Review

B-cell receptor signaling as a driver of lymphoma development and evolution

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ABSTRACT

The B-cell receptor (BCR) is essential for normal B-cell development and maturation. In an increasing number of B-cell malignancies, BCR signaling is implicated as a pivotal pathway in tumorigenesis. Mechanisms of BCR activation are quite diverse and range from chronic antigenic drive by microbial or viral antigens to autostimulation of B-cells by self-antigens to activating mutations in intracellular components of the BCR pathway. Hepatitis C virus infection can lead to the development of splenic marginal zone lymphoma, while Helicobacter pylori infection is associated with the development of mucosa-associated lymphoid tissue lymphomas. In some of these cases, successful treatment of the infection removes the inciting antigen and results in resolution of the lymphoma. Chronic lymphocytic leukemia has been recognized for decades as a malignancy of auto-reactive B-cells and its clinical course is in part determined by the differential response of the malignant cells to BCR activation. In a number of B-cell malignancies, activating mutations in signal transduction components of the BCR pathway have been identified; prominent examples are activated B-cell-like (ABC) diffuse large B-cell lymphomas (DLBCL) that carry mutations in CD79B and CARD11 and display chronic active BCR signaling resulting in constitutive activation of the NF-κB pathway. Despite considerable heterogeneity in biology and clinical course, many mature B-cell malignancies are highly sensitive to kinase inhibitors that disrupt BCR signaling. Thus, targeted therapy through inhibition of BCR signaling is emerging as a new treatment paradigm for many B-cell malignancies. Here, we review the role of the BCR in the pathogenesis of B-cell malignancies and summarize clinical results of the emerging class of kinase inhibitors that target this pathway.

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1. Introduction

The B-cell receptor (BCR) signaling pathway, critical to the development and maturation of normal B cells, is emerging as a valuable target for the treatment of B-cell malignancies [1–5]. Several mechanisms activating this pathway have been identified in different B-cell malignancies; ranging from chronic antigenic drive due to hepatitis C virus (HCV) infection in splenic marginal zone lymphoma (SMZL) [6,7], over autostimulatory activation of the BCR in chronic lymphocytic leukemia (CLL) and follicular lymphoma (FL) [4,8–11], to antigen-independent signaling due to CARD11 mutations in a subset of activated B-cell like diffuse large B-cell lymphomas (ABC-DLBCL) [12]. Emerging data from clinical trials indicate that interruption of BCR signaling has substantial antitumor activity in a number of B-cell malignancies. In particular, kinase inhibitors directed against SYK [13], BTK [14–18] and PI3K [19], central “hubs” in the signal transduction network downstream of the BCR, are effective in CLL [19,20], mantle cell lymphoma (MCL) [15], follicular lymphoma (FL) [14], Waldenström’s macroglobulinemia (WM) [14], and in a subset of ABC-DLBCL [16]. In this review, we will concentrate on components of the BCR pathway that are targeted in clinical or preclinical trials for B-cell malignancies. We will give a brief overview of BCR signaling in normal B cells, discuss the evidence for its role in the pathogenesis of select B-cell malignancies, and summarize the available clinical experience (see Table 1 for an overview).

2. The BCR signaling pathway

The BCR consists of two immunoglobulin (Ig) heavy and two Ig light chains forming the extracellular, antigen binding part of the BCR (mIg). These are similar to the secreted antibodies of mature plasma cells and encoded by the same gene loci. During B-cell differentiation, genes encoding the Ig heavy variable region (IgHV) and Ig light variable region undergo recombination and somatic hypermutation, forming the multitude of distinct BCRs and antibodies that B cells are capable of expressing [21]. The extracellular
antigen recognizing domain is complexed with CD79A and CD79B (Igs and Igβ, respectively) that form the cytoplasmic tail of the BCR.

While the intracellular signaling cascades downstream of the BCR are well characterized, the mechanism by which antigen binding triggers the cellular response is still a matter of investigation. In particular, the conformational state of the BCR in resting B cells awaits further elucidation. One model describes the BCRs of resting B cells as inhibited oligomers floating in the plasma membrane that, upon antigen binding, assume an open conformation conducive for signal propagation [22]. In contrast, another model proposes oligomerization upon antigen binding as the initial trigger [23].

Within the cytoplasmic part of the BCR, Immunoreceptor Tyrosine-based Activation Motifs (ITAMs) are critical for intracellular signal generation. ITAMs are highly conserved peptide motifs within CD79A and CD79B of the BCR complex [24]. ITAMs are also found in other immunoreceptors, for example in the CD3 component of the T-cell receptor and in Fc-receptors [25]. Binding of antigen to the extracellular part of the BCR activates upstream kinases that phosphorylate ITAMs, which then serve as docking sites for additional kinases and adaptor molecules. SRC family kinases, in particular LYN and SYK, are important for these initial steps of BCR activation [23].

