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Targeting the B cell receptor signaling pathway in B lymphoid malignancies

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Abstract

PURPOSE OF REVIEW—Normal B cells that failed to productively rearrange immunoglobulin V region genes, encoding a functional B cell receptor (BCR) are destined to die. Likewise, the majority of B cell malignancies remain dependent on functional BCR signaling, while in some subtypes BCR expression is missing and, apparently, counterselected. Here we summarize recent the experimental evidence for the importance of BCR signaling and clinical concepts to target the BCR pathway in B cell leukemia and lymphoma.

RECENT FINDINGS—While the dependency on pre-BCR signaling in pre-B acute lymphoblastic leukemia (ALL) seems to be limited to few ALL subtypes (e.g. *TCF3-PBX1*), most mature B cell lymphomas rely on BCR signaling provided by different stimuli e.g. tonic B cell signaling, chronic (auto)-antigen exposure, and self-binding properties of the BCR. The finding that in chronic lymphocytic leukemia (CLL), BCRs bind to an epitope on the BCR itself unravels a novel concept for CLL pathogenesis.

SUMMARY—Targeting of the B cell receptor tyrosine kinases SYK, BTK, and PI3K achieve promising clinical responses in various mature B cell malignancies and might also be useful in defined subsets of ALL. However, further understanding of the BCR signal integration in the different disease groups are required to accurately predict, which groups of patients will benefit from BCR pathway-inhibition.

Keywords

(pre-)B cell receptor signaling; acute lymphoblastic leukemia; lymphoma

Introduction

Mature B cells are stringently selected for the continuous expression of a functional B-cell receptor (BCR) throughout their life and loss of BCR expression leads to rapid cell death [1,2]. The majority of B cell malignancies remain dependent on while others lack BCR expression, either due to their maturation state or to selection pressure against the expression

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of a functional BCR (Table 1) [3–10]. While the BCR is thought to contribute to malignant transformation in most BCR-expressing malignancies, it may serve as a tumor suppressor in BCR-negative B cell malignancies [11–13]. Developing B cells can be classified into different stages according to the expression of differential surface markers (Hardy Fraction A–F) [14]. In Fraction C', the pre-B cell receptor (pre-BCR) is expressed after successful rearrangement of the μ heavy chain and pairing with the surrogate light chain (VPREB and λ5). The expression causes self-aggregation and results in clonal proliferation and downregulation of pre-BCR components [15]. After further maturation and rearrangement of the light chain, the surrogate light chains of the pre-BCR are replaced by κ or λ light chains, which together with the μ heavy chain form the BCR on mature B cells.

BCR signaling

The pre-BCR and BCR utilize very similar signaling components, particularly within the proximal signaling compartment. While both the pre-BCR and the BCR exhibit ligand-independent, 'tonic' signaling, mature B cells are typically activated by engagement of the BCR by antigen, for instance in germinal centers. Despite their structural similarities, the outcome of pre-BCR and BCR signaling can be divergent and may depend on expression levels and cellular organization of signaling components, expression of downstream mediators (i.e. transcription factors), and the chromatin structure [16–19].

In mature B cells, monovalent antigens fail to activate the BCR. Therefore, activation was assumed to rely on crosslinking of BCRs in order to create clusters [7]. However, recent evidence indicates that in the absence of stimuli, BCRs are clustered in an autoinhibitory conformation and antigen binding disperses these clusters [20,21]. Consequently, Src kinases Lyn, Fyn, and Blk phosphorylate the now accessible immunoreceptor tyrosine-based activation motifs (ITAMs) on Igα (CD79A) and Igβ (CD79B), thereby create a docking site for the tyrosine kinase SYK [22–24]. Activated SYK then phosphorylates direct targets such as the phospholipase $C\gamma_2$ ($PLC\gamma_2$), Bruton's tyrosine kinase (BTK), and the adaptor molecules BLNK and BCAP. Together with the phosphorylated co-receptor CD19, BCAP recruits PI3K to the plasma membrane to generate phosphatidylinositol-3,4,5-trisphosphate (PIP_3). This creates a docking site for BTK that, together with SYK, activates $PLC\gamma_2$. Activated $PLC\gamma_2$ hydrolyses phosphatidylinositol-4,5-bisphosphate (PIP_2) to inositol triphosphate (IP_3) and diacylglycerol leading to Ca^{2+} influx and PKCβ activation. PKCβ phosphorylates and activates CARD11 that together with MALT1 and BCL10 activate NF-κB [25]. PKCβ-mediated phosphorylation of RasGRP3 promotes GTP acquisition of RAS, which activates Raf1 and subsequently the MAP kinases ERK 1/2. Finally, BCR activation results in transcription factor activation including NFAT, NF-κB, and MYC [26]. Further modulation of the signaling outcome is mediated by co-receptors of the BCR that can either dampen signaling by recruitment of inhibitory phosphatases that oppose tyrosine kinase function, i.e. CD22, PECAM1 or promote BCR-downstream signaling through amplifiers of signal transduction (e.g. MEK1-ERK1/2).

