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# Targeting of B-cell receptor signaling in B-cell malignancies

Mats Jerkeman[1], Michael Hallek[2], Martin Dreyling[3], Catherine Thieblemont[4], Eva Kimby[5], Louis Staudt[6]

[1]Department of Oncology, Lund University, Lund, Sweden; [2]Department I of Internal Medicine, Cologne University Hospital, Cologne, Germany; [3]Department of Medicine III, University of Munich, Munich, Germany; [4]Hémato-Oncologie, Hôpital Saint-Louis, Assistance Publique-Hôpitaux de Paris (AP-HP), Paris, France; [5]Hematology Center, Department of Medicine, Karolinska Institute, Stockholm, Sweden; [6]Lymphoid Malignancies Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA.

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Corresponding author:

Mats Jerkeman
Department of Oncology
Lund University
SE-221 85 Lund, Sweden
Phone +46 46 177520
Fax +46 46 176080
E-mail: mats.jerkeman@med.lu.se

# Abstract

Pharmacological agents that inhibit enzymes of the B-cell receptor (BCR) pathway are of increasing importance in the treatment of B-cell malignancies. These include inhibitors of Bruton tyrosine kinase (BTK), phosphatidylinositol 3-kinase (PI3K), splenic tyrosine kinase (SYK) and protein kinase Cβ (PKCβ). Two agents are already approved in US and Europe, ibrutinib, a BTK inhibitor, for chronic lymphatic leukemia (CLL), mantle cell lymphoma (MCL), and Waldenström's macroglobulinemia (WM), and idelalisib, a PI3Kδ inhibitor, for CLL and follicular lymphoma (FL). In addition, their role in diffuse large B-cell lymphoma (DLBCL), and marginal zone lymphoma (MZL) is under development, as single agents and in combination with chemotherapy. In CLL, both ibrutinib and idelalisib have an established role as first line therapy in patients with del(17p), and in MCL, ibrutinib is a standard option for patients relapsing after chemoimmunotherapy. Unexpected toxicities have been encountered when combining these potent new agents with other drugs, including chemotherapy and lenalidomide, and in light of this experience, the risks and benefits of novel combinations has to be evaluated carefully.

In this review we summarize results on efficacy and safety with these inhibitors, discuss novel combinations that are under study, and the future role of BCR inhibitors in these disorders.

# Novel classes of targeted therapy

Lymphomas are malignancies of the adaptive immune system. The role of this system is to provide maximum diversity in targeting a multitude of foreign antigens, and the malignant counterparts of lymphocytes have inherited the complexity of this system. Around 90% are derived from B-cells, and are the topic of this review. A unique property of B-cells is the presence of the membrane-bound B-cell receptor (BCR), which is encoded by the immunoglobulin genes[1]. In several subtypes of B-cell lymphoma, the lymphoma cell is dependent on stimulation signals propagated through the BCR, making this pathway an Achilles' heel of the lymphoma cell, that can be targeted by pharmacological agents. As the BCR is specific for B-cells, there is also a possibility of developing drugs with high specificity. It is almost 10 years since the first patient was treated with the splenic tyrosine kinase (SYK) inhibitor fostamatinib[2], and there has been a huge increase in small pharmaceutical molecules that are known to target kinases in the BCR pathway. In this review, we will mainly discuss published data on the efficacy of BCR inhibitors in a select number of B-cell malignancies: chronic lymphatic leukemia (CLL), mantle cell lymphoma (MCL), diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), Waldenström's macroglobulinemia (WM), and marginal zone lymphoma (MZL). There are currently 4 classes of BCR inhibitors in clinical trials, which inhibit Bruton's tyrosine kinase (BTK), phosphatidylinositol 3-kinase (PI3K), spleen tyrosine kinase (SYK), or protein kinase Cβ (PKCβ) (Figure 1).

