B-Cell Receptor Signaling in Chronic Lymphocytic Leukemia and Other B-Cell Malignancies

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Abstract: B-cell receptor (BCR) signaling has emerged as a key pathway for the expansion of neoplastic B-cell clones in several B-cell malignancies. The mechanisms that activate BCR signaling differ substantially among subtypes of B-cell lymphoma and leukemia. These include BCR stimulation by foreign or self-antigens, or the acquisition of mutations in components of the BCR pathway that result in autonomous or enhanced antigen-induced BCR signaling. Targeting BCR signaling with selective inhibitors of the BCR-associated kinases Bruton’s tyrosine kinase, spleen tyrosine kinase, and phosphoinositide 3-kinase δ induces high response rates in patients with chronic lymphocytic leukemia, mantle cell lymphoma, Waldenström macroglobulinemia, and diffuse large B-cell lymphoma of the activated B-cell–like subtype and is currently transforming the therapeutic landscape in these diseases. Here we review the mechanisms of BCR activation that govern growth and survival of malignant B cells. We also summarize recent clinical trials of BCR inhibitors, with a focus on the most clinically advanced agents.

B-Cell Receptor Expression and Signaling in Normal B Cells

The B-cell receptor (BCR) is a unique, defining feature of B lymphocytes. The purpose of B-cell development in the bone marrow and secondary lymphatic organs is to generate a diverse set of mature B cells, each equipped with a unique BCR. These unique BCRs allow B cells to recognize foreign antigens and mount specific antibody responses while sparing host (auto) antigens. B cells lacking a functional BCR rapidly undergo apoptosis. The BCR consists of 2 identical immunoglobulin heavy (IgH) chains and 2 identical immunoglobulin light (IgL) chains. The variable domains of IgH and IgL chains are products of gene rearrangements at the pro-B (IgH) and pre-B (IgL) cell stage and define the antigen specificity of the BCR. The transmembrane domain anchors the BCR to the cell membrane,
where each BCR molecule associates noncovalently with a heterodimer of Igα (CD79a) and Igβ (CD79b). The Igα/Igβ heterodimer constitutes the signaling subunit of the BCR complex. Within their cytoplasmic tails, Igα and Igβ harbor 2 conserved tyrosine residues as part of a 26 amino acid–long sequence, also referred to as an immunoreceptor tyrosine–based activation motif (ITAM). Phosphorylation of these ITAM tyrosine residues through Src kinases, such as Lck/Yes-related novel protein tyrosine kinase (LYN), FYN, B-lymphoid kinase (BLK), or spleen tyrosine kinase (SYK), marks the first step in signal transduction from the BCR to the nucleus.

Two distinct means of BCR activation have been described: antigen-induced and antigen-independent autonomous (tonic) BCR signaling. Engagement of the BCR by antigen induces membrane movement and aggregation of BCR components, ITAM phosphorylation, and consequently the recruitment of SYK to the BCR by antigen induces membrane movement and aggregation of BCR components, ITAM phosphorylation, and consequently the recruitment of SYK to the BCR complex. Within their cytoplasmic tails, Igα and Igβ (Figure 1). In proximity of the BCR, SYK gets activated through ITAM binding, and SYK phosphorylation occurs through Src kinases and autophosphorylation. Active SYK, together with the Src kinase LYN, phosphorylate the adaptor proteins CD19, B-cell adaptor for phosphoinositide 3-kinase (BCAP), and B-cell linker protein (BLNK), which are detrimental to the proper activation of BCR downstream signaling. CD19 and BCAP each recruit phosphoinositide 3-kinase 3-kinase (PI3K) to the plasma membrane, where PI3K acts in concert with BLNK to activate Bruton’s tyrosine kinase (BTK) and its crucial downstream target phospholipase C (PLC). PLC ties BCR engagement to the activation of several signaling cascades. These include calcium mobilization, mitogen-associated protein (MAP) kinase and Ras activation, and activation of protein kinase Cβ and CARD11, causing recruitment of BCL10 and MALT1 into a multiprotein CBM complex that activates IkB kinase, thereby initiating nuclear factor κB (NFκB) signaling. Collectively, these signaling events promote B-cell survival and proliferation.