Divergent outcomes of pre-B cell receptor signaling at the time of transformation to acute lymphoblastic leukemia

Pre-B cells within the bone marrow represent the normal counterpart of ALL in the vast majority of cases (~90%) [27]. This is surprising because pre-B cells represent a very short-lived transitional subset during early B cell development: Unless rescued through survival signals from a successfully assembled pre- BCR, pre-B cells are destined to die within days [28,29]. During normal early B cell development, the pre-BCR has a dual function in that it first promotes survival and proliferation of large cycling pre-B cells (Fraction C') and subsequently induces differentiation in small resting (Fraction D) pre-B cells (Figure 2) [14,30]. To which extent these signaling processes cooperate with the different oncogenic lesions in B cell precursors is still under investigation.

Genetic defects in the pre-BCR pathway are very common in most ALL subsets – exceptions

The high frequency of defects in the pre-BCR-related signaling molecules in ALL cells identified by us and others suggests that the pre-BCR may counteract malignant transformation. Focal deletions of *EBF1*, cryptic translocations, point mutations, and monoallelic deletions of *PAX5* result in reduced expression of BCR-related genes such as *CD19, CD79A, BLNK* and *CD72*. Besides, deletions of *BLNK, VPREB1,* and *IGLL1* as well as *SYK, SLP65, CD79B, IRF4,* and *BTLA* are described [3,31]. On the other hand, the pre-BCR also delivers critical survival and proliferation signals and its expression is required for abnormal lymphoproliferation [32]. In addition, previous work demonstrated that the pre-BCR-related tyrosine kinase Syk is required for Myc-mediated transformation of pre-B cells [33]. Our group previously demonstrated that the pre-B cell receptor-related signaling molecule BTK plays a central role in oncogenic signaling of leukemia cells [34]. Based on these findings, it is currently unclear whether pre-BCR signaling is required to enable malignant outgrowth in ALL or functions to suppress leukemogenesis.

The (pre-)BCR tyrosine kinases SYK and BTK as therapeutic targets in B cell malignancies

Future studies to validate (pre-) BCR-related signaling molecules as therapeutic targets are of immediate clinical relevance, because data from four major clinical trials in 2013 demonstrated that targeting of the (pre-) B cell receptor tyrosine kinases SYK and BTK achieves durable clinical responses in various mature B cell malignancies (discussed below). Despite the critical role of pre-BCR signaling in ALL, the clinical successes of Ibrutinib (BTK) and Fostamatinib/GS-9973 (SYK) in mature B cell lymphoma could not be recaptitulated in pre-clinical models for ALL. While ALL cells from some patients are extremely sensitive to BTK/SYK inhibition, ALL cells from other patients are completely resistant to Ibrutinib (BTK) and Fostamatinib/GS-9973 (SYK). Kinase-independent adaptor function as described for BTK in pre B cells may account for this discrepancy [35]. These findings suggest that critical additional information on pathway-specific targeting of pre-BCR signaling molecules is needed to effectively use these and other agents in the treatment of B cell lineage ALL.