## BTK inhibitors

BTK is a cytoplasmic protein-tyrosine kinase. Its corresponding gene was isolated and identified in the early 1990s as point mutations in the *BTK* gene was shown to cause X-linked (or Bruton's) agammaglobulinemia (XLA)[3]. Given the phenotype of affected patients, namely the lack of B-lymphocytes and plasma cells, and an inability to mount humoral immune responses, it was originally anticipated that BTK inhibitors would be used in autoimmune diseases. In 2010, data on the first BTK inhibitor, ibrutinib, was reported[4], which is now an approved drug both in the United States and Europe. It is an irreversible, covalent inhibitor of BTK. Both *in vitro* and in patients, ibrutinib has been shown to potently inhibit BCR signaling, to prevent lymphocyte adhesion and homing, andalso to inhibit protective effects of the microenvironment. However, it is also associated with unwanted off-target activity on other kinases, such as EGFR, interleukin-2 inducible tyrosine kinase (ITK), and JAK3. This may explain some of the adverse effects of this agent, such as inhibition of platelet function and induction of atrial fibrillation.

During the first few weeks of therapy, all BCR inhibitors, not only inhibitors of BTK, are able to cause transient lymphocytosis due to redistribution of lymphoma cells from the tissue to peripheral blood[5]. Normally, the lymphoma cells circulate in the peripheral blood, where they are attracted to tissue stromal cells by a chemokine gradient, where the CXCL12-CXCR4 axis is predominant for bone marrow homing. BCR inhibitors interfere with these homing mechanisms, leading to the egress of lymphoma cells from tissue sites into the peripheral blood, resulting in increased lymphocytosis. Lymphocytosis usually occurs concomitantly with reduction in lymph node size and clinical improvement. Importantly, the transient

lymphocytosis should not be confused with disease progression and should not lead to discontinuation of the drug.

Through inhibition of ITK present in NK cells, ibrutinib has an inhibitory effect on rituximab-induced ADCC *in vitro*[6], which is a potential disadvantage in the design of combination regimens. A number of second-generation BTK inhibitors are under development, with higher *in vitro* selectivity for BTK—including acalabrutinib (previously known as ACP-196)[7], spebrutinib (CC-292)[8], ONO/GS-4059[9], and BGB-3111.

## PI3K inhibitors

Phosphatidylinositol 3-kinase (PI3K) is a widely-expressed kinase linking several signaling pathways to cellular growth, proliferation, and survival. There are four different isoforms: α, β, γ, and δ. The γ and δ subunits are restricted to hematopoietic cells, and the δ subunit is necessary for antigen-induced BCR signaling. In addition, PI3Kα can participate in BCR signaling and stimulate cell survival[10].

Idelalisib (CAL-101), a selective PI3Kδ inhibitor taken orally, is the first approved drug of its class[11]. A number of other PI3K inhibitors with other specificities are under development for lymphoid malignancies, including duvelisib (IPI-145; targeting PI3Kγ and δ)[12], copanlisib (BAY 80-6946; a PI3K α + δ inhibitor)[13], and buparlisib (a pan-PI3K inhibitor)[14].

Common toxicities with PI3K inhibitors include elevation of liver transaminases, diarrhea, and neutropenia, but also more serious side effects including late-onset colitis and pneumonitis. Recently, an increased risk of *Pneumocystis jiroveci* pneumonia and reactivation of cytomegalovirus infection has been reported.

## SYK inhibitors

Upon antigen binding to the BCR, LYN phosphorylates SYK, which in turn amplifies the initial BCR signal, upstream of BTK and PI3K, and activates the downstream signaling cascade[1]. In addition, SYK is involved in chemokine, integrin, and Fc (Fragment, crystallizable)-receptor signaling. The first SYK inhibitor in clinical development was fostamatinib[2], and a number of other compounds with higher selectivity have since been developed, mainly in rheumatology, including entosplenetib and cerdulatinib. Currently, there is most extensive clinical experience with entosplenetib (also known as GS-9973)[15]. Common toxicities with SYK inhibitors are diarrhea, fatigue, neutropenia, thrombocytopenia, hypertension, and nausea, but serious toxicity has rarely been encountered.

## PKCβ inhibitors

Protein kinase C-β (PKC-β) is an immediate downstream target of BTK and PLCγ. Currently, there are 2 PKC-β-inhibitors in clinical development for lymphoma: enzastaurin[16] and sotrastaurin (AEB071)[17]. Of these two agents, there is more clinical experience with enzastaurin, and in terms of safety, adverse effects have been mostly mild, including diarrhea, chromaturia and neutropenia[18].