2.1. LYN

Initiation of BCR signaling through phosphorylation of ITAMs on the cytoplasmic part of CD79A and CD79B involves LYN (see Fig. 1). LYN and two other SRC family kinases (FYB and BLK) are essential for BCR mediated pro-survival signaling during early B-cell development, with some redundancy between the three kinases [26]. LYN directly phosphorylates SYK that is essential for further signal propagation. At the same time, LYN activates phosphatases that in turn inhibit signal transduction through the BCR [2]. Through this dual mode of action, LYN both activates and terminates BCR signaling. LYN deficient mice have reduced numbers of B cells that are less responsive to antigenic BCR activation. However, these mice eventually develop a lupus-like autoimmune disease due to the accumulation of self-reactive IgM. Thus, LYN plays an essential role in downregulating BCR activation and limiting the expansion of autoreactive B cells [27,28]. LYN can be inhibited by dasatinib (approved for the treatment of chronic myeloid leukemia, CML) that also targets a number of other kinases including BTK [29,30]. In a subgroup of CLL patients, LYN has been shown to phosphorylate and thereby activate hematopoietic cell-specific Lyn substrate-1 (HS1). HS1 is a cytoskeletal importer in the BCR pathway and its phosphorylation has been correlated with poor outcome. In mice transplanted with splenocytes from the Eμ-TCL1 transgenic model, dasatinib was effective in inhibiting the expansion of the tumor cells [31]. Due to the different kinases inhibited by dasatinib, its activity in clinical trials of CLL and against ABC-DLBCL cell lines in vitro may or may not be due to LYN inhibition [32,33]. Thus, validation of the clinical significance of targeting LYN will require further study.

2.2. SYK

SYK is a non-receptor tyrosine kinase essential for BCR signaling [34]. It is closely related to ZAP70, which is essential for T-cell receptor signaling, and whose expression in CLL cells is an adverse prognostic marker indicating more rapid disease progression [35]. Mice with a genetic deletion of SYK have a severe impairment of B-cell development at the pro-B cell to pre-B cell transition and lack mature B cells [36,37]. SYK binds directly to the phosphorylated ITAMs of CD79A/B via tandem SH2 domains. Binding of two phosphorylated ITAMs is necessary for maximal activation of SYK through autophosphorylation [38]. In addition, SYK can be phosphorylated by activated LYN. Thus, a SYK dependent amplification of the initial BCR signal promotes the activation of downstream signaling cascades (see Fig. 1, and recent reviews [34,39]).

The therapeutic value of SYK inhibition has been evaluated in a phase I/II study with fostamatinib that included patients with different B-cell malignancies [13]. The response rate in CLL was the highest (55%), ahead of DLBCL (22%), MCL (11%), and FL (10%). Confirming the drug’s target effects, inhibition of BCR signaling, and a decrease in cellular activation and proliferation was demonstrated in tumor cells of CLL patients treated with fostamatinib [40]. A study of fostamatinib in DLBCL and late stage clinical trials in rheumatoid arthritis are ongoing [41]. Fostamatinib also inhibits FLT3 and JAK1, which could result in clinical effects due to inhibition of multiple signaling pathways. Preclinical data on two other SYK inhibitors (PRT318 and P505–15) that seem to be more selective have recently been published [42].
Fig. 1. The B-cell receptor (BCR) and its downstream pathways. The arrow indicates direction of signaling from plasmamembrane toward effectors. Antigen binding or cell autologous interaction activates BCR, resulting in phosphorylation of ITAMs in the cytoplasmic domains of CD79A and CD79B. SYK amplifies the initial signal by autophosphorylation and further phosphorylation of ITAMs (the initial amplifying complex is marked in green). LYN has a double function in initiating and terminating BCR signaling depending on interaction with CD19 (inhibitory molecules marked purple, bifunctional molecules light purple). SYK also activates the PI3K arm of the pathway (marked in yellow). Phosphatidylinositol 4,5-bisphosphate (PIP2) is phosphorylated by PI3K to phosphatidylinositol 3,4,5-triphosphate (PIP3). PIP3, AXL, and BLNK form a signaling hub that recruits the upper part of the BCR pathway to the plasmamembrane. Inhibitory mechanisms include FcγRIIB that inhibits BCR signaling upon binding to immune complexes at the BCR. SHIP-1 and Pten phosphatases inhibit the PI3K arm of the pathway by hydrolysis of PIP3. Akt and mTOR relay PI3K activation further to downstream targets and cell cycle regulation. The BTK arm of the pathway (marked in red) is initiated by recruitment of BTK to the plasmamembrane signaling hub. PLCγ2 is activated downstream of BTK, leading to subsequent activation of PIP3. PKCβ phospholipidates IκB to activate NF-κB transcription factors that regulate gene expression of several survival factors. The complex of CARD11, MALT1, and BCL10 is an important part of the pathway activating NF-κB whereas A20 is a negative regulator of NF-κB. The downstream effectors can be modulated toward the pro-apoptotic NF-κB – ERK arm or the pro-survival NF-κB arm depending on balancing of the signaling cascades. Please see the sections on specific parts of the signaling pathways and activation in B-cell malignancies for further details.