Dasatinib selectively kills *TCF3*-rearranged ALL cells

Dasatinib is widely used as ABL1 kinase inhibitor in Ph^+ ALL and CML, but unexpectedly showed very strong activity in all *TCF3*-rearranged cases of ALL (Type 1 ALL). The effect of Dasatinib in *TCF3*-rearranged ALL cells is not ABL1-dependent because other ABL1 kinase inhibitors (Imatinib, Nilotinib) have no effect on these ALL cells. Interestingly, *TCF3*-rearranged ALL cells are also sensitive to SYK (GS-9973) and BTK (Ibrutinib) inhibitors, suggesting that these ALL cells are pre-BCR-dependent [36]. Given that TCF3 may directly promote the expression of IgM and κ-LC and reduce the expression of the negative regulator PTPN6, this signaling pathway may be of importance [37]. Recent work demonstrated that besides its activity on BCR-ABL1 and ABL1, Dasatinib is a powerful inhibitor of the pre-BCR tyrosine kinases BTK and the SRC family kinases LYN and BLK [38]. A recent study suggested that the therapeutic effect of Dasatinib on *TCF3*-rearranged ALL is indeed owing to selective inhibition of BTK and SRC [29,39] (Figure 3). These findings are potentially relevant because (1) they suggest that understanding of the pre-BCR pathway in ALL will allow to identify patients that will benefit from drugs that target pre-BCR signaling (e.g. GS-9973, Ibrutinib). (2) In addition, the FDA-approved ABL1 kinase inhibitor Dasatinib unexpectedly kills Non-Ph^+ Type 1 ALL cells that express an active pre-BCR. It will be interesting to determine which other subsets of ALL exhibit a similar dependency on pre-BCR signaling as *TCF3-PBX1* ALL.

BCR and its function in B cell lymphoma

The majority of mature B cell lymphoma express a functional B cell receptor. In Burkitt's lymphoma (BL), BCR expression is required to provide tonic signaling [37,40]. Activating mutations in TCF3 or deleterious lesions of its negative regulator ID3 in BL are associated with increased expression of the BCR, and knockdown of CD79A and SYK was shown to reduce cell survival [37].

For most types of lymphoma, there is strong evidence that the BCR signaling pathway is specifically activated and contributes to pathogenesis (e.g. follicular lymphoma (FL), chronic lymphocytic leukemia CLL, activated B cell type- diffuse large B cell lymphoma (ABC-DLBCL), marginal zone lymphoma (MZL), mantle cell lymphoma (MCL) [7]). These are characterized by the usage of stereotyped, non-random Ig V_H segments and chronic activation of the BCR pathway, and for some, ongoing somatic hypermutation during clonal evolution [7,41]. Several different mechanisms contribute to the activation of the BCR signaling pathway in these lymphoma: chronic exogenic antigen stimulation (hepatitis C virus in splenic MZL [42]), chronic auto-antigen stimulation (FL, CLL, mucosa-associated lymphoid tissue lymphoma (MALT) [43–46]), autonomous BCR signaling (CLL [47]), as well as mutations that activate the pathway downstream of the BCR itself (CD79B and CARD11 mutations in ABC-DLBCL [40,48]). Further augmentation of BCR signaling in ABC-DLBCL has been attributed to high expression levels of BCL6, which increases SYK activity by repressing expression of the phosphatase PTPROt [49]. Importantly, removing the BCR stimulus, e.g. by antiviral or antibacterial treatment, results in regression of the lymphoma [50,51], underlining the importance of BCR stimulation in lymphoma development.

Self-recognition in CLL and other lymphoma entities

Recently, novel insight in the signaling from BCR in CLL has led to further understanding of the importance of this pathway in B cell lymphomagenesis. Similar to the pre-BCR or the BCR in non-selected, transitional B cells, CLL BCRs confer autonomous signals by recognizing a peptide within the framework region 2 (FR2) of surface Ig itself [47]. A previous study using phage display libraries identified a peptide (WNWPLPPVRQFS) that was recognized -with different affinity- by the BCR of all tested CLL samples irrespective of IGHV gene use or mutational status but did not bind to the control samples [52]. Homologies between this epitope and a sequence within the BCR itself has led to the identification of a self-bound epitope in FR2 [47]. For CLL, further studies using phage display identified alternative epitopes for autostimulatory mechanisms in CLL [53]. The finding that poly-reactivity of CLL BCRs correlates with inferior clinical outcome, indicates that further stimulation of CLL via autoantigen (in addition to its self-binding) may modulate signaling outcome, i.e. result in increased NF-κB activation and thereby increase the proliferation capacity [52]. A recent study suggests that a subgroup of FL-derived BCR bind themselves, however, whether this is via a similar mechanism – on a single cell level- remains to be determined as well as its clinical impact [45,54].

Treatment options targeting the BCR signaling pathway

Despite clinical improvement, particularly indolent B cell Non-Hodgkin's lymphoma (B-NHL) lack persistent response rates to current therapies and remain mainly incurable. Given the importance of the BCR signaling pathway in many B-NHLs, drug discovery efforts have focused on the BCR-pathway and several molecular targets have been evaluated in different clinical trials.