# Chronic lymphatic leukemia

## Ibrutinib in CLL

Chronic lymphatic leukemia (CLL) appears to be a disorder that is particularly sensitive to BCR inhibition due to an enhanced up-regulation of the BCR pathway related genes in bone marrow and lymph nodes. In the first (larger) study with ibrutinib in CLL, a phase-1b/2 study in relapsed/refractory (R/R) CLL, the overall response rate (ORR) was 71% by 2008 IWCLL criteria, with a complete response (CR) in 2 patients; an additional 18% of the patients achieved a partial response (PR) with lymphocytosis (PRL)[19] (Table 1). The ORR was comparable across different risk groups, including del17p. At 26 months, the estimated progression-free (PFS) and overall survival (OS) were 75% and 83%, respectively. For patients with del17p (n = 28), the estimated rates of PFS and OS at 26 months were slightly lower: 57% and 70%, respectively. In the phase-3 RESONATE trial, 391 patients with relapsed or refractory CLL or small lymphocytic leukemia (SLL) were randomly assigned to receive daily ibrutinib or ofatumumab[20]. The ORR for ibrutinib was 43% and for ofatumumab it was 4.1%. An additional 20% of ibrutinib-treated patients had PRL. The PFS with ibrutinib was 88% at 6 months, as compared to 65% with ofatumumab ($P < 0.001$). Ibrutinib also significantly improved OS at 12 months, to 90% as compared to 81% in the ofatumumab arm.

Ibrutinib as single agent has also been compared to chlorambucil in patients ≥65 years with untreated CLL in a phase 3 trial (RESONATE-2)[21]. Overall, ibrutinib resulted in significantly higher ORR (86% vs. 35%, $P<0.001$), longer PFS (median not reached vs. 18.9 months), as well as prolongation of overall survival (2-year OS: 98% vs. 85%).

Initial data have suggested that CLL patients who progress or relapse while receiving

ibrutinib have a very poor outcome with a median OS from discontinuation of only 3.1 months[22].

## Ibrutinib combinations in CLL

In a phase-2 trial, combining ibrutinib with rituximab for 6 cycles, followed by ibrutinib until disease progression in R/R CLL, the ORR was 95%, with CR in 8%[23]. Similar results have been obtained by adding ibrutinib to bendamustine and rituximab (I-BR). In a trial involving 33 patients, ORR was 93% (with 13% CR), and at a median follow-up of 8.1 months the PFS was 90%[24]. The response was compared with a historical ORR of 59% with bendamustine and rituximab (BR) alone. These results led to the design of the phase-3 HELIOS trial, comparing I-BR with BR + placebo. The results from this trial showed a clear improvement in PFS for the I-BR combination[25]. PFS at 18 months was 79% (95% CI 73–83) in the ibrutinib group and 24% (18–31) in the placebo group.

Intriguingly, when performing an indirect comparative analysis of data from the RESONATE and HELIOS trials, adjustment for prognostic factors suggested that the addition of BR to ibrutinib may not improve PFS or OS compared with single-agent ibrutinib[26].

Currently, there are 34 trials listed in clinicaltrials.gov with different ibrutinib combinations for untreated or pretreated CLL, including combinations with CD20 antibodies, lenalidomide, PD1 inhibitors, and the BCL2 inhibitor venetoclax. For the latter combination, *in vitro* data

suggest synergism[27], and one combination of ibrutinib and venetoclax will also be investigated in untreated fit CLL patients in the phase-3 CLL13 trial, comparing standard chemoimmunotherapy (FCR or BR) with 3 chemotherapy-free combinations: venetoclax+rituximab, venetoclax-obinutuzumab, and venetoclax + obinutuzumab + ibrutinib.

From whole-exome sequencing of patients with late relapse, acquired mutations have been found in *BTK* at the binding site of ibrutinib (C481), and in the gene for phospholipase Cγ2 (*PLCG2*), the kinase immediately downstream of BTK, where multiple mutations were identified[28]. Functional characterization of these mutations has demonstrated that *BTK* C481S reduces the affinity of binding of ibrutinib to BTK and allows only reversible inhibition of BTK. Because of the relatively short half-life of ibrutinib, this results in only transient inhibition of BTK, and it has been confirmed that patients with the C481S mutation who have relapse show expression of phosphorylated BTK that is not inhibited by administration of ibrutinib[28, 29]. The mutations identified in *PLCG2* have all been found to be potentially gain-of-function, allowing activation in the presence of inactive BTK[28, 30]. In many cases, clinical progression on ibrutinib is very rapid, but more gradual progression has also been encountered, suggesting that there may be different mechanisms of resistance. Primary resistance to ibrutinib has been observed only rarely and no mechanism has been identified yet, although it is possible that activating mutations downstream of BTK, such as the mitogen-activated protein kinase [MAPK] pathway,the PI3K pathway, or the non-canonical NFkB pathway, may reduce sensitivity to ibrutinib.