2.3. PI3K (phosphatidylinositol 3 kinase)

PI3Ks are composed of a regulatory p85 subunit that binds via SH2 domains to phosphorylated tyrosine motifs, for example in the ITAMs, and a catalytic p110 subunit that phosphorylates phosphatidylinositol substrates, thus generating PIP3. The PI3Kβ isoform that is primarily expressed in leukocytes, and the ubiquitously expressed PI3Kε isoform are essential for B-cell development (both are class 1a PI3Ks that can generate PIP3, see So et al. for a recent review [43]). Mice lacking PI3Kε and δ show severe defects in B-cell development [44]. The importance of the PI3K pathway downstream of the BCR is further evidenced by the observation that resting B cells lacking BCR expression can be rescued from apoptosis by constitutively active PI3Kε [45,46]. Whether signaling through PI3Kβ vs. PI3Kα elicits qualitative or only quantitatively different responses remains to be elucidated [43].

PIP3 is a pivotal scaffold in the plasma membrane that recruits key components of the functional signaling complex downstream of the BCR including BTK, PLCγ2, and Akt (see Fig. 1). Hence, a hub for the initial phase of BCR signaling is formed at the plasmamembrane that in addition to PIP3 involves AXL and BLNK [47]. AXL is a receptor tyrosine kinase recently identified to be constitutively phosphorylated in CLL microvesicles [48]. AXL forms a complex with several molecules of the BCR pathway including LYN, SYK, PI3K, and PLCγ2 [49]. Similarly, BLNK (SLP65) functions as a multivalent adaptor molecule that also interacts with numerous components of the pathway including LYN, SYK, BTK, and PLCγ2 [50]. BLNK depends on interactions with CIN85 for plasma membrane translocation and the transmembrane protein CMTM7 for recruitment to the IgM class BCR [51]. Lack of any of these molecules due to mutations or inhibition seems to impair both B-cell development and BCR signaling. These molecules are thus potential candidates for the development of novel BCR inhibitors. While BTK (see below) can be tyrosine phosphorylated in the absence of PI3Kβ, downstream effects such as calcium mobilization and cell proliferation are severely impaired in the absence of PI3K [52]. Furthermore, the PI3K pathway regulates migration of B-cells, thus pointing toward an important role in the interaction of CLL cells with the microenvironment [43,44]. The significant preclinical and clinical results of PI3K inhibition in different B-cell malignancies are detailed in a later section.

2.4. BTK (Bruton’s Tyrosine Kinase)

BTK is a member of the Tec kinase family that also includes Tec (B-cells/T-cells/liver cells), ITK (IL2 inducible T-cell kinase), and BMX/ETK (bone marrow, endothelia, epithelia). Loss of BTK causes X-linked agammaglobulinaemia with absence of mature peripheral B cells and low serum immunoglobulin levels [53,54]. BTK is a non-receptor tyrosine kinase recruited early in the BCR signaling cascade in conjunction with SYK and PI3Kδ [55]. Upon activation of the BCR pathway, BTK attaches to the plasma membrane through its pleckstrin homology domain that binds to PIP3 [56]. BTK appears to be essential only in B cells and is required for BCR-induced calcium release, cell proliferation, and activation of the NF-κB pathway (see Fig. 1) [57,58]. BTK regulates actin dynamics and antigen processing during BCR activation [59]. An important downstream target of BTK is PKCβ, which in turn phosphorylates IκB kinase (IKK) resulting in release and translocation of NF-κB transcription factors to the nucleus [60]. BTK is also involved in B-cell trafficking mediated by the chemokine receptors CXCR4 and CXCR5 [61]. Results from
clinical trials of BTK inhibitors in a range of B-cell malignancies are summarized in a later section.

2.5. mTOR (mammalian Target of Rapamycin)

mTOR is an ubiquitously expressed serine/threonine kinase. It is a downstream mediator of BCR signaling (through PI3K/AKT, see Fig. 1) as well as a cell cycle regulator at the transition from G1 to S phase [62,63]. Rapamycin (sirolimus, used as an immuno suppressant in organ transplants, isolated from Streptomyces hygroscopicus), was initially identified as a fungicide and later as an anti-tumor substance [64]. mTOR was identified as the primary Target of Rapamycin [65] and mTOR inhibition has effects on multiple cell cycle regulators [66]. The mTOR inhibitors everolimus and temsirolimus (indicated for treatment of some solid tumors), which have improved stability and oral availability compared to rapamycin, have been evaluated in FL, MCL, DLBCL, and CLL [67–72]. The overall response rate (OR) has been between 20 and 40%. While these agents have been tolerable, the immunosuppressive effect and especially the high frequency of respiratory infections have been a concern [70,72].