In contrast to the transient nature of antigen-induced BCR signaling, tonic BCR activation is characterized by low-level signals continuously emerging from the BCR complex independent of antigenic stimulation. The presence of tonic BCR signaling was first noted when inducible ablation of BCR expression in mature B cells resulted in the induction of apoptosis and their disappearance from the periphery. Subsequently, an elegant study by Srinivasan and colleagues revealed that BCR-deficient mature B cells are rescued by constitutive PI3K signaling but not by NFκB or ERK activation, thus suggesting a crucial role for PI3K and protein kinase B (AKT) in tonic BCR signaling and the BCR-dependent survival of mature B cells. However, the mechanisms leading to tonic BCR activation remain controversial, potentially involving the self-aggregation of BCR molecules, an altered balance of positive and negative regulators of BCR signaling, or the hijacking of the BCR complex molecules by other receptors, such as the B-cell activating factor of the tumor necrosis factor (TNF) family (BAFF) receptor. In light of the importance of the BCR in normal B cells, it is not surprising that BCR signaling is also involved in the pathogenesis of a variety of B-cell malignancies. Indeed, the majority of B-cell lymphomas retain BCR expression despite their genomic instability, and the presence of active BCR signaling (antigen-induced, tonic, or both) has been confirmed for several B-cell lymphomas/leukemias, including chronic lymphocytic leukemia (CLL), diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma (MCL), hairy cell leukemia (HCL), and Burkitt leukemia/lymphoma (BL).

**Chronic Lymphocytic Leukemia**

Several lines of evidence suggest the importance of BCR signaling in the pathophysiology of CLL. Markers associated with active BCR signaling, such as zeta chain–associated protein kinase 70kDa (ZAP-70) expression and increased expression of the T-cell–attracting chemokines CCL3 and CCL4, are strong predictors of CLL progression and time to treatment. Moreover, the structure of the BCR itself strongly influences progression of the disease. Based on the degree of somatic hypermutation within the BCR antigen–binding site, CLL patients can be classified as unmutated if they have 98% or more homology with the germline sequence, or mutated if they have less than 98% sequence homology. Mutated CLL is typically associated with slower disease progression and better overall survival (OS), whereas unmutated CLL progresses faster, resulting in a shorter time to treatment and shorter survival.

The nature of BCR engagement in CLL B cells remains controversial, with evidence for the presence of both tonic- and antigen-induced BCR activation. The importance of tonic BCR stimulation is supported by constitutive phosphorylation of BCR pathway components in primary CLL B cells, such as LYN, SYK, ERK, and subunits of NFκB. Furthermore, overexpression of MYC in murine B cells leads to a CLL-like disease in the absence of antigenic stimulation. A highly restricted IGHV gene repertoire, culminating in the presence of CLL BCRs with virtually identical (stereotyped) complementarity-determining region 3 (CDR3) sequences, argues for the necessity of antigenic stimulation during CLL pathogenesis. Consistently, BCRs from unmutated CLL exhibit polyreactivity against a variety of ubiquitous autoantigens, such as nonmuscle...
myosin heavy chain 2A and vimentin, as well as the Fc tail of immunoglobulin G, single-stranded DNA, double-stranded DNA, lipopolysaccharides, apoptotic cells, insulin, and oxidized low-density lipoproteins.\textsuperscript{43-48} Foreign antigens also have been implicated in the selection and expansion of CLL cells with certain BCR specificities. For instance, CLL BCRs harboring \textit{IGHV1-69} or \textit{IGHV3-21} interact with the cytomegalovirus-derived superantigen pUL32.\textsuperscript{49} Likewise, cases of mutated CLL with \textit{IGHV3-07} and short heavy-chain complementarity-determining region 3 (HCDR3) sequences (V3-7Sh) were shown to bind with high affinity to \(\beta(1,6)\)-glucan, a major antigenic determinant of yeast and filamentous fungi.\textsuperscript{50} A recent study demonstrated antigen-independent cell-autonomous activation of CLL BCRs, resulting in constitutive Ca\(^{++}\) signaling.\textsuperscript{51} Thus, CLL cells appear to depend

\textbf{Figure 1.} Key constituents of the B-cell receptor signaling pathway.