## Acalabrutinib in CLL

In a phase-1/phase-2 multicenter study, 61 patients with R/R CLL received oral acalabrutinib at a dose of 100–400 mg once daily in the dose-escalation (phase-1) part of the study and 100 mg twice daily in the expansion (phase-2) part[31]. With a median follow-up period of 14.3 months, the overall response rate was 95%, including 85% with a partial response and 10% with a partial response with lymphocytosis; the remaining 5% of patients had stable

disease. Of the patients with del(17p), the overall response rate was 100%. Although less data are available than with ibrutinib, the safety data indicate that there are possibly fewer side effects leading to interruption of therapy with acalabrutinib. A direct comparison of acalabrutinib and ibrutinib in previously treated CLL is now ongoing (NCT02477696).

## Other BTK inhibitors in CLL

For BTK inhibitors other than ibrutinib and acalabrutinib, less data are available. An overall response rate of 53% was observed with spebrutinib (CC-292) in 84 patients with CLL or SLL; this was apparently lower than with ibrutinib, and with shorter response duration[32]. More encouraging response rates have been seen in a phase-I trial with ONO/GS-4059, where 24 of 25 evaluable CLL patients (96%) responded[33], and similarly with BGB-3111, showing an ORR in CLL of 93% (13/14 patients)[34], but data on PFS and response duration are not yet available.

## Idelalisib in CLL

In the phase-I trial involving idelalisib, including patients with various B-cell malignancies, 54 patients with R/R CLL were included[35]. Of these, the ORR according to the 2008 IWCLL criteria was 39%, with an additional 33% of patients having PRL. The median PFS for all the CLL patients enrolled was 16 months, and it was 32 months for those receiving continuous dosing with idelalisib ≥ 150 mg twice daily. Although patients with a del(17p) or *TP53* mutation responded to treatment, the median PFS in these patients was only 5 months, as compared to 41 months in the remaining patient population.

In a following study , 220 frail patients with relapsed CLL were randomized to either idelalisib with rituximab or rituximab with placebo[36]. The DSMB recommended early termination of the trial due to an excess of events in the placebo group.. The ORR (all PR) was 81% in the idelalisib group and 13% in the placebo group. The benefit of idelalisib and rituximab was similar in groups based on the presence of del(17p), *TP53* mutations, and IGHV mutations. As in monotherapy with idelalisib, serious adverse events as pneumonitis and diarrhea were encountered more often with the idelalisib combination.

More recently, a phase-3 trial showed that idelalisib plus bendamustine + rituximab (IDELA + BR) increased PFS and OS compared to BR alone in R/R CLL[37]. In the intention-to-treat analysis, there were 207 patients in the IDELA + BR arm and 209 patients in the BR + placebo arm. Del17p/*TP53* mutation was present in 32.9% of patients and unmutated *IGHV* in 83.2%, and 29.8% had refractory disease. The median PFS of IDELA + BR was 23 months, as compared to 11 with BR + placebo (HR = 0.33; 95% CI 0.24–0.45), irrespective of the presence of high-risk features. In addition, OS was significantly longer in the IDELA + BR group (HR = 0.55; 95% CI 0.36–0.86).

Duvelisib has also been studied in combination with rituximab and with BR[38]. In a phase-1/2 trial, the ORR in CLL with the latter combination was encouraging (92%), but information about PFS and response duration has not yet been presented.

