Targeting B-Cell Receptor Signaling – Changing the treatment landscape of B-Cell Lymphoma

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Abstract:
Introduced in 1998, the anti-CD20 monoclonal antibody rituximab, with its unique mechanism of action, was the first agent to improve survival in patients with B-cell lymphoma (BCL) treated with chemotherapy. Laboratory investigation of the B-cell receptor signaling pathway identified the critical nature of this pathway for normal B-cell development, survival and proliferation. Further investigation showed that lymphoma cell lines were also dependent upon this pathway and hence small molecule inhibitors of critical proteins in the pathway were synthesized and shown to be cytotoxic. Subsequent translation to the clinic has shown impressive activity in some types of B-cell lymphoma. The aim of this article is to provide an overview of the constituents of the BCR signaling pathway, to illustrate how addiction to this pathway is critical for survival of some BCL, and to summarize the clinical experience with novel small molecule inhibitors of specific proteins in the BCR pathway. We speculate that combination of these agents with newer drugs, each with a unique mechanism of action might lead to improved therapy and the eventual elimination of standard chemotherapy from our therapeutic arsenal.

Keywords: B-cell lymphoma, B-cell receptor, Small molecule inhibitors

In the decade that began 1980, several phase 2 clinical trials suggested superiority of more intensive chemotherapy regimens over the standard CHOP (Cyclophosphamide/Adriamycin/Vincristine/Prednisone) regimen for the treatment of B-Cell Lymphoma (BCL)¹. However, when tested in a randomized phase 3 controlled clinical trial, Fisher and colleagues demonstrated that CHOP provided equivalent outcome to the more intensive regimens with less toxicity². The outcome with CHOP chemotherapy was improved with the addition of rituximab to the CHOP regimen³. This improved outcome without significantly increased toxicity changed the treatment landscape of BCL and signaled the beginning of targeted therapy in oncology⁴. Identification of CD20, a glycosylated phosphoprotein expressed on most B lymphocytes, and demonstration of its suitability as a target for monoclonal antibody therapy led to the development of rituximab. Rituximab, a chimeric monoclonal antibody directed against CD20 is believed to exert its cytotoxic effect by triggering apoptosis, mediating antibody dependent cellular cytotoxicity (ADCC) and initiating complement dependent cytotoxicity (CDC)⁵. Although, the addition of rituximab or other CD20 directed monoclonal antibodies to conventional combination chemotherapeutic agents has significantly improved progression free and overall survival, it is not uncommon for patients with BCL to develop toxicity related to cytotoxic chemotherapy. The appearance of resistance to therapy leading to relapse of lymphoma or transformation to aggressive type leaves patients with limited curative treatment options and frequently results in their demise⁶.

Recent understanding of the B-Cell receptor and its signaling mechanism is rapidly changing the treatment landscape of BCL. The focus of this article is to
briefly discuss BCR signaling and present an overview of some of the novel drugs targeting BCR signaling pathways.

**Molecular Biology of the BCR**

B cell development requires successful rearrangement and expression of the immunoglobulin heavy (IgH) and light (IgL) chain genes which together form the BCR on the cell surface\(^7\). BCR engagement signals downstream pathways and promotes development, expansion and survival of normal B cells\(^8,9\) (Figure 1). These same pathways are used by malignant B cells to drive proliferation, growth and survival\(^10\). Engagement and activation of the BCR occurs by diverse mediators and ranges from autologous stimulation from self-origin antigens, microbial derived antigens and constitutive signaling occurring as a result of somatic mutation of genes with immunoreceptor tyrosine-based activation motif (ITAM) in the signaling modules of CD79A and CD79B\(^9\). The activated BCR leads to a cascade of well described phosphorylation events involving multiple kinases such as the Src family kinases (SFK, Lyn), spleen tyrosine kinase (SYK), Bruton Tyrosine Kinase (BTK) and Phosphoinositide 3-kinase (PI3K). These proximal tyrosine kinase pathways in turn activate distal signaling pathways involving such molecules as NFkB, MAPK, AKT/mTOR that drive proliferation, growth and survival\(^11\). Understanding the components of these pathways as prospective targets for inhibition by small molecule inhibitors (SMI) has resulted in discovery of novel targeted agents.