SYK inhibition—SYK is a key element of the BCR pathway [55]. The first clinically relevant SYK inhibitor Fostamatinib (also called R406, or the oral form R788; AstraZeneca Pharmaceuticals) is an ATP-competitive inhibitor that has been shown to inhibit BCR signaling in CLL and ABC-DLBCL models [56–58]. In a phase 1 study patients with relapsed hematologic malignancies, fostamatib induced objective response rates (ORR) of 55% in CLL, 24% in DLBCL, 11% in MCL, and 10% in FL [59]. In this study all patients with CLL experienced an increase in circulating lymphocytes following initial treatment, a phenomenon that has also been noted in patients treated with other BCR pathway inhibitors whcih might be due to common signaling events after microenvironmental stimuli [55,60]. However, *in vitro* kinase assays demonstrated that fostamatinib can also inhibit kinases i.e. FLT3, KIT, Lck, JAK1, and JAK3, with comparable potencies [61]. Other more specific SYK inhibitors such as GS-9973 (Gilead Sciences) [62] and PRT062607 (Portola Pharmaceuticals) are currently tested in clinical trials. *In vitro* data shows anti-proliferative activity against DLBCL cell lines and inhibition of the BCR signaling pathway [63,64].

BTK inhibition—Ibrutinib (PCI-32765) is an orally available, highly selective kinase inhibitor that irreversibly binds to the C481 residue of BTK and has low off target effects. In Februrary 2013, it was designated a "breakthrough therapy" by the United States Food and Drug Administration (FDA) for the treatment of patients with relapsed or refractory mantle cell lymphoma and Waldenström macroglobulinemia. A Phase 1 study revealed safety and

an ORR of 60% in relapsed/refractory aggressive and indolent lymphoma at escalating oral doses [65,66]. In elderly, previously untreated CLL/SLL patients Ibrutinib was found to be safe and effective (71% ORR) in a phase 1b/2 trial [67]. Interestingly, similar to the SYK inhibitor [59], most patients treated with Ibrutinib experienced an initial lymphocytosis as a consequence of a lymphocyte egress from nodal compartments [68], suposedly due to common signaling events form stimuli from the microenvironment [69]. For indolent lymphoma the results are reviewed in [70]. In aggressive lymphoma, ibrutinib revealed a ORR of 68% in MCL [71] and showed substancial response rates in the relapsed, refractory ABC DLBCL (ORR=40%). In contrast, the ORR for GCB DLBCL was only 5%, consistent with the concept that this subgroup is not dependent on BCR-signaling [48]. In addition, combination of Ibrutinib with either Rituximab alone or both, Rituximab (R) and Bendamustine (B) have revealed very promising initial ORRs (Ibrutinib+R 85%; Ibrutinib +BR 92%) [72–74].

For Ibrutinib, mutation of the C481 BTK binding site (BTK^{C481S}) within the tyrosine kinase domain was found in 4 of 13 patients with acquired resistance [75,76]. However, only some of the clinically evident ibrutinib resistant cases can be attributed to mutations in BTK or other genes of the BCR signaling pathway, such as CARD11 [48]. Further mutations in genes that confer resistance to necroptosis RIPK1 Q375* or constititive activation of KRAS via Q61H confered resistance to Ibrutinib *in vitro* [77].

PI3K inhibition—The PI3K pathway is a promising therapeutic target as it is downstream target of the BCR and the SYK kinase [78]. In addition, its negative regulator PTEN is frequently deleted or deregulated in B-NHL [79]. Initial *in vitro* studies have indicated that different PI3K inhibitors were promising in CLL [80], ABC DLBCL [48,81] and MCL [82]. In addition, there is evidence that tonic BCR signaling mainly utilized the PI3K pathway, supporting a rationale for PI3K inhibition in Burkitt‘s lymphoma [13,37,83].