AKT, protein kinase B; BCAP, B-cell adaptor for phosphoinositide 3-kinase; BCR, B-cell receptor; BLNK, B-cell linker protein; BTK, Bruton’s tyrosine kinase; CBM, CARD11/BCL10/MALT1 complex; IgH, immunoglobulin heavy chain; IgL, immunoglobulin light chain; LYN, Lck/Yes-related novel protein tyrosine kinase; NF\(\kappa\)B, nuclear factor \(\kappa\)B; PI3K, phosphoinositide 3-kinase; PKC\(\beta\), protein kinase C\(\beta\); PLC\(\gamma\)2, phospholipase C\(\gamma\)2; SYK, spleen tyrosine kinase.
on continuous and intermittent BCR signaling that drives cell survival and expansion.

**Diffuse Large B-Cell Lymphoma**

Similar to CLL, the *IGHV* gene repertoire in DLBCL is highly biased, with 3 *IGHV* gene segments (*IGHV4-34, IGHV3-23, and IGHV4-39*) accounting for one-third of DLBCL BCRs.52-55

Gene expression profiling studies revealed the existence of 2 subtypes of DLBCL, activated B-cell–like (ABC) and germinal center B-cell–like (GCB) DLBCL, based on their resemblance to either activated or germinal center B cells.56,57 It was initially noted that ABC-type DLBCL cells are dependent on the BCR downstream target NFκB.58 Retroviral suppression of NFκB signaling, as well as short hairpin RNA-mediated knockdown of the NFκB pathway components IKKβ, CARD11, MALT1, and BCL10, induce cell death selectively in ABC-type DLBCL.59 In 10% of ABC DLBCL cases, constitutive NFκB activation is caused by mutations in the coiled-coil domain of CARD11.60 In the remaining cases, NFκB activation results from chronic active BCR signaling, which is characterized by surface BCR clustering and resembles antigen-induced BCR activation.14 Consistently, ABC DLBCL cells harboring wild-type *CARD11* were sensitive to knockdown of the BCR pathway components CD79A/B, IgM, Igκ, SYK, and BTK.14

In GCB DLBCL, direct evidence for the involvement of BCR signaling in disease pathogenesis is missing. However, Chen and colleagues recently reported the dependency of certain GCB DLBCL cell lines on SYK, suggesting a role for the BCR in subgroups of GCB DLBCL.61,62 In contrast to ABC DLBCL, GCB DLBCL cells do not depend on constitutive NFκB signaling, indicating that BCR signaling in GCB DLBCL mimics tonic rather than antigen-induced BCR activation. Consistently, expression of a constitutively active form of AKT successfully rescues SYK-deficient GCB DLBCL cells.62

Recurrent somatic mutations in essential components of the BCR signaling cascade are another sign of the important role of BCR signaling in DLBCL. In addition to carrying mutations in the *CARD11* gene, about 21% of ABC DLBCL patients carry mutations in either *CD79A* (2.9%) or *CD79B* (18%).14,63 The majority of *CD79A* mutations cause deletions of large parts of the ITAM region, including the second ITAM tyrosine residue.14 *CD79B* mutations are almost exclusively missense mutations resulting in the replacement of the first ITAM tyrosine by a variety of other amino acids.14 Functionally, *CD79A/B* mutations were shown to increase BCR surface expression levels and to attenuate the autoinhibitory function of LYN, all likely leading to enhanced BCR pathway activation.14

Supporting the presence of tonic BCR signaling in GCB DLBCL, Pfeifer and colleagues recently reported the loss of tumor suppressor phosphatase and tensin homolog (PTEN) expression in 55% of GCB DLBCL cases.64 This was in part owing to genomic alterations, including deletions and mutations in the *PTEN* gene. PTEN-deficient GCB DLBCL cells are dependent on PI3K signaling and MYC, and are selectively sensitive to the PI3K inhibitor LY294002.64