**Bruton Tyrosine Kinase (BTK) Pathway Inhibitors**

BTK is a cytoplasmic tyrosine kinase essential for BCR signaling. BTK is expressed in all B cells from early precursor to mature forms (except plasma cells) and is essential for B cell survival. Following active BCR signaling, BTK is phosphorylated by SYK and in-turn leads to activation of downstream pathways such as protein kinase C beta, CARD11 and NF-kB\(^12\). Ibrutinib is an orally bioavailable selective kinase inhibitor that covalently binds to the Cys-481 residue of BTK\(^13\). In early clinical trials, Ibrutinib demonstrated clinical antitumor activity, near-complete target binding based on fluorescent studies with a mild to moderate toxicity profile. There was no reported cumulative hematological or non-hematological toxicity on prolonged dosing. Responses were seen in multiple histological variants of BCL. These results prompted phase 2 studies of Ibrutinib in patients with relapsed/refractory indolent and aggressive BCL\(^14,15\). Given higher response rates in Mantle Cell Lymphoma (MCL) (ORR 68%) and Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL) (ORR 71%) when compared to other BCL subtypes, Ibrutinib was approved for the treatment of relapsed/refractory MCL and CLL/SLL\(^14,15\). Ibrutinib has also received approval as front line therapy for patients with CLL who harbor the 17p deletion following demonstration of an ORR of 68% in these patients. The presence of the 17p- makes cells resistant to cytotoxic chemotherapeutic agents\(^15\). When tested in relapsed diffuse large B cell lymphoma (DLBCL) higher responses were seen in the activated B-cell (ABC) subtype at 40% compared to the germinal center B cell (GCB) subtype at 5%\(^16\). This was as expected due to the frequent presence of a BCR activating mutation leading to constitutive BCR signaling seen in the ABC subtype of DLBCL but not in the GCB subtype\(^17\). Identification of the MYD88 mutation and its involvement in aberrant BCR signaling led to testing of ibrutinib in patients with relapsed refractory Waldenstrom’s macroglobulinemia who frequently harbor the MYD88 mutation\(^18\).
In a phase 2 multicenter study involving 63 patients with relapsed refractory Waldenstrom’s Macroglobulinemia harboring the MYD88 mutation, treatment with ibrutinib resulted in rapid reduction in serum IgM levels and improvement in hematological parameters. These responses were durable with 87% of patients continuing on treatment at a median of 9 months\textsuperscript{19}. With the demonstration of efficacy, durable response and a mild toxicity profile in the relapsed refractory setting, investigators have designed clinical trials with ibrutinib either as a single agent or in combination with other therapeutic agents as frontline therapy for the treatment of BCL. (See Table I).

At the time of writing Ibrutinib is the only commercially available drug, but there are other BTK inhibitors in various stages of development. Examples include CC292 (Onyx Pharmaceuticals) and ONO-WG-307 (ONO Pharma UK), where early studies have demonstrated significant BTK
Table I: BTK Inhibitor combination studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Phase</th>
<th>N</th>
<th>Histology</th>
<th>ORR</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ibrutinib+Rituximab</td>
<td>2</td>
<td>50</td>
<td>MCL</td>
<td>85%</td>
<td>Wang et al 45</td>
</tr>
<tr>
<td>Ibrutinib + R-CHOP</td>
<td>3</td>
<td>TBD</td>
<td>Non GCB-DLBCL</td>
<td>Ongoing</td>
<td>NCT01855750</td>
</tr>
<tr>
<td>Ibrutinib+ R-CHOP</td>
<td>1b</td>
<td>33</td>
<td>Aggressive NHL</td>
<td>100%</td>
<td>Younes et al 43</td>
</tr>
<tr>
<td>Ibrutinib+ Benadmustine+Rituximab (BR)</td>
<td>1</td>
<td>11</td>
<td>NHL</td>
<td>38%</td>
<td>Blum et al. 39</td>
</tr>
<tr>
<td>Ibrutinib + BR and Ibrutinib + R CHOP</td>
<td>1 / 2</td>
<td>30</td>
<td>CLL</td>
<td>90%</td>
<td>O’Brien et al. 36</td>
</tr>
<tr>
<td>Ibrutinib+ Rituximab</td>
<td>3</td>
<td>TBD</td>
<td>iNHL</td>
<td>Ongoing</td>
<td>NCT01974440</td>
</tr>
<tr>
<td>Ibrutinib+Rituximab and Ibrutinib + R CHOP</td>
<td></td>
<td></td>
<td>WM</td>
<td>Ongoing</td>
<td>NCT02165397</td>
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Phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) Inhibitors

