all of whom experienced normalization upon temporary interruption of GS-1101 therapy ⁶¹.

Trials are ongoing to evaluate the combination of GS-1101 with other therapies. Preliminary data from 27 patients with relapsed or refractory CLL treated on a phase I study of GS-1101 with rituximab and/or bendamustine was presented in abstract form ⁶³. With combination therapy, most patients appear to have rapid lymph node reductions and less prominent lymphocytosis than patients treated with GS-1101 alone. Objective response rates were greater than 80%, and toxicities were consistent with those expected with rituximab and/or bendamustine, including pneumonia (10-14%) and neutropenia (10% for the GS-1101 with rituximab group, and 60% for GS-1101 with bendamustine group). All of the initial 3 patients with relapsed/refractory iNHL or MCL treated on the same protocol achieved objective responses ⁶⁴. Finally, preliminary data of 11 patients with multiply-relapsed CLL treated with GS-1101 and ofatumumab demonstrated an 82% response rate with mitigation of the lymphocyte mobilization phenomenon, and a favorable safety profile ⁶⁵. Phase 3 registration trials with CAL-101 for CLL are ongoing.

Ibrutinib (PCI-32765)

Ibrutinib is a small molecule that can be taken orally to yield plasma concentrations that inhibit Btk. This agent forms a covalent bond with a cysteine that is near the kinase domain of Btk, irreversibly inhibiting its enzymatic activity with an IC₅₀ of 0.5 nM ⁶⁶. Although its pharmacologic half-life is short (1.5-2.7 h), covalent binding to its target allows for prolonged irreversible activity ⁶⁷. Preclinical assays demonstrated inhibition of BCR but not T-cell receptor signaling ⁶⁶, and direct cytotoxicity in DLBCL and MCL cells and primary leukemia cells from patients with CLL ⁵⁸. Inhibition of chemokine and integrin mediated signaling and cell migration have also been demonstrated by in vitro and in vivo models of CLL ^{10, 68}.

Initial clinical trials included patients with relapsed or refractory CLL, DLBCL, MCL, Waldenström macroglobulinemia, and follicular and marginal zone lymphomas, and demonstrated a favorable safety profile at doses that achieved greater than 95% enzyme occupancy and yielded an overall response rate of 44% ⁶⁷. Phase II studies have shown response rates of 70% in relapsed/refractory CLL, 67% in relapsed/refractory MCL, and 73% in previously untreated patients with CLL ⁶⁹⁻⁷¹. Serious adverse events related to ibrutinib occurred in 10-20% of patients, most commonly diarrhea, fatigue, nausea, and ecchymosis. Grade 3 or higher infections and cytopenias have also been reported in 10% of patients, though considered only potentially attributable to ibrutinib due to underlying and preexisting cytopenias ⁷¹. An egress of malignant B-cell from lymphoid tissues into the blood occurred in patients with either CLL or MCL ⁷². The combination of ibrutinib and ofatumumab in patients with relapsed or refractory CLL, has preliminarily indicated safety, tolerability, and an impressive 100% ORR in the initial 23 patients ⁷³. Studies are also ongoing in relapsed/refractory ABC-DLBCL, in which preliminary data showed a 25% rate of complete response and 37% rate of stable disease. It is too early to determine if response is dependent on, or correlates with the BCR-activating mutations described previously, though it appears that chronic active BCR-signaling may occur in the absence of known mutations ⁷⁴.

AVL-292

Structure-based drug design identified AVL-292 as another inhibitor of Btk, capable of specific and durable silencing by irreversibly covalently binding the Cys481 residue. Preclinical studies demonstrated inhibition of Btk in biochemical and cellular assays, and companion pharmacodynamics assays confirmed greater than 98% target occupancy at safe and tolerable dosages ⁷⁵. Preliminary clinical data from the Phase Ib study showed that all 8 patients with CLL had stable disease (most had some reduction in lymph node size, though not yet meeting criteria for objective response) and 2 of 3 evaluable patients with lymphoma

also had stable disease⁷⁶. It should be cautioned that these results are very preliminary; the median time on treatment was only 65 days and the majority of patients continue on treatment⁷⁶. The most frequent adverse events have included transient diarrhea, rash, skin infection, upper respiratory infection, nausea, and fatigue. The only grade 4 adverse event was thrombocytopenia in a single patient with follicular lymphoma⁷⁶.

Sorafenib

The rapidly activated fibrosarcoma (RAF)/ MEK/ extracellular-signal-regulated kinase (ERK) pathway is also activated by BCR signaling⁷⁷. This pathway can be blocked by sorafenib, a small molecule that can inhibit Raf and that already has been approved for the treatment of patients with renal cell and hepatocellular carcinoma. Sorafenib is selectively cytotoxic for primary CLL cells in vitro, an effect that is not mitigated by survival factors elaborated by supportive stromal cells^{78, 79}. For this reason, clinical trials also are ongoing testing the activity of this agent in the treatment of patients with CLL (NLM identifier: NCT01510756).

FUTURE DIRECTIONS

The biological rationale and initial clinical success of BCR inhibiting therapies for B-cell lymphoma has garnered much excitement and momentum. As the clinical trials listed above near completion and peer-review publication, phase III registration trials are under way in CLL, with the expectation that these compounds will gain FDA approval in the foreseeable future. But based on the preclinical and clinical results, certain issues can also be raised that, while speculative, warrant investigation as part of the development of these compounds.

While each of the aforementioned compounds was designed and confirmed to inhibit their target enzymes, potential additional effects have not been fully explored. As summarized previously, Btk and PI3K function not only in the BCR pathway, but also integrate diverse extracellular chemokine, cytokine, and integrin-mediated signals that are not captured in the simplified schema in Figure 1. The complex interactions of the various intracellular signaling networks likely result in pleomorphic cellular effects from even the most targeted inhibitors. For instance, quantitative proteomic analysis of prostate cancer cell lines following inhibition of PI3K in vitro determined changes in levels of nearly 100 unique phosphopeptides, many of which are not considered substrates of PI3K⁸⁰. Conceivably, similar off-target effects could be experienced in patients with lymphoma who are treated with any one of the various inhibitors described above. The complex interactions with other pathways may also provide for compensatory signaling that may result in relapse or resistance. These may be circumvented or prevented with other agents, such as compounds with even more broad activity such as

However, the pleomorphic effects of these compounds are not necessarily to their detriment, and may in fact contribute to their efficacy. The BCR and other signaling pathways are broadly up-regulated in most lymphomas, and thus inhibitors with multiple targets may be most effective. It has been noted that ibrutinib can irreversibly inhibit kinases other than Btk at drug concentrations that are required to inhibit Btk¹⁴. Identification of these effects can validate new therapeutic targets and inform rational drug combinations. For instance, in DLBCL cell cultures, ibrutinib synergizes with lenalidomide by combined inhibition of pro-survival transcription factors⁸¹. Therefore, the full impact of these compounds may extend far beyond their intended effect.

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Figure 1.

Schema for B-cell receptor (BCR) mediated signaling in B-cell lymphoma. The BCR complex consists of surface Ig and accessory molecules CD79a and CD79b, which are phosphorylated in response to antigen binding, and recruit Syk to the activated complex. Syk activates downstream adapter proteins and molecules such as BLNK, Btk, and PLCγ2, which ultimately promotes cell survival and proliferation by various downstream pathways. BCR activation also triggers Lyn dependent phosphorylation of CD19, which provides a docking site of PI3K, leading to activation of AKT, which modulates pathways including mTOR and NF-κB. Molecules that play an inhibitory role include FcγRIIB, which recruit and activate SHIP and other phosphatases that reset the activation cascade.