**Mantle Cell Lymphoma**

In keeping with the requirement for antigenic stimulation during MCL pathogenesis, MCL BCRs are characterized by the use of a biased *IGHV* gene repertoire. Stereotyped HCDR3 regions are present in 10.4% of MCL cases.65 Based on somatic hypermutation status, MCL can be further subdivided into mutated and unmutated MCL, with 1 study reporting a more favorable outcome for mutated MCL cases (5-year OS: 59% for mutated MCL vs 40% for unmutated MCL).66 Further suggesting a crucial role for the BCR in subgroups of MCL, gene expression profiling and phosphoproteomic analysis revealed the presence of active BCR signaling in several MCL cell lines.67,68

Despite the strong preclinical evidence for the involvement of antigen and BCR signaling in MCL pathogenesis, the significance of the BCR pathway as a therapeutic target was fully appreciated after clinical trials with the BCR inhibitor ibrutinib (Imbruvica, Pharmacyclics/Janssen), resulting in clinical responses in a majority of MCL patients.69 Subsequently, Rahal and colleagues unraveled the importance of canonical NFκB signaling for the BTK-dependent survival of MCL cell lines, thus providing a biologic rationale for the clinical success of BCR pathway inhibition in MCL.25 It remains to be determined whether this is a consequence of chronic active BCR signaling, as seen in ABC-type DLBCL, or whether MCL cells utilize other means of NFκB activation.

**Hairy Cell Leukemia**

The most solid evidence for the involvement of the BCR and BCR signaling in HCL pathogenesis arises from structural analyses of HCL BCR molecules. These reveal a biased *IGH* and *IGL* variable gene segment repertoire, as well as the existence of mutated and unmutated HCL cases.70,71 Unmutated HCL and the usage of the *IGHV4-34* gene segment are considered poor prognostic markers.72,73 Suggesting an involvement of BCR signaling in HCL pathogenesis, Weston-Bell and colleagues reported that BCRs of HCL cells respond to antibody-mediated cross-linking with an increase in cellular calcium levels, ERK phosphorylation, and apoptosis.74 On the contrary, we recently reported on the ability of BCR cross-linking to protect primary HCL...
cells from undergoing spontaneous apoptosis in vitro. Importantly, pretreatment with the BTK inhibitor ibrutinib completely abrogated these effects, suggesting therapeutic relevance of the BCR pathway in HCL.

**Burkitt Leukemia/Lymphoma**

BL joined the list of BCR-dependent B-cell malignancies recently, after a genomic screen of sporadic, endemic, and HIV-associated BL cases identified mutations in the transcription factor gene $TCF3$ and its negative regulator gene $ID3$ in more than 70% of cases. Extensive functional analysis revealed that both types of mutations entail the activation of TCF3 transcriptional activity, ultimately affecting the expression of several BCR pathway components, including upregulation of IgH and IgL and the suppression of SHP-1, a key negative regulator of BCR signaling. Consequently, mutations in $TCF3$ and $ID3$ were shown to enhance tonic BCR signaling, which, in cooperation with the deregulation of MYC, fuels proliferation and survival of BL cells and serves as a potential target for therapeutic intervention.

**BCR Signaling Inhibitors in B-Cell Malignancies**

The critical role of the BCR pathway in the pathogenesis of autoimmune disorders and several B-cell malignancies led to the development of kinase inhibitors that target BCR signaling. All currently available BCR inhibitors belong to the group of small-molecule tyrosine kinase inhibitors, and several of these are being evaluated in clinical trials for B-cell malignancies.
inhibitors, and can be classified according to their target specificity in BTK, PI3K, and SYK inhibitors (Figure 2). In the following sections, we discuss their mechanism of action and their activity, with a focus on the clinically most advanced agents (see Table).

**Bruton’s Tyrosine Kinase**

BTK belongs to the TEC family of nonreceptor tyrosine kinases, and constitutes an essential part of the BCR signaling cascade. BTK is primarily expressed in hematopoietic cells, particularly in B cells and not in T cells or plasma cell lines. Its inhibition by ibrutinib has shown promising results in the treatment of B-cell malignancies, with a particularly high activity in chronic lymphocytic leukemia (CLL) and mantle cell lymphoma (MCL).

