Hot Topic

Targeting the B-cell receptor pathway in diffuse large B-cell lymphoma

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ABSTRACT

Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous diagnostic category with different molecular subtypes defined by distinct gene expression patterns and divergent mechanisms of oncogenic activation. Several studies have suggested an inferior survival for patients of the activated B-cell-like (ABC) versus the germinal center B-cell-like (GCB) DLBCL subtype which has led to increasing interest in investigating pharmacological inhibition of signaling pathways which contribute to lymphomagenesis and that are specifically utilized by ABC DLBCL cells. One of these signaling cascades is the B-cell receptor (BCR) pathway and several approaches in clinical trials to target this cascade have demonstrated promising therapeutic activity. This review discusses our current understanding of the role of BCR signaling in different DLBCL subtypes, including primary central nervous system lymphoma (PCNSL), a subgroup of DLBCL that is particularly dependent on BCR signaling. One specific aim of this review is to highlight novel approaches to therapeutically target BCR signaling in DLBCL.

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Introduction

Diffuse large B-cell lymphoma is an aggressive malignant lymphoma that constitutes approximately 30%-40% of all cases of non-Hodgkin lymphoma (NHL), making it the most common type of lymphoma. While once considered to be a single disease entity, many distinct clinical and morphological variants are now recognized [1]. Additionally, over recent years, gene expression profiling and other molecular high resolution technologies have revealed that DLBCL is genetically heterogeneous. According to gene expression profiling, most cases can be divided into one of two major molecular subtypes, according to their cell of origin (COO) – a germinal center B-cell (GCB) subtype or an activated B-cell (ABC) subtype [2]. Each of the two subtypes corresponds to a different stage of B-cell differentiation, is characterized by distinct oncogenic activation mechanisms and is associated with clinical and morphologic variants [2–4]. While most patients with DLBCL are cured with R-CHOP (rituximab in addition to cyclophosphamide, doxorubicin, vincristine and prednisone) chemotherapy, a sizeable proportion of patients fails R-CHOP and a critical step in improving outcomes is accurately identifying upfront patients with low curability [5].

Outcome of ABC DLBCL and GCB DLBCL patients

While rituximab has augmented the cure rate for DLBCL patients by 10–15% and has been the most significant advance in DLBCL therapeutics over the past 30 years, a significant proportion of patients are not cured with current standard therapies [6]. To augment curability, there is an urgent need to better understand the molecular mechanisms responsible for treatment failure. Clinical prognostic characteristics such as those incorporated in the international prognostic index (IPI) have been validated in several studies with standard approaches but do not take into account tumor biology and the molecular heterogeneity of DLBCL [7–10]. Several studies, both in the pre and post rituximab eras have demonstrated that ABC DLBCL patients have a significantly inferior outcome following standard therapy [3,9,11–13]. Recently, the British Columbia Cancer Agency (BCCA) assessed the outcome of 344 patients with DLBCL uniformly treated with R-CHOP [9]. Cell of origin was determined by the Lymph2CX assay and very high concordance with conventional gene expression profiling was demonstrated. The five year progression-free survival was 48% versus 73% for ABC versus GCB DLBCL patients verifying once more the inferior outcome of ABC DLBCL patients [9].

In the 2008 World Health Organization (WHO) Classification of Lymphoid Tumors, while the GCB and ABC DLBCL subtypes were recognized, the sub-classification of DLBCL was considered optional. However, due to an improved understanding of the

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molecular pathogenesis of these two subgroups and recognition of the necessity to investigate selective strategies that may mitigate the inferior outcome of ABC DLBCL patients, a recent update of the classification in 2016 required the identification of the two subtypes at diagnosis [14].

Role of B-cell receptor signaling in ABC DLBCL and PCNSL

ABC DLBCL samples express many of the genes that are induced after antigen engagement of the B-cell receptor (BCR) in normal B-cells [2]. Over recent years, there has been significant progress in molecularly defining its biology [15–21]. In contrast to GCB DLBCL, constitutive activation of NF-κB as a driver of lymphomagenesis is characteristic for virtually all cases of the ABC DLBCL subtype [22,23]. Several genomics studies have provided very helpful insights by identifying various mutations and driver pathways that activate NF-κB in ABC DLBCL and BCR signaling in particular, has been shown to play a critical role (Fig. 1) [16,23–26]. CARD11 as part of the BCR signaling cascade is a cytoplasmic scaffolding protein that is more highly expressed in the ABC DLBCL subtype compared to other DLBCLs. Following activation of the upstream BCR receptor, it critically regulates NF-κB activity by coordinating the activation of the IκB kinase (IKK) complex [27]. Approximately 10% of ABC DLBCLs harbor activating mutations in the coiled-coil domain of CARD11, leading to constitutive NF-κB activation [26]. In ABC DLBCL cases that lack CARD11 mutations, signaling resulting from BCR activation upstream of CARD11 may engage wild type CARD11 to activate NF-κB [16,26]. Approximately 20% of ABC DLBCL cases have mutations in the genes encoding the CD79B or CD79A subunits of the BCR [16]. Toll-like receptor (TLR) signaling may also play an important role in NF-κB activation in ABC DLBCL [25]. TLRs are a class of proteins that play critical roles in mechanisms underlying innate immunity where in response to antigen stimulation, they recruit signaling complexes composed of the MYD88 protein and IRAK1/IRAK4 kinases to ultimately activate NF-κB via type 1 interferon pathways. MYD88 gain-of-function mutations occur in roughly 40% of ABC DLBCL cases with approximately 30% of cases harboring the L265P MYD88 mutation (Fig. 1) [25]. Due to the combination of poor therapeutic outcome and recognition that ABC DLBCLs are enhanced in promising