The PI3k/AKT pathway is well described and is part of proximal intracellular signal transduction pathways affecting many intracellular biological functions critical for metabolism, growth and proliferation. Although existence of multiple isoform has been described, p110 delta and p110 gamma isoforms are predominantly expressed in cells of hematopoietic origin and hence represent a potential target for therapy in hematological malignancy. Aberrant activation of this pathway has been implicated in initiation and maintenance of tumors. Inhibition of this pathway has demonstrated antitumor activity via suppression of cell signaling and angiogenesis. Currently, Idelalisib an orally administered selective inhibitor of PI3K delta isoform, is the only approved drug commercially available for treatment of relapsed refractory CLL and Follicular Lymphoma (FL). In a phase 1 trial involving 54 heavily pretreated relapsed/refractory CLL patients, Idelalisib as a single agent had substantial activity with a 72% overall response rate (54% in patients with 17p deletion) with a median time to response of 1 month. As a continuous oral therapy, the drug was well tolerated and had a durable response with treatment extending > 3 years in some patients. In a phase 2 open label trial of 125 patients with history of relapsed/refractory indolent BCL (Follicular, Marginal zone, lymphoplasmacytic and small lymphocytic
lymphoma) Idelalisib had similar activity as in CLL with ORR of 57% and an acceptable safety profile\textsuperscript{28}. Based on these findings there are many clinical trials in various phases exploring the potential of PI3K inhibitors in the treatment of relapsed BCL (Table II).

Duvelisib (IPI-145) is another novel PI3K inhibitor which inhibits both delta and gamma isoform of PI3K. In a phase 1 trial involving 32 patients with indolent BCL, duvelisib demonstrated an ORR of 65% (with 25% CR) and was well tolerated\textsuperscript{29}.

**Spleen Tyrosine Kinase (SYK) Inhibitor**

SYK is another proximal kinase critically involved in the BCR pathway promoting cell proliferation and survival. Inhibition of SYK has been shown to abrogate BCR signaling and induce apoptosis\textsuperscript{30}. Overexpression of SYK demonstrated in various BCL’s is believed to be one of the underlying aberrations responsible for malignant transformation of B lymphocytes and provided additional rationale for targeting SYK\textsuperscript{31}. Fostamatinib competitively inhibits SYK in-vitro and down regulates BCR signaling. In a phase 1/2 clinical trial, oral Fostamatinib has shown both safety and efficacy in a heavily pretreated relapsed refractory BCL cohort of 68 patients. Overall rates of response seen in CLL/SLL were 55%, DLBCL 22%, FL 10% and MCL 11%. Toxicity was mild and included diarrhea, fatigue and cytopenia\textsuperscript{32}.

**The Phenomenon of “Redistribution Lymphocytosis”**

Patients treated with Ibrutinib (BTK inhibitor), PI3K and SYK inhibitors often show rapid reduction of lymphadenopathy that is accompanied by peripheral lymphocytosis. This phenomenon known as “redistribution lymphocytosis” represents a compartmental shift of malignant lymphocytes from the bone marrow and lymph nodes into the peripheral circulation\textsuperscript{15, 33}. In most patients, initial brisk lymphocytosis is transient and is followed by a return to pre-treatment baseline values usually within few months (< 8 months).