2.6. Inhibitory regulators of the BCR pathway and anergy

In addition to the dual role of LYN, which both promotes and inhibits BCR signaling (as outlined above) there are several additional mechanisms to limit and/or turn off BCR signaling. One inhibitory molecule is Fcγ receptor IIB (FcγRIIB). Upon antigen binding to the BCR, the immunoglobulin constant region is bound to FcγRIIB that inhibits further activation. The inhibitory effect of activated FcγRIIB is in part mediated by interaction with LYN and inhibition of BCR oligomer formation early in the signaling pathway [73].

Internalization of the BCR is another mechanism to dampen antigen-dependent activation of B cells. Repeat antigen stimulation results in decreased surface IgM expression and consequently reduced BCR signaling. BTK interacts with the actin cytoskeleton during BCR signaling causing both internalization of the BCR and migration of B cells [59]. Increased turnover of BCRs upon repeat antigen activation results in preferential expression of surface IgM molecules that are not fully N-glycosylated [2]. Recent findings demonstrate that BCR internalization is indeed involved in fine tuning the response to antigen. Inhibition of BCR internalization was shown to result in dysregulated gene expression due to hyperphosphorylation of kinases [74].

Chronic BCR activation promotes anergy of B-cells characterized by the monophosphorylation of ITAMs and loss of SYK activation [75,76]. Phosphorylation and thereby activation of the phosphatase Src homology-2-containing inositol 5-phosphatase (SHIP-1) also contributes to anergy. SHIP-1 hydrolyses PIP3, thus inhibiting signaling through the PI3K arm and presumably the BTK arm of the BCR pathway (see Fig. 1). Furthermore, activation of PTEN can also contribute to turning off the PI3K arm of the BCR pathway [75]. For a recent review of molecular mechanisms of anergy, see Yarkoni [77].

2.7. Downstream from BTK toward NF-κB in the BCR pathway

PKCβ (Protein Kinase C β) functions downstream of BTK in the BCR pathway relaying signals further downstream through IKK to activation of NF-κB transcription factors. PKCβ has been targeted in preclinical and clinical trials for different B-cell malignancies by enzastaurin. However, the clinical results have been disappointing and no further clinical trials with this agent are expected [78]. Nevertheless, clinical and preclinical lessons from targeting PKCβ may prove helpful in further exploring the BCR pathway and guide combination therapy approaches [79]. Studies in mice demonstrate that CLL development in the murine TCL1 model is dependent on PKCβ expression [80]. However, whether PKCβ expression is required in B cells or stromal cells or both was not addressed. More recently, an extension of the initial study demonstrated that disruption of PKCβ in stromal cells was sufficient to inhibit CLL cell survival in the murine TCL1 model [81].

As outlined in the next section and illustrated in Fig. 1, CARD11, BCL10, and MALT1 form a complex downstream of PKCβ that propagates signaling toward the NF-κB effectors [12,82,83] and plays a prominent role in B-cell malignancies. IKK is acting in conjunction with this complex just upstream of NF-κB. However, despite strong preclinical rationale for targeting IKK, only preclinical results with no successful translation into clinical studies have been reported [84].

NF-κB is constitutively activated in many B-cell malignancies. In part this may be due to NF-κB being a major downstream effector in the BCR pathway that conveys pro-survival and proliferation signaling [2,85]. NF-κB upregulates anti-apoptotic BCL2-family members [60], whose function is now also directly targeted with specific antagonists in clinical trials [86]. PBS-1086, a pan-Rel inhibitor that decreases DNA binding of subunits in both the canonical (p65, p50, Rel-C) and non-canonical (p52, Rel-B) NF-κB pathway was recently shown in vitro and in xenograft models of multiple myeloma to inhibit NF-κB activity. Apoptosis was induced in the models and synergism with bortezomib was demonstrated, in vitro effect was also demonstrated in CLL cells [85,87]. However, due to the ubiquitous role of NF-κB signaling, targeting this pathway directly could result in unwanted effects in other organ systems.

3. Antigen driven BCR activation in B-cell malignancies

Among the different types of B-cell malignancies, several different tactics are applied for activation of the BCR pathway. Even though they convey on the same pathway, understanding where the pathway is modulated helps unravel which B-cell malignancies would likely respond to which kind of targeted therapies.

Evidence for a causal relationship between HCV infection, activation of the BCR by viral antigen, and lymphomagenesis is found in a subset of SMZL. Some of these lymphomas express a BCR that binds the E2 envelope protein of HCV (see Fig. 2a) [6,88], suggesting that a subgroup of these lymphomas arise as an expansion of HCV-reactive B cells. Consistently, antiviral treatment results in complete responses (CR) in about 75% of HCV positive non-Hodgkin lymphoma (NHL) patients, whereas no responses are seen in HCV negative NHL patients [89]. Thus, antigen-dependent BCR activation appears to be the driver of lymphomagenesis for some SMZL cases; and removal of the antigen can lead to clinical remissions in these patients.