### Table. Summary of Published BCR Inhibitor Studies in Various B-Cell Malignancies

<table>
<thead>
<tr>
<th>Target</th>
<th>Reference</th>
<th>Drugs</th>
<th>Patients (n)</th>
<th>Disease Characteristics</th>
<th>ORR, %</th>
<th>CRR, %</th>
<th>Median PFS, mo</th>
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<tbody>
<tr>
<td>BTK</td>
<td>Advani</td>
<td>ibrutinib</td>
<td>56</td>
<td>R/R CLL and B-NHL</td>
<td>60</td>
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<td>13.6</td>
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<td></td>
<td>Wang</td>
<td>ibrutinib</td>
<td>111</td>
<td>R/R MCL</td>
<td>68</td>
<td>21</td>
<td>13.9</td>
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<td>Byrd</td>
<td>ibrutinib</td>
<td>86</td>
<td>R/R CLL and SLL</td>
<td>71</td>
<td>1.7</td>
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<td></td>
<td>O’Brien</td>
<td>ibrutinib</td>
<td>29</td>
<td>Untreated CLL and SLL, age ≥65 y</td>
<td>71</td>
<td>13</td>
<td>NR</td>
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<tr>
<td></td>
<td>Byrd</td>
<td>ibrutinib vs ofatumumab</td>
<td>391</td>
<td>R/R CLL and SLL</td>
<td>42.6 vs 4.1</td>
<td>2 vs 1</td>
<td>NR vs 8.1</td>
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<td>Younes</td>
<td>ibrutinib + R-CHOP</td>
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<td>CD20+ B-NHL</td>
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<td>Burger</td>
<td>ibrutinib + rituximab</td>
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<td>HR CLL</td>
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<td>8</td>
<td>NR</td>
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<td>Maddocks</td>
<td>ibrutinib + BR</td>
<td>48</td>
<td>Untreated and R/R B-NHL</td>
<td>72</td>
<td>52</td>
<td>NR</td>
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<td>Farooqui</td>
<td>ibrutinib</td>
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<td>CLL with TP53 aberrations</td>
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<td>0</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td>Brown</td>
<td>ibrutinib + BR or ibrutinib + FCR</td>
<td>33</td>
<td>(30 ibrutinib + BR; 3 ibrutinib + FCR)</td>
<td>93.3 (ibrutinib + BR)</td>
<td>16.7 (ibrutinib + BR)</td>
<td>100 (ibrutinib + FCR)</td>
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<td></td>
<td>Wilson</td>
<td>ibrutinib</td>
<td>80</td>
<td>R/R DLBCL</td>
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<td>10</td>
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<td></td>
<td>Burger</td>
<td>ibrutinib vs chlorambucil</td>
<td>269</td>
<td>Untreated CLL and SLL, age ≥65 y</td>
<td>86 vs 35</td>
<td>4 vs 2</td>
<td>NR vs 18.9</td>
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<tr>
<td>PI3Kδ</td>
<td>Furman</td>
<td>Idelalisib + rituximab vs rituximab</td>
<td>220</td>
<td>Relapsed CLL</td>
<td>81 vs 13</td>
<td>0 vs 0</td>
<td>NR vs 5.5</td>
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<td>Idelalisib</td>
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<td>Idelalisib</td>
<td>40</td>
<td>R/R MCL</td>
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<td>Fostamatinib disodium</td>
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<td>Recurrent B-NHL</td>
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<td>Sharman</td>
<td>GS-9973</td>
<td>41</td>
<td>R/R CLL</td>
<td>61</td>
<td>0</td>
<td>13.8</td>
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B-NHL, B-cell non-Hodgkin lymphoma; BR, bendamustine and rituximab; BTK, Bruton’s tyrosine kinase; CLL, chronic lymphocytic leukemia; CRR, complete remission rate; DLBCL, diffuse large B-cell lymphoma; FCR, fludarabine, cyclophosphamide, and rituximab; HR, high-risk; MCL, mantle cell lymphoma; mo, months; NR, not reached; ORR, overall response rate; PFS, progression-free survival; PI3Kδ, phosphoinositide 3-kinase δ; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone; R/R, relapsed/refractory; SLL, small lymphocytic lymphoma; SYK, spleen tyrosine kinase; y, years.
cells. Upon BCR activation, increasing levels of the PI3K product phosphatidylinositol (3,4,5)-triphosphate recruit BTK to the plasma membrane via its pleckstrin homology domain. LYN and SYK subsequently activate BTK in concert through phosphorylation, ultimately resulting in activation of the transcription factor NFkB, B-cell proliferation, and B-cell survival. In addition to its involvement in BCR signaling, BTK has been shown to transduce signals from chemokine (CXCR4 and CXCR5) and integrin receptors, thus making it also a crucial component in the regulation of B-cell migration and tissue homing.