<table>
<thead>
<tr>
<th>Study</th>
<th>Phase</th>
<th>N</th>
<th>Histology</th>
<th>ORR</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Idelalisib + rituximab</td>
<td>1/2</td>
<td>30</td>
<td>Indolent NHL (iNHL)</td>
<td>77%</td>
<td>Fowler et al.\textsuperscript{44}</td>
</tr>
<tr>
<td>Idelalisib + rituximab</td>
<td>3</td>
<td>390</td>
<td>CLL</td>
<td>Ongoing</td>
<td>NCT011539512</td>
</tr>
<tr>
<td>Idelalisib + bendamustine +/- rituximab</td>
<td>1/2</td>
<td>46</td>
<td>iNHL</td>
<td>77% - 85%</td>
<td>Fowler et al.\textsuperscript{44}</td>
</tr>
<tr>
<td>Duvelisib</td>
<td>2</td>
<td>TBD</td>
<td>iNHL</td>
<td>Ongoing</td>
<td>NCT01882803</td>
</tr>
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</table>
However in certain patients lymphocytosis may be persistent lasting > 12 months. It is important to recognize this phenomenon and not interpret it as progressive disease or treatment failure. Interestingly extended follow-up studies have actually suggested a favorable prognosis in patients exhibiting persistent lymphocytosis.

**Future trends**

The elucidation of the components of the BCR signaling pathway has allowed the creation of small molecule inhibitors that target specific constituents of this critical pathway. Despite the demonstration of efficacy by multiple agents targeting the BCR pathway, susceptibility to these agents is not uniform across all BCL histological subtypes. As evidenced by the differential response to ibrutinib in DLBCL of ABC type versus the GBC type, there is much yet to learn about these signaling pathways. The molecular basis of this difference offers the possibility of identifying new targets for drug development. The long term toxicity profile, mechanisms of resistance and degree of immunosuppression are largely unknown. The potential mechanisms of resistance include the acquisition of mutations in CARD11, BTK binding residue mutations and activation of alternate pathways that might bypass BCR signaling. Thus early identification by molecular testing of resistance to BCR inhibitors would avoid prescription of ineffective therapy. However, given the advantage of oral availability, mild toxicity profile and unique mechanism of action of these drugs, they are particularly attractive for combination with other anticancer agents. The ideal combinations would be synergistic leading to reduced toxicity and a higher complete response rate ultimately improving overall survival. There are preclinical data targeting multiple kinases within BCR pathway that suggests augmented antitumor activity.

Several studies are underway exploring these options. Several small studies involving the combination of Idelalisib and Ibrutinib plus bendamustine with or without rituximab have shown efficacy and safety in the treatment of relapsed/refractory indolent BCL. In addition to drugs targeting BCR signaling there are other novel agents being developed or integrated into the treatment of BCL. Agents such as ABT-199 targeting BCL2, Nivolumab an anti-PD1 receptor antibody, and SGN CD19A an anti-CD19 antibody drug conjugate are actively being studied either as single agents or in combination with existing drugs for treatment of BCL. These novel agents are particularly attractive to combine with BCR pathway inhibitors due to their different mechanisms of action and offer the possibility of eliminating traditional chemotherapy and its attendant toxicity from the treatment paradigm. With the rapid expansion of our therapeutic armamentarium an integrated approach to therapy based upon traditional clinical trial methodology might be outpaced by our understanding of molecular events upon which these diseases are dependent. Thus such platforms as next generation sequencing might allow a more definitive prediction of efficacy of not only single agents but also their rational combination. It behooves the informed practitioner to stay abreast of these advances as the design of treatment strategy becomes ever the more individualized.

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Conflict of interests- Nil Acknowledgements- Nil

Date of submission: 11-03-2015 Date of acceptance: 17-06-2015

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