Chronic exposure to microbial antigens has been implicated in the pathogenesis of other types of B-cell malignancies as well (Fig. 2b). For gastric mucosa associated lymphoid tissue (MALT) lymphomas, a clear association with Helicobacter pylori infection has been established. At least in early cases, remission of the lymphoma can be achieved by eradication of the helicobacter infection alone [90]. However, a direct link to the BCR pathway has not been demonstrated, and inflammation as well as direct antigenic drive may contribute to lymphomagenesis. Furthermore, CD4+ Th2 cells and follicular dendritic cells have been shown to be important for tumor formation in a murine model of H. pylori driven MALT lymphoma [91]. Thus, different pathways seem to contribute to lymphomagenesis in this case [90].

B-cell maturation involves somatic recombination and mutation of the IGHV genes that encode the antigen binding domains of the BCR. One of the first indications that antigen selection may
play a role in the pathogenesis of B-cell malignancies was provided by observations that CLL cells use a restricted repertoire of IGHV genes, [92,93]. Furthermore, some cases express virtually identical BCRs, so called “stereotyped BCRs” that are predicted to recognize distinct antigens [11,94]. Furthermore, clinical disease progression is variable depending on whether the expressed IGHV gene has undergone somatic hypermutation (M-CLL) or not (U-CLL) [95]. The U-CLL subtype is more rapidly progressive and often expresses ZAP-70, a paralogue of SYK that has been shown to enhance BCR signaling in vitro and that may contribute to an increased responsiveness of the U-CLL subtype to BCR activation [96–98].

An increasing number of antigens bound by the BCR on CLL cells have been identified (Fig. 2c) including autoantigens expressed on dying cells [99,100] as well as viral [101], bacterial [100], and fungal antigens [102]. Comparing purified CLL cells isolated concomitantly from the peripheral blood, bone marrow, and lymph node of patients; we recently showed that CLL cells in the lymph node contain increased levels of activated SYK and express genes upregulated in response to BCR activation. This indicates that antigenic signaling continues throughout the disease course and that the BCR is engaged primarily in the lymph node [98]. While both CLL subtypes showed evidence of antigen signaling in vivo, the more progressive U-CLL subtype had stronger BCR activation, higher expression of MYC and increased proliferation compared to M-CLL suggesting that BCR signaling may contribute to the clinical progression of CLL. Consistent with chronic antigen contact in vivo is the observation of a reversible down-modulation of surface IgM expression on CLL cells and the resemblance of these cells to anergic B-cells [103,104].

Recently, it has been shown that the BCR of many CLL cells recognizes an epitope that is part of the CLL BCR itself (Fig. 2d) leading to autostimulation on a single cell level [9]. Similarly for heavy chain diseases, evidence suggesting autologous misfolding-induced signalling through the BCR has been presented [105]. In vivo, a number of additional elements may shape the cellular response, including the degree to which a BCR can react with multiple antigens (polyreactive BCRs are associated with more aggressive disease [106]), the strength of the intracellular response to antigenic activation (intracellular responses are stronger in disease subsets with inferior outcome [96,98,107]), the availability of co-stimulatory signals in the tissue microenvironment (BCR signaling is stronger in CLL cells in the lymph node than in the peripheral blood [98]) and the extent to which the cells have been anergized by chronic stimulation resulting in a dampened response to BCR activation [103,104].

In summary, several lines of evidence indicate that BCR signaling plays a pivotal role in the pathogenesis of CLL and it has recently become the target of some of the most promising therapeutic advances in this disease [4,108,109,2,5,110]

Initial antigen selection during transformation or continued antigenic drive appears to play a role in additional B-cell malignancies, including MCL [111,112], FL [8], WM [113], and HCL [114,115]. In MCL, similar to what has been described in CLL, there is a bias toward usage of certain IGHV genes and expression of stereotyped receptors [111], albeit often with usage of IGHV genes distinct from those used in CLL [116]. While for about 25% of FL self-reactivity has been shown (Fig. 2e) [8], a more common mechanism of BCR activation may be crosslinking of the BCR by mannose-binding lectins in the tissue microenvironment due to increased N-glycosylation [117].

4. Combining extrinsic and intrinsic mechanisms of BCR activation in B-cell malignancies

While in most cases of B-cell malignancies specific disease promoting antigens have remained elusive, it is remarkable that most mature B-cell malignancies express an IgM type BCR, irrespective of pre- or post-germinal center origin. Given that IgM signaling promotes primarily NF-κB activation and cell proliferation, while IgG signaling promotes plasmacytic differentiation, a plausible explanation for this observation is that these malignancies derive a selection bias from antigen-dependent BCR signaling [1]. In ABC-DLBCL, acquired mutations in the IGH switch regions prevent isotype switching and thereby maintain the expression of an IgM class BCR on the tumor cells [118]. Further evidence for a selective pressure toward retaining a functional IgM type BCR is exemplified by FL, which is characterized by the chromosomal translocations t(14;18). In addition to the translocated allele that leads to overexpression of BCL-2, these cells have a productively rearranged Ig heavy chain locus that encodes the IgM BCR expressed on the cell surface [119–121]. Thus, there appears to be a strong selective pressure for these malignant B-cells to maintain a functional IgM-type BCR in addition to intrinsic genetic abnormalities.