Clinically tested PI3K inhibitors are Idelalisib and IPI-145. Idelalisib (formally called GS-1101 or CAL-101) reversibly inhibits the PI3Kδ isoform of the p110 catalytic subunit. Phase I trial in relapsed/refractory CLL revealed that Idelalisib was well tolerated and reduced lymphadenopathy and phosphorylation of the downstream molecule AKT. As described for Fostamatinib and Ibrutinib, Idelalisib also caused a initial increase in lymphocyte count as consequence of a regress form the protective microenvironment. A phase 2 study of Idelalisib in heavily pretreated indolent lymphoma patients revealed a response rate of 57% [84,85]. IPI-145 (formerly INK-1147; Infinity Pharmaceuticals) is another selective PI3K inhibitor that targets both, PI3K-δ and PI3K-γ isoforms and has been evaluated in CLL [86]. The PI3K-γ isoform has additional functions in mediating mast cell activation and chemokine-induced cell trafficking [87,88].

MALT1 inhibition—A compound screen for the ability to inhibit the cleavage function of a dimeric, active form of MALT1 resulted in identification of novel MALT1 inhibitors. These molecules decreased NF-κB signaling and reduced target gene expression and induced apoptosis at low concentrations in ABC-DLBCL but not GC-like DLBCL *in vitro* and *in vivo* [89,90]. As some of the used components have already been used in clinical

setting for neurological symptoms, they might be useful for the treatment of ABC-DLBCL and other MALT1-dependent lymphomas in the near future.

Conclusion

In summary, recent studies identified tyrosine kinases (SYK, BTK), PI3K, and MALT1 within the BCR pathway as a novel class of therapeutic targets in B cell lymphomas. While the BCR pathway is integrated in oncogenic signalling in the majority of mature B cell lymphomas, this is only the case for a small fraction of B cell lineage acute lymphoblastic leukemia (ALL). Future studies will elucidate the mechanistic basis of divergent outcomes of BCR signaling in B cell malignancies.

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(*) special interest

(**) for outstanding interest

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Key points

Reflecting its key role in B cell survival and selection, the majority of B cell malignancies remain dependent on BCR function.

B cell malignancies can be divided into BCR-addicted tumors and malignancies in which the BCR plays a tumor-suppressive role.

Mechanisms contributing to oncogenic activation of the BCR pathway include chronic stimulation by exogenous antigen, autonomous BCR signaling, as well as mutations that activate the pathway downstream of the BCR itself.

Recent clinical trials validated tyrosine kinases (SYK, BTK) and PI3K within the BCR-pathway as a novel class of therapeutic targets in a number of mature B cell malignancies.