Ibrutinib, formerly called PCI-32765, is the most clinically advanced BTK inhibitor. Ibrutinib blocks the enzymatic activity of BTK through covalent binding to a conserved cysteine residue (Cys-481) in the active site of BTK. In samples from patients with CLL, ibrutinib has been shown to block BCR-derived survival signals and downregulate the secretion of the BCR-dependent chemokines CCL3 and CCL4 in vitro, as well as in CLL patients receiving ibrutinib. Ibrutinib also antagonizes the effects of the prosurvival factors CD40 ligand, BAFF, and interleukin 6 on CLL cells. Apart from BCR signaling, ibrutinib has been shown to interfere with integrin-mediated CLL cell adhesion and with the migration of CLL cells towards the tissue homing factors CXCL12 and CXCL13. Alongside the compelling in vitro data, ibrutinib also effectively thwarted disease progression in the TCL1 mouse model of CLL.

In DLBCL, ibrutinib blocks BTK-dependent NFkB activation, resulting in the selective killing of ABC-type DLBCL cells. These effects were augmented by the addition of lenalidomide (Revlimid, Celgene), resulting in enhanced cytotoxicity and superior effects in xenograft models of ABC-type DLBCL. In subsets of MCL, ibrutinib was shown to exert its effects in a similar fashion, depriving MCL cells from crucial NFkB survival signals and inducing apoptosis.

In clinical trials, ibrutinib exhibited activity in CLL, MCL, Waldenström macroglobulinemia, and ABC DLBCL patients. In CLL, Byrd and colleagues reported that single-agent ibrutinib induced an overall response rate (ORR) of 71% in relapsed or refractory patients. An additional 15% to 20% of patients had a partial response with lymphocytosis. The response was independent of clinical and genomic risk factors present before treatment. At 26 months, the estimated progression-free survival (PFS) rate was 75% and the OS rate was 83%. O'Brien and colleagues assessed the safety and efficacy of single-agent ibrutinib in treatment-naive patients aged 65 years and older. ibrutinib was well tolerated, with the most common side effects being mild to moderate diarrhea, nausea, fatigue, and hypertension. The objective response rate in this cohort of patients was 71%, with an additional 13% achieving a partial response with lymphocytosis. In a phase 3 clinical trial of ibrutinib vs the anti-CD20 antibody ofatumumab (Arzerra, GlaxoSmithKline) in previously treated CLL, the ORR was 42.6% in the ibrutinib group and 4.1% in the ofatumumab group. At a median follow-up of 9.4 months, ibrutinib also significantly improved PFS and OS. The results of these studies led to FDA approval of ibrutinib for the treatment of CLL patients who received at least 1 prior therapy and for all CLL patients with the 17p deletion. In a recent study, we investigated the activity and safety of the combination of ibrutinib with the anti-CD20 antibody rituximab (Rituxan, Genentech/Biogen Idec) in high-risk CLL. All participants had high-risk cytogenetic abnormalities (deletion 17p, deletion 11q, or TP53 mutations) or a PFS of less than 36 months after first-line chemoimmunotherapy. Despite these high-risk features, the 18-month PFS and OS of ibrutinib plus rituximab were 78% and 84%, respectively.

Further evaluating the activity of ibrutinib in high-risk CLL, Farooqui and colleagues recently reported a 92% objective response rate (50% partial response rate; 42% rate of partial response with lymphocytosis) in previously untreated and relapsed/refractory patients with CLL who had TP53 aberrations. In a 3-year follow-up study, single-agent ibrutinib was well tolerated, and prolonged ibrutinib therapy was associated with durable remissions that improved in quality over time. Disease progression was rare and mainly occurred in subgroups of patients characterized by extensive prior therapy and high-risk cytogenetic features (deletion 17p and deletion 11q).