Activation of BCR signaling through a combination of extrinsic signaling, by antigenic drive, and intrinsic signaling, due to acquired mutations, is observed in a subset of ABC DLBCLs. This lymphoma is characterized by constitutive activation of the NF-κB pathway. Using an RNAi screen it was found that most ABC-DLBCL cell lines depend on expression of a functional BCR and genetic knockdown

Fig. 2. Different mechanisms of BCR activation by antigen in B-cell malignancies. (a) An HCV epitope may activate the BCR in SMZL. (b) Helicobacter pylori infection causing antigenic and/or inflammatory drive in gastric MALT lymphoma. (c) Stereotyped BCRs in CLL and MCL that recognize autoantigens or common microbial antigens provide an antigenic drive. (d) BCR of CLL cells binds to epitope within the same or adjacent BCR, resulting in autostimulation. (e) The conservation of a functional BCR in FL despite a translocation to the IGH locus is suggestive of a role for BCR activation. Indeed, a fourth of FL specimens have BCRs that recognize self-antigen. Please see text for further details.
of BCR components (IGH, CD79A, CD79B) or BCR signal transduction components (SYK, PI3K, BTK) effectively killed these cells [32]. This dependency on activation of the BCR pathway with or without extrinsic antigenic involvement in ABC DLBCL has been called “chronic active BCR signaling” (Fig. 3a). About a fifth of primary ABC DLBCL cases and several cell lines derived from this lymphoma carry a mutation of a critical tyrosine residue in the ITAM of CD79B. Interestingly, these mutations are not sufficient to initiate BCR activation but rather increase the signaling response by preventing BCR internalization and by interfering with activation of LYN. In this subset of ABC DLBCL cells, PI3K and BTK signaling remain essential for NF-κB activation [122]. In addition to CD79B mutations, activating mutations of CARD11, a key scaffolding protein that connects BCR activation to NF-κB signaling, have been identified in about a tenth of ABC DLBCL cases. This mutation is sufficient to intrinsically activate survival signaling in the malignant B cells and obviates the need for upstream BCR signaling in a subset of ABC DLBCL [12]. Also, loss of function mutations in a negative regulator of NF-κB, the tumor suppressor A20, contributes to NF-κB pro-survival signaling in 24% of ABC DLBCL cases [123].

Another example of a transition from a dependence on extrinsic BCR activation to intrinsic activation has been well described in MALT lymphomas, which, as described above, likely arise in the setting of chronic antigen stimulation and, may be cured by elimination of the infectious agent. However, in more advanced cases recurrent chromosomal translocations are observed; t(11;18) results in a fusion transcript of API2-MALT1 and t(1;14) leads to overexpression of BCL10 under the control of the Ig heavy chain locus [124–126]. MALT1, a paracaspase, BCL10, and CARD11 form a complex that recruits IKKβ to activate the classical NF-κB pathway.

The MALT1 fusion protein and BCL10 overexpression can activate NF-κB independent of upstream BCR signaling (Fig. 3b) [82,127]. Consequently, the presence of these translocations, or overexpression of nuclear BCL10 by immunohistochemistry identifies a subset of MALT lymphomas that often fail to regress after eradication of the underlying infection [128,129].

While most cases of B-cell malignancies discussed so far depend on antigenic activation of the BCR resulting in activation of the NF-κB pathway, tonic survival signaling through PI3K may play a role in Burkitt’s lymphoma (BL). The hallmark of BL is a translocation of MYC to the Ig heavy chain locus. However, MYC has strong pro-apoptotic effects and requires activation of pro-survival signaling through the PI3K pathway. In BL activation of PI3K resembles the tonic signaling in normal resting B cells (Fig. 3c) [130,131]. Consistently, BL cells are sensitive to genetic knockdown of CD79A or SYK and pharmacologic inhibition of PI3K but are not affected by knockdown of BTK [130].

Disinhibition or direct activation of the PI3K arm of the BCR pathway is also implicated in some B-cell malignancies. In a murine model, it is shown that concomitant deletion of SHIP-1 and PTEN (both inhibitors of the PI3K arm of the BCR pathway) induces lethal B-cell malignancies resembling marginal zone lymphoma or follicular lymphoma [132]. In a study of DLBCL specimens, 37% of the samples showed reduction or lack of PTEN whereas 8% of the samples showed activating mutations in the PIK3CA domain of PI3K [133]. In another study, loss of PTEN was solely seen in the GCB subtype of DLBCL (11% of the GCB samples) [134]. In a Hodgkin lymphoma cell line, deletion of one of the SH2 domains of PI3K that results in PI3K activation has also been identified [135]. Thereby, GCB DLBCL that is independent of BCR signaling still seems to
depend upon intrinsic activation of the PI3K pathway (Fig. 3d). It is important to mention that the PIK3CA mutations are not inhibited by idelisib, which is specific for PI3Kδ (see below). Thus, PI3Kδ or pan PI3K inhibitors may be of importance for some B-cell malignancies [43]