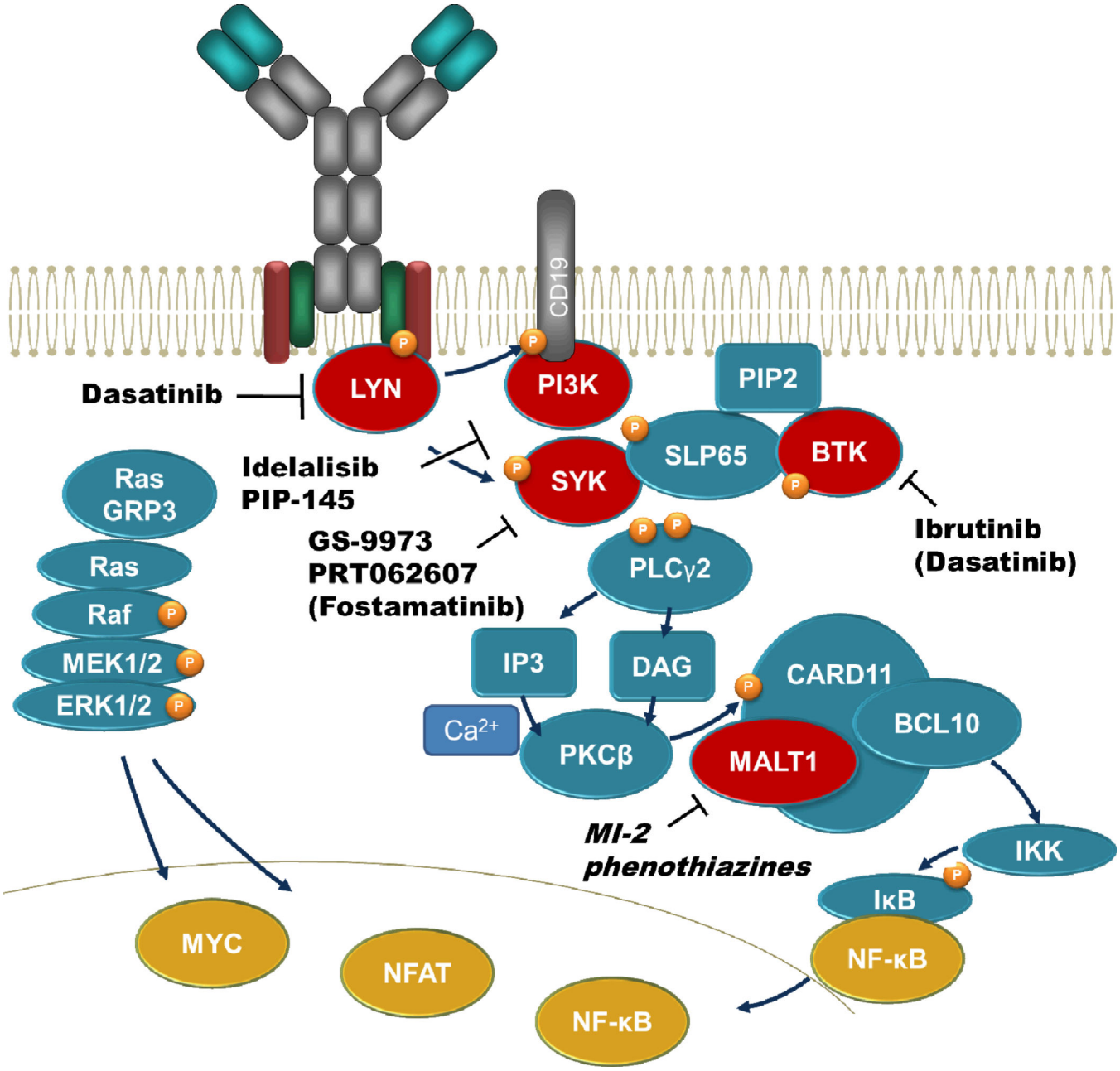

Figure 1. The BCR signaling pathway

After ligation of BCRs, Src kinases phosphorylate ITAMs, where SYK binds and is also activated by phosphorylation. SYK then passes the signal further downstream by phosphorylating SLP65, BTK, and PLCγ_2. Furthermore, the PI3K and MAPK signaling pathways are activated. Finally, BCR receptor stimulation leads by sequential phosphorylation events to activation of MYC, NF-κB and NFAT.