In addition to showing benefits in patients with CLL, the initial clinical evaluation of ibrutinib also suggested that MCL patients may benefit from ibrutinib therapy. In a subsequent phase 2 study, Wang and colleagues reported on single-agent efficacy of ibrutinib in patients with relapsed or refractory MCL, with a complete response rate of 21% and a partial response rate of 47%. The estimated median duration of response was 17.5 months, the estimated median PFS was 13.9 months, and the estimated OS rate was 58% at 18 months. The FDA consequently granted accelerated approval to ibrutinib for the treatment of MCL patients who have received at least 1 prior therapy.

Of note, in CLL and MCL patients, ibrutinib induces the rapid egress of tumor B cells from lymphatic tissues into the peripheral blood. Clinically, this is associated with a rapid reduction in lymph node size and peripheral blood lymphocytosis. The extent of lymphocytosis is variable among patients, is asymptomatic, and usually resolves during the first months of therapy. In a recent study by Woyach and colleagues, prolonged lymphocytosis (>12 months) in CLL patients receiving ibrutinib did not predict an inferior
outcome. Similar effects on lymph node size and peripheral blood lymphocyte counts have been observed with PI3K and SYK inhibitors, suggesting that this is a BCR inhibitor–specific phenomenon, most likely due to their effects on cell migration and tissue homing.

Wilson and colleagues recently reported the results of a phase 1/2 clinical trial of single-agent ibrutinib in relapsed/refractory DLBCL patients. ibrutinib therapy resulted in complete or partial responses in 37% of ABC-type but only in 5% of GCB-type DLBCL, corroborating the preclinical data of ibrutinib in DLBCL. Patients responded frequently in ABC-type cases with BCR mutations (5/9), as did patients with combined BCR and MYD88 mutations (4/5). CARD11 mutations were associated with ibrutinib resistance.

**Phosphoinositide 3-Kinase δ**

PI3Ks are key regulators of proliferation and survival in various cell types. Based on sequence homology and substrate specificity, PI3Ks can be divided into 3 classes: I, II, and III. Class I PI3Ks, which are particularly involved in cancer, comprise PI3Kα, PI3Kβ, PI3Kγ, and PI3Kδ. The PI3Kα and PI3Kβ isoforms are ubiquitously expressed, whereas the PI3Kγ and PI3Kδ isoforms are limited to hematopoietic cells. PI3Kδ has a role in T-cell activation, and PI3Kδ plays a critical role in B-cell homeostasis and function. Lacking a functional PI3Kδ isoform are characterized by a highly dysfunctional B-cell compartment resulting in reduced numbers of B1 and marginal zone B cells, low levels of immunoglobulins, poor responses to immunization, and defective BCR and CD40 signaling. In B-cell malignancies, an array of cell surface receptors have been shown to activate PI3Ks, including the BCR and CXCR4.

Idelalisib (Zydelig, Gilead), previously called CAL-101 or GS-1101, is a highly selective PI3Kδ inhibitor with preclinical activity in a variety of B-cell malignancies. Idelalisib was shown to effectively block constitutive and BCR-induced PI3K activation in several B-cell lymphomas, including CLL and MCL. In addition to its effect on the BCR signaling cascade, idelalisib was also shown to counteract the effects of BAFF, TNF-α, and fibroblast stimulation on primary CLL cells.