Fig. 1. Schematic depiction of BCR signaling in ABC DLBCL and PCNSL. Both DLBCL subtypes are characterized by activated BCR signaling leading to the activation of downstream pathways like the NF-κB or the PI3K/AKT signaling cascade. These pathways involve components that can be used as potential therapeutic targets for ABC DLBCL and/or PCNSL patients. Selected drugs that have been tested in clinical trials are depicted. Asterisks indicate genes that are frequently mutated.
therapeutic targets, this DLBCL subtype has become a showcase for novel drug investigation and many encouraging strategies are being studied.

Interestingly, one rare subtype of DLBCL – primary central nervous system lymphoma (PCNSL) – that is also characterized by adverse prognosis, has very recently been shown to be sensitive to inhibition of BCR signaling as well [28]. Due to its rarity and the technical challenges to obtain sufficient tissue from the central nervous system (CNS), the biology of PCNSL has not yet been well elucidated but genomic studies thus far have demonstrated similarities to systemic ABC DLBCL [29,30]. Interestingly, DNA sequencing studies revealed a much higher proportion of CD79B ITAM and MYD88 L265P mutations compared to systemic ABC DLBCL with over three quarters of cases having one or both of these genetic events suggesting that interruption of BCR and MYD88 signaling may be particularly effective strategies in this subtype of DLBCL (Fig. 1) [29,31,32].

Initial studies on ABC DLBCL treatment

Prior to the initiation of specific clinical trials in ABC DLBCL patients, pre-clinical work validated NF-κB as a therapeutic target [33]. An inhibitor of IkB kinase, which is critical for the activation of NF-κB, was tested in both GCB and ABC DLBCL cell line models and demonstrated differential activity, paving the way for the clinical investigation of agents targeting NF-κB [33]. One early study demonstrated differential activity of bortezomib in combination with chemotherapy in ABC versus GCB DLBCL [34]. Several other studies are either currently running or have investigated drugs that interfere with constitutive NF-κB signaling. The results of these studies are critically awaited.

Studies with Bruton’s tyrosine kinase inhibitors

Bruton’s tyrosine kinase (BTK) is a kinase linking BCR signaling to NF-κB activity and hence a rationale target for inhibition in ABC DLBCL (Fig. 1). Ibrutinib is a selective, covalent, and irreversible BTK inhibitor that has high activity in other lymphoid malignancies that are dependent on BCR signaling such as mantle cell lymphoma (MCL) or chronic lymphocytic leukemia (CLL) [35–37]. It furthermore induces toxicity in ABC DLBCL models by reduction of NF-κB pathway activation [16]. Based on these findings, with the hypothesis that ibrutinib would be active predominantly in ABC and to a lesser degree in GCB DLBCL patients, a multicenter study of 80 patients with relapsed and refractory DLBCL was initiated (Table 1) [38]. Patients received ibrutinib on a once daily basis (560 mg) and treatment was continued until disease progression or ibrutinib intolerance. Gene expression profiling was performed on lymphoma patient samples to determine the molecular subtype. This analysis demonstrated that 37 patients had ABC DLBCL, 20 patients GCB DLBCL and 17 patients were ‘unclassifiable’ DLBCLs. Clinical characteristics and prior treatment histories were similar in ABC and GCB DLBCL patients. In ABC DLBCL, 37% of patients responded to ibrutinib compared to just 5% (1 patient) in the GCB DLBCL group. Although the median progression free survival was short with 2.02 and 1.31 months in the ABC and GCB DLBCL groups respectively, ABC DLBCL patients had a significantly longer overall survival compared to GCB DLBCL patients (10.32 versus 3.35 months) [38]. Interestingly, in a subset of patients, mutational analyses on tumor tissue were performed to investigate for the presence of BCR and MYD88 mutations. Their presence, absence or co-existence was correlated with clinical outcome. Albeit rather small numbers, the patient group with lymphomas harboring gain of function mutations of CD79B had a response rate of 55%, while patients that were wildtype for CD79B responded in 31% of cases. The patient group that had both a CD79B and a MYD88 mutation had a response rate of 80% indicating functional cooperation between these signaling pathways. As suggested by preclinical data, those cases with CARD11 mutations did not respond, highlighting the dominance of downstream signaling in this group [38]. These clinical findings with ibrutinib suggest preferential activity of BTK inhibition in ABC compared to GCB DLBCL patients. To elucidate if ibrutinib has a role in the upfront treatment of DLBCL, a randomized trial of R-CHOP versus BTK in combination with ibrutinib in newly diagnosed non-GCB DLBCL patients has been fully recruited and results are awaited. Other BTK inhibitors such as acalabrutinib, which may be less toxic than ibrutinib, are under investigation, but at this time, their efficacy in DLBCL is not clear [39].