5. BCR pathway inhibitors: PI3K targeting agents

The PI3Kδ isoform specific kinase inhibitor Idelisib (GS-1101, CAL-101) has shown clinical efficacy in B-cell malignancies, particularly for CLL patients. In vitro, it decreases AKT and ERK phosphorylation downstream in the BCR pathway and induces apoptosis in ALL, MCL, and CLL primary cells as well as in MCL, FL, and DLBCL cell lines [136,137]. In addition to direct effects in the BCR pathway, idelisib inhibits microenvironmental signals through CD40L, BAFF, TNFα, and fibronectin [138,139]. Moreover, idelisib inhibits secretion of cytokines and chemokines in a dose dependent manner. In vivo, CCL3 and CCL4 that are upregulated in CLL cells in a BCR dependent manner, show a rapid decrease in CLL patients treated with idelisib [139,140,98]. T cell viability is not affected by idelisib, although T cell secretion of some inflammatory and anti-apoptotic cytokines seems to be inhibited [138]. Thus, T cell modulation may also contribute to the effect of idelisib against B-cell malignancies.

Safety and activity of idelisib in hematologic malignancies were evaluated in a phase I study enrolling fifty four patients with CLL. The OR by IWCLL criteria [141] was 26% [19,142]. However, 80% of patients had a reduction in lymphadenopathy by ≥50%. Many of these patients did not meet criteria for response by IWCLL criteria due to a transient increase in the absolute lymphocyte count. Initial lymphopenia has been seen with several targeted therapies for CLL in otherwise responding patients without any sign of progressive disease [13,17,18]. As discussed recently by Cheson et al., the peripheral lymphopenia seen with most targeted drugs in CLL may warrant amendment of the response criteria for CLL [143]. Progression free survival (PFS) was not reached at >11 months and responses were independent of classic risk factors, including responses in patients with 17p deletion. Grade ≥3 adverse events included pneumonia (24%), neutropenia (24%), thrombocytopenia (7%), neutropenic fever (7%), anemia (6%), and increased liver enzymes (6%). Studies in which idelisib is combined with bendamustine and/or rituximab, fludarabine, ofatumumab, chlorambucil, and chlorambucil + rituximab maintenance are currently ongoing. Preliminary results from some of these studies include OR up to 87% and 1 year PFS rates up to 88% [144,145]. No limiting safety concerns have hitherto been identified for any of the combinations.

Several PI3K inhibitors with different isotype specificities are in preclinical and early clinical studies in hematologic malignancies. Isotype specific PI3K inhibitors seem to confer different effects, i.e. the PI3Kδ inhibitors PIK-90 and PI-103 are more effective than PI3Kδ or PI3Kβ/δ specific inhibitors at inhibiting CLL cell migration to CXCCL12 and in antagonizing stromal cell mediated survival signals [146]. Rigosertib, a PI3Kα/β inhibitor in phase III studies for myelodysplastic syndrome, induces apoptosis in CLL cells cultured in contact with stromal cells [147]. A pan-PI3K inhibitor (NVP-BKM120) is cytotoxic for Burkitt’s lymphoma cell lines that depend on pro-survival PI3K activation [130]. SAR245408 is another pan PI3K inhibitor that is well tolerated in patients with solid tumors [5,148]. In addition to idelisib, other PI3Kδ specific inhibitors are in preclinical and early clinical trials in CLL, including IPI145 (ongoing phase 1), PWT143, and TGR1202 [149,150]. As mentioned previously, a mutant constitutively active PIK3CA subunit that could confer resistance to idelisib was identified in a subset of ABC DLBCL [133]. Thus, results from clinical trials of PI3Kδ-specific or pan-PI3K inhibitors are awaited for these conditions. Taken together, isotype specific PI3K inhibitors may be attractive for treatment of B-cell malignancies. A caveat with highly selective inhibitors may be that activation or upregulation of other PI3K isoforms that are not targeted may give rise to drug resistance [151].

6. BCR pathway inhibitors: BTK targeting agents

The BTK inhibitor Ibrutinin (PCI-32765) is a BCR inhibitor with very promising results, especially in MCL [152], WM, the ABC subset of DLBCL [153], and CLL [18]. It binds covalently to the cysteine Cys-481 of BTK and thereby irreversibly inactivates the kinase [57,154]. In addition to blocking BCR signaling and integrin-mediated adhesion, migration of primary CLL cells to CXCL12, CXCL13, and CCL19 is inhibited by ibrutinib in vitro [155]. The off-target effect is reflected in the downregulation of BCR regulated genes and by decreased NF-κB activity in tumor cells from both peripheral blood and lymph nodes of CLL patients treated with ibrutinib [156]. Herman et al. show that ibrutinib not only inhibits BCR signaling but also disrupts the protective effect of stromal cells, and inhibits CD40, BAFF, TLR, and cytokine signaling [157]. Furthermore, ibrutinib inhibits adhesion to stromal elements such as fibronectin and VCAM1 [158]. ABC DLBCL cell lines that depend on constitutive active BCR signaling can be killed by genetic knock down of BTK as well as by ibrutinib treatment [32]. Primary ALL and HCL cells/cell lines also show decreased proliferation and increased apoptosis upon ibrutinib treatment [159,160]. Studies in a mouse model of autoimmune disease demonstrates reduction of circulating autoantibodies and objective clinical responses have been described in dogs with spontaneous non-Hodgkin lymphoma [58]. Inhibition of BCR signaling with no effect on T-cell receptor signaling has been demonstrated in these models.