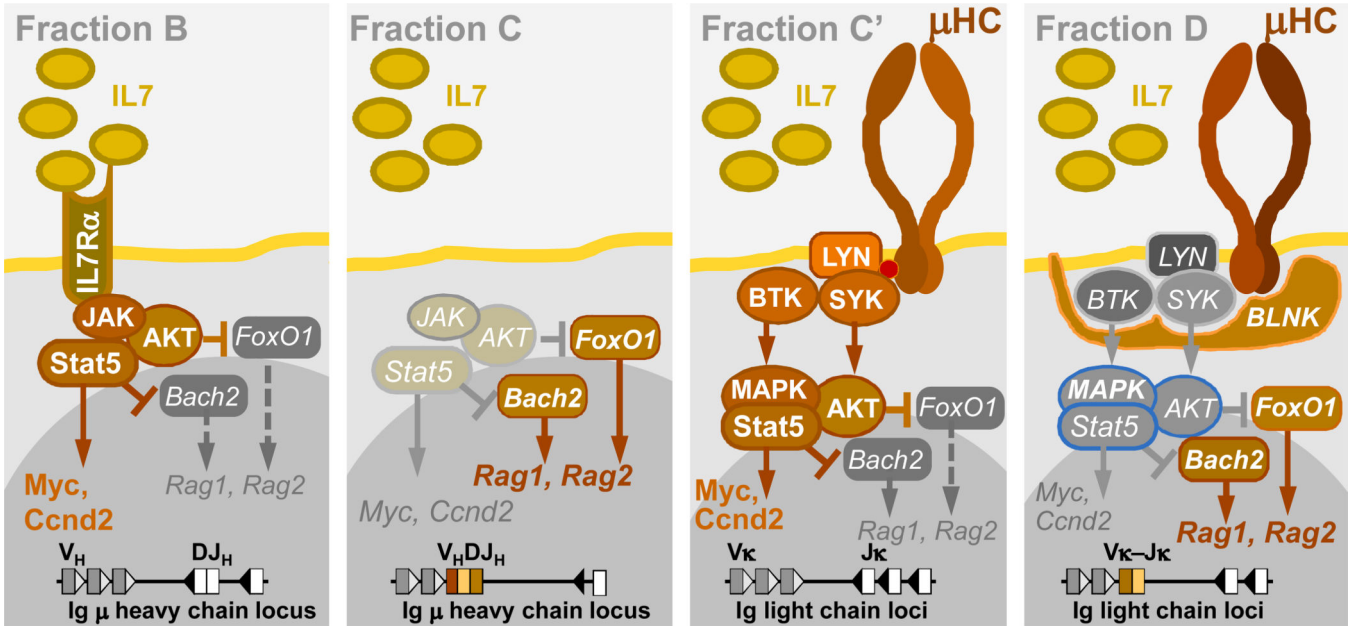

Figure 2. Pre-BCR checkpoint control during early B cell development

Pro-B cells (Fraction B) proliferate under the influence of the IL7 receptor (activation of JAK-STAT5, AKT) until they rearrange Ig VH-DJH gene segments to express a functional μ heavy chain (μ HC) as part of the pre-BCR (Fraction C'). Upon initial cell surface expression (Fraction C'), autonomous pre-BCR signaling induces strong proliferation via activation of SRC kinases (Lyn), SYK, BTK and multiple phosphorylation events in STAT5, AKT and MAPK pathways. Studies in the initial project period demonstrated that subsequent engagement of the pre-BCR linker molecule SLP65 results in global de-phosphorylation in the STAT5, AKT and MAPK pathways, cell cycle arrest and differentiation of pre-B into mature B cells [25].

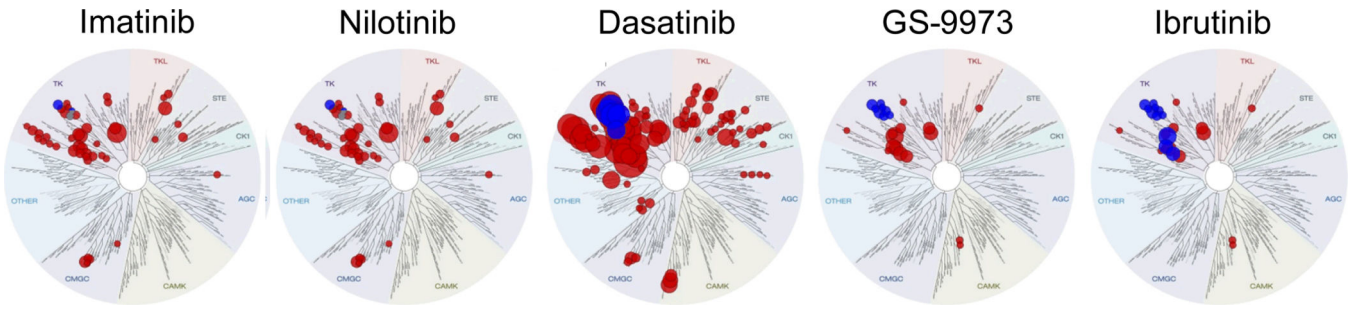

Figure 3. Spectrum of Dasatinib-targets compared to narrow inhibitors of ABL1 kinase and pre-BCR signaling

Dendrograms of target-kinases were generated with the TreeSpot software (KinomeScan). Sizes of circles depict inverse Kd values for each kinase target. Red circles are all targets of the individual compound. Among these targets, kinases within the pre-BCR pathway (SYK, BTK, LYN, BLK, SRC) are highlighted in blue.

Table 1

BCR expressing and non-expressing B cell malignancies

BCR-expressing B cell malignancies	**BCR-negative B cell malignancies**
TCF3-PBX1 acute lymphoblastic leukemia (ALL) [3]	Ph^+ and MLL-AF4 ALL [3]
Chronic lymphocytic leukemia (CLL) [4–6]	Hodgkin's lymphoma [8]
Diffuse large B cell lymphoma [7]	Immunoblastic lymphoma [7]
Mantle cell lymphoma [7]	Post-transplant lymphoma [7]
Splenic marginal zone lymphoma [7]	Primary effusion lymphoma [7]
Hairy-cell leukemia [7]	Primary mediastinal B cell lymphoma [9]
Prolymphocytic leukemia [7]	AIDS-related lymphoma [10]
Burkitt's lymphoma [7]	Multiple Myeloma [7]
Follicular lymphoma [7]	