Ibrutinib treatment in PCNSL

Since PCNSL is a very rare subtype of DLBCL that almost never manifests outside the CNS, its treatment approach differs significantly to that of systemic DLBCL and has relied on agents that cross the blood-brain barrier as well as whole brain radiation [40]. Its outcome is significantly inferior to that of systemic DLBCL and its biology has been poorly understood for a long time. Just recently it has been appreciated that it closely resembles the ABC subtype

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<th>Table 1</th>
<th>Efficacy of selected novel compounds in the treatment of systemic DLBCL and PCNSL.</th>
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<td>Agent</td>
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<td>Ibrutinib</td>
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<td>Lenalidomide</td>
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<td>Everolimus</td>
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Abbreviations: Rel/ref = relapsed/refractory; UniT = untreated.
of DLBCL and is characterized by specific somatic mutations [29–32,41]. Interestingly, it is particularly dependent on BCR and MYD88 signaling and studies that have investigated the presence of CD79B and MYD88 mutations have found one or the other or both of these genes in the majority of cases to be mutated, at a rate much higher compared to systemic ABC DLBCL. For these reasons, strategies that target BCR and MYD88 signaling are very interesting to consider in this disease (Fig. 1). There are now three studies that have investigated the use of ibrutinib in PCNSL (Table 1)[28,42]. In the first of these, ibrutinib was tested in a ‘window’ for 14 days before the administration of any immunochemotherapy (combined with ibrutinib) to test its single agent activity in the disease [42]. In 18 patients with relapsed/refractory or untreated PCNSL, the PR rate was 83% and cerebrospinal fluid pharmacokinetic studies confirmed its entry into the CNS. In combination with immunochemotherapy, ibrutinib was highly effective inducing long-term remissions in several patients with relapsed and refractory disease although toxicity was high with a high rate of aspergillosis infections. Two other ongoing studies are also testing the efficacy of single agent ibrutinib in both relapsed primary CNS lymphoma and relapsed systemic lymphoma with CNS involvement (secondary CNS lymphoma [SCNSL]). In one of the studies with 20 enrolled patients, there were responses in 75% of patients [28]. In the second study, an interim analysis of 18 evaluable patients showed objective responses in 56% of cases [43]. In these single-agent ibrutinib studies, invasive aspergillosis was also observed. As the results of these 2 latter studies read out, it will be interesting to see if there is differential activity of ibrutinib in PCNSL versus SCNSL.

Studies using phosphatidylinositol-3-kinase (PI3K) inhibitors

Activation of the phosphatidylinositol-3-kinase (PI3K)/protein kinase B (AKT) pathway has been detected in a significant number of primary DLBCL samples [44,45]. Activated PI3K isoforms phosphotyrosyl phosphatidylinositol-4,5-bisphosphate to phosphatidylinositol-3,4,5-trisphosphate (PIP3) which activates AKT, whereas the tumor suppressor PTEN, as a major negative regulator of PI3K/AKT, dephosphorylates PIP3 [46,47]. PI3K/AKT signaling has a critical role in mediating survival of mature B-cells. Specifically, studies that have looked at the genetic ablation of the BCR in B-cells demonstrate that the survival of BCR deficient mature B-cells can be rescued by downstream PI3K signaling, with the FOXO1 transcription factor playing a critical central role [48]. Studies in ABC DLBCL, have demonstrated that PI3K activation and the downstream kinase PDK1 are essential for survival of cell lines that have CD79B mutations [49]. Furthermore, recent preclinical studies suggested that inhibition of the PI3K isoforms alpha and delta is highly active both in vitro and in vivo models of ABC DLBCL mainly due to interference with the BCR signaling cascade and subsequent inhibition of NF-kB activation (Fig. 1) [46,50].