Based on the encouraging preclinical data, Gopal and colleagues conducted a single-group, open-label phase 2 study of idelalisib monotherapy in relapsed/refractory indolent non-Hodgkin lymphomas. The study included patients with follicular lymphoma (72 patients), small lymphocytic lymphoma (28 patients), marginal-zone lymphoma (15 patients), and lymphoplasmacytic lymphoma with or without Waldenström macroglobulinemia (10 patients). The response rate was 57%, with similar response rates across all subtypes. The most common grade 3 or higher adverse events were neutropenia (27%), elevations in aminotransferase levels (13%), diarrhea (13%), and pneumonia (7%). The results of this study led to the accelerated approval of idelalisib by the FDA for the treatment of patients with relapsed follicular B-cell non-Hodgkin lymphoma or relapsed small lymphocytic lymphoma. Furman and colleagues evaluated the efficacy of idelalisib in combination with rituximab vs rituximab plus placebo in relapsed CLL. This multicenter phase 3 trial enrolled 220 patients who were randomly assigned to receive rituximab and either 150 mg of idelalisib or placebo twice daily. Owing to the superior efficacy of idelalisib, the study was terminated early at the first interim analysis. ORRs for patients receiving idelalisib vs placebo were 81% and 13%, respectively. OS at 12 months was 92% for rituximab plus idelalisib and 81% for rituximab plus placebo. Serious adverse events occurred in 40% of the patients receiving idelalisib and rituximab and in 35% of those receiving placebo and rituximab. The FDA consequently granted approval of idelalisib for the treatment of patients with relapsed CLL.

**Spleen Tyrosine Kinase**

SYK is a nonreceptor tyrosine kinase that belongs to the family of SYK/ZAP-70 protein tyrosine kinases. SYK is predominantly expressed in hematopoietic cells and is crucial for the function of an array of cell surface receptors, including the BCR, integrin receptors, Fc receptors, and pattern recognition receptors of the innate immune system. Homozygous inactivation of SYK in mice is consistently associated with perinatal lethality, and selective deletion of SYK in the hematopoietic system severely impairs B-cell differentiation, with a block at the pro-B cell to pre-B cell transition. Moreover, in vivo studies recently demonstrated that SYK is critical for survival and maintenance of mature normal and malignant B cells.

The SYK inhibitor fostamatinib disodium (R788, FosD) was the first BCR inhibitor under clinical evaluation for the treatment of BCR-dependent malignancies. The initial phase 1/2 study of fostamatinib in recurrent non-Hodgkin lymphoma patients reported an objective response rate of 22% for all cases, and 55% for CLL patients in particular. In vitro, fostamatinib kills DLBCL and CLL cells. Fostamatinib also blocks migration towards the tissue-homing chemokines CXCL12 and CXCL13 in CLL cells. Despite these encouraging results, further development of this drug has focused on rheumatoid arthritis, and most recently has focused on idiopathic thrombocytopenic purpura. Alternative SYK-specific inhibitors are under development and have demonstrated promising preclinical and clinical activity. Sharan and colleagues recently reported results of a phase 2 clinical trial of the SYK-specific inhibitor entospletinib
Lymphoma models, is currently being evaluated in a trial with promising in vitro activity in DLBCL and Burkitt lymphoma models, is currently being evaluated in a phase 1 dose-escalation study in CLL and non-Hodgkin lymphoma patients.

Concluding Remarks

BCR inhibitors are currently transforming the therapeutic landscape in several B-cell malignancies. The induction of durable remissions, irrespective of established risk factors, alongside the favorable toxicity profile—especially a lack of myelotoxicity—has led to a wide use of BCR inhibitors in patients with CLL, MCL, and follicular lymphoma. Response rates and durability are particularly high in patients with CLL, whereas response rates and/or duration appear to be lower in other B-cell malignancies. The clinical success of these novel agents has fueled a series of translational studies to study the consequences of BCR inhibition in humans. As with any major discovery, these new concepts are challenging us with new questions. For instance, the available data on the efficacy of BCR inhibitors in B-cell malignancies are mostly derived from single-agent studies in high-risk and heavily pretreated patient populations, and do not take into account potential synergisms between BCR inhibitors and established therapeutic agents. However, with the FDA approval of ibrutinib and idelalisib, follow-up clinical trials are now testing the benefit of combination regimens to better define the optimal use of BCR signaling inhibitors in patients with B-cell malignancies.

Acknowledgements

This work was supported in part by a Leukemia & Lymphoma Society Scholar in Clinical Research Award to Dr. Burger, by the MD Anderson Cancer Center Moon Shots Program, and by a Cancer Center Support Grant (CA016672) from the National Cancer Institute.

Disclosures

Dr. Burger has received research funding from Pharmacycides and Gilead. Dr. Koehrer has declared no competing financial interests.

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