In the first clinical trial reported with ibrutinib, an OR of 54% across different B-cell malignancies was reported, 7/9 for MCL, 11/16 for CLL, 6/16 for FL (later updated with response in 11/16 patients), 2/7 for DLBCL (no data on subtype), and 3/4 for WM [14,161]. Another study for DLBCL patients showed responses for ABC but not GCB subtypes in agreement with the preclinical data on the importance of BTK signaling preferentially in ABC DLBCL (two partial responses (PR) and one CR out of 10 patients) [153]. More recently, OR of 71% for treatment naïve CLL patients, 67% for relapsed or refractory patients, and 50% for high risk patients has been reported [18]. If PR with lymphocytosis is included according to the proposed amendment to CLL response criteria [143], the response rates increase to 81%, 87%, and 79% respectively. The estimated PFS at 26 months was 75% for the relapsed/refractory cohort and 96% for treatment naïve patients, demonstrating a remarkable duration of response with single agent therapy. In addition, patients with high risk CLL, including those with del17p, benefit from ibrutinib treatment with rapid and apparently persistent disease control [162]. Preliminary results from combination therapy with ibrutinib and rituximab, ofatumumab, or bendamustine in high risk CLL patients show OR rates approaching 100%. Adverse events are reported to be manageable [163–165]. A recent report from a phase II study in relapsed MCL (n = 111, 48 previously bortezomib treated) showed OR of 68% and CR of 21%; after a median of 15.3 months follow up the estimated PFS was 13.9 months [152].

Another selective, orally available BTK inhibitor, AVL-292, has been tested in early clinical trials. Preliminary data showed stable disease in 8 of 8 CLL patients with median decrease in lymph node size of 28% and initial augmented peripheral blood lymphocytosis in most patients [166]. Less effect of AVL-292 compared to ibrutinib may in part be due to differences in kinase specificity, in part due to differences in pharmacokinetics. Preliminary data indicates higher
OR in patient groups treated with higher doses or twice daily dosing [167]. Several other BTK inhibitors are in preclinical testing (GDC-0834, LFM-A13, AVL-101) [168] with no clinical studies registered at clinicaltrials.gov.

7. Conclusion

The BCR pathway has emerged as the driving pathway of lymphoma development and evolution in many mature B-cell malignancies. In contrast to the homogenous, monogenetic disease entity of CML, where the development of tyrosine kinase inhibitors for the BCR-ABL fusion protein of the Philadelphia chromosome t(9;22) has been a turning point in the management of the disease, the genetic background of these B-cell malignancies is heterogeneous and may pose multiple challenges for the successful translation of targeted therapy. This heterogeneity non-withstanding, BCR signaling appears to be a “hub” of such importance that successful translation of targeted approaches to many of these diseases appears within reach.

The different mechanisms of BCR activation in different B-cell malignancies testify to the proto-oncogenic nature of this pathway, which regulates proliferation, survival, and differentiation of B-cells. Different aspects of BCR activation may be viewed as stages in a multistep process that increasingly corrupt the normal function of the pathway. Chronic antigenic drive as seen in SMZL, CLL, and other lymphomas may be part of a first stage in the development of an antigen-dependent malignancy (Fig. 4, in green), whereas the acquisition of mutations in CD79B that enhance chronic active BCR signaling in ABC DLBCL may be viewed as a second event (Fig. 4, in blue). Gain of function mutations in downstream BCR components (Fig. 4, in red) and/or loss-of-function mutations in pathway inhibitors (Fig. 4, in yellow) then represent further hits that may promote disease entities with antigen/BCR independent proliferation and pro-survival signaling. While such a multistep process in the development of the different entities is hypothetical, it may serve well to stratify treatment approaches. Entities strongly dependent on extrinsic antigenic signaling appear to be sensitive to pathway inhibitors targeting PI3K and BTK, while an entity such as BL, that depends on tonic signaling, is not sensitive to BTK inhibitors, and lymphomas that have activating mutations in downstream signaling molecules, such as ABC-DLBCL with CARD11 mutations are resistant to both BTK and PI3K inhibitors.

For many patients, BCR pathway inhibitors are rapidly becoming the preferred option for treatment. In recognition of this, the FDA has granted breakthrough designation to ibrutinib for patients with WM, MCL, and CLL with 17p deletion. Challenges will be to individualize treatment goals and approaches, to define optimal combination therapies, and to integrate molecular characterization for response prediction.

Conflict of interest

None.

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