However, PI3K signaling also appears to be important in the biology of GCB DLBCLs as a fraction of cases seems to rely on activation of PI3K by antigen independent BCR signaling [51]. One study in 248 DLBCL primary samples demonstrated that loss of PTEN was detectable in 55% of GCB DLBCL cases and its loss was inversely correlated with activation of the PI3K/AKT pathway [47]. Interestingly, predominantly GCB DLBCL models with loss of PTEN were highly sensitive to specific AKT inhibition [46]. Therefore it is believed that PI3K signaling plays an important role in the molecular pathogenesis of both major DLBCL subtypes and thus might represent a promising target for future therapies.

Various studies are currently investigating the clinical efficacy of different PI3K inhibitors. Buparlisib is currently under investigation in clinical studies for relapsed/refractory lymphoma patients including DLBCL and results are awaited. Furthermore, activity of buparlisib has been analyzed in PCNSL patients but due to limited clinical response, the trial was closed prematurely [52]. Just recently a phase II study in relapsed and/or refractory DLBCL patients treated with the PI3K alpha/delta inhibitor copanlisib was completed. As suggested by the preclinical data, PI3K alpha/delta inhibition was predominantly active in ABC DLBCL patients implying that this pathway plays an important role in the biology of these lymphomas [53].

Studies with other inhibitors of BCR signaling

Several other BCR pathway inhibitors targeting different kinases of the cascade have been and are currently being tested in clinical trials. As protein kinase C beta (PKCβ) plays a critical role in the BCR pathway, enzastaurin, an oral inhibitor of PKCβ was tested in a number of B-cell malignancies. Though a series of Phase II studies demonstrated promising activity in different B-cell lymphomas, a phase III study investigating its activity in prevention of relapses was stopped early, as it did not reach its primary end-point [54–57].

The SYK inhibitor fostamatinib has shown activity in DLBCL in a small phase II study. However due to a missing molecular classification of patients, it is unclear if there was preferential activity in ABC DLBCL patients [58]. Additionally, the clinical activity of fostamatinib was not confirmed in other clinical studies [59]. mTOR is also a critical downstream target of BCR signaling, but so far mTOR inhibitors in systemic DLBCL have only been moderately active [60,61]. The PILLAR-2 trial tested the efficacy of adjuvant everolimus in poor-risk DLBCL but early results demonstrated no improvement in disease-free survival [62]. Considering the high dependency of most PCNSLs on BCR signaling, the results of a single agent study with the mTOR inhibitor temsirolimus are interesting in this disease. Thirty-seven patients with relapsed and refractory PCNSL were treated with the drug in a phase II trial and 54% of patients had a response, albeit short-lived (median progression-free survival 2.1 months) in most patients (Table 1) [63].

Lenalidomide is a second-generation immunomodulatory imide drug (IMiD) that is also interesting to consider with respect to targeting BCR signaling in DLBCL (Fig. 1). A retrospective analysis of patients with relapsed/refractory DLBCL demonstrated higher activity in non-GCB versus GCB DLBCL patients leading to its in vitro investigation in ABC DLBCL [64]. In these analyses, lenalidomide induced toxicity in ABC DLBCL models by decreasing expression of the two transcription factors IRF4 and SPIB that transactivate CARD11 and downstream NF-kB signaling [65]. In a small study of newly diagnosed non-GCB DLBCL patients in which lenalidomide was combined with R-CHOP (R2-CHOP), this combination overcame the negative prognostic impact of the non-GCB subtype compared to a historical control [66]. Based on this finding, an ongoing randomized study of R-CHOP versus R2-CHOP in newly diagnosed patients with ABC DLBCL (defined by the Lymph2CX assay) was initiated [11,67]. Interestingly, lenalidomide (both alone and in combination with rituximab) has also demonstrated good activity in relapsed/refractory PCNSL (Table 1) [68,69].

Future directions

The role of BCR signaling and its interaction with other oncogenic signaling pathways in DLBCL is intriguing and much remains to be discovered as we move forward with developing rationale-based mechanistic clinical studies. Recent advances in our understanding of the molecular biology of PCNSL have
demonstrated that these lymphomas are particularly dependent on BCR signaling which is reflected by high response rates to BCR pathway inhibitors. Though various classes of inhibitors and immunomodulatory drugs target BCR signaling in DLBCL, BTK inhibitors in particular have demonstrated very good activity. However, in both, systemic DLBCL and PCNSL, duration of responses is unfortunately rather short suggesting early ability of lymphoma cells to circumvent specific pathway inhibition. This underscores the necessity to find approaches that will act synergistically such as immunomodulation in combination with BTK inhibition which has displayed synergy in in vivo models [70]. At this point in time, it is unknown if specific inhibitors of BCR signaling have a role in the up-front therapy of DLBCL patients, but randomized studies will clarify this question in the very near future.

Conflict of interest statement


References