## SYK inhibitors in CLL

The first clinical trial of fostamatinib included patients with R/R B-cell lymphoma and CLL[2]. In 11 patients with CLL, the ORR was 55%. The subsequent development of fostamatinib was focused on rheumatoid arthritis, but it was ultimately halted due to negative results in phase-3 trials and dose-limiting adverse effects, including a high incidence of hypertension, gastrointestinal effects, and neutropenia—which have been attributed to off-target kinase activities. Entospletinib is a more selective SYK inhibitor, and in a phase-2 study, patients with CLL previously treated with a BCR inhibitor were included[39]. In 8 patients, early responses were seen with entospletinib (3 PR, 1 stable disease, and 1 PD; 3 patients were too early to evaluate). PR occurred in 1 patient previously treated with a BTK inhibitor and in 2 patients previously treated with PI3K inhibitors.

On account of synergistic preclinical activity with PI3Kδ and SYK inhibition, a phase-2 study has evaluated the safety and efficacy of the combination of idelalisib and entospletinib[40]. Eligible patients with relapsed or refractory CLL or NHL individually underwent dose escalation with each agent. With a median exposure to treatment of 10 weeks, 60% and 36% of patients with CLL or follicular lymphoma (FL), respectively, had objective responses. However, the study was terminated early because of treatment-emergent pneumonitis in 18% of the patients (which was severe in 11 of 12 cases). Although most patients recovered with supportive measures and systemic steroids, 2 died due to pneumonitis.

# Mantle cell lymphoma

## Ibrutinib in MCL

In competition with CLL, mantle cell lymphoma (MCL) is the foremost success story of BCR inhibition. In the initial phase-1 study of ibrutinib, 9 patients with MCL were enrolled and responses were observed in 7, including 3 complete responses[41] (Table 2). These results led to a multicenter phase-2 trial of ibrutinib in patients with R/R MCL[42]. In this study, the ORR was 68% (21% CR), with no differences based on previous bortezomib exposure. These patients were heavily pretreated, having received a median of 3 previous therapies; 45% were refractory to their last regimen and 49% were high-risk by the simplified MCL International Prognostic Index (MIPI). After a median follow-up of 27 months, the median response duration and median progression-free survival (PFS) was 17.5 and 13 months, respectively. Grade-3 to -4 toxicities were relatively uncommon, and consisted of neutropenia (16%), thrombocytopenia (11%), diarrhea (6%), dyspnea (4%), and rash (2%). The efficacy of ibrutinib in MCL has subsequently been confirmed in the phase-3 RAY trial, where patients with R/R MCL were randomized to ibrutinib or the mTOR inhibitor temsirolimus[43]. The primary efficacy analysis showed significant improvement in PFS for patients treated with ibrutinib as opposed to temsirolimus (HR = 0.43; 95% CI 0.32–0.58). ORR was significantly higher for ibrutinib (72%) than for temsirolimus (40%) ($p < 0.0001$), with a CR rate of 19%  as opposed to 1.4%.

Although the data are limited, the initial data suggest that MCL patients who progress or relapse while receiving ibrutinib have a very poor outcome. In a series of 114 patients with

MCL, either refractory to or with relapse after ibrutinib, ibrutinib was given for a median of 4.7 months, and the patients had a median OS of only 2.9 months after cessation of the drug. Using whole-exome sequencing, 2 patients with partial responses to ibrutinib of 14–30 months duration were found to have *BTK* C481S mutations in samples obtained at relapse[44]. In contrast, these mutations were not present in serial blood and lymph node samples in 6 patients who failed to respond to ibrutinib or who had responses of < 5 months duration. In 25 refractory patients enrolled on the multicenter phase-2 ibrutinib trial with disease progression at the first response assessment, 1 patient had an identifiable *PLCG2* mutation and none of the 25 had a C481S mutation in pretreatment samples[45]. Thus, patients with MCL who fail to respond to or progress despite ibrutinib have very poor outcome, with limited response to subsequent salvage therapy, and very short survival. The mechanisms of ibrutinib resistance in MCL are less well understood compared to CLL, as most patients do not have identifiable mutations in *BTK* or *PLCG2*, and development of novel strategies for treatment after ibrutinib-based therapy is necessary. A novel mechanism of ibrutinib resistance in MCL may be through mutations of *CCND1*. In vitro studies on cell lines and on primary MCL cells have shown that *CCND1* mutations increase the stability of CCND1 protein and cause ibrutinib resistance[46]. More data on the mechanisms of resistance are expected to be gained from patients who are progressing on ibrutinib in the RAY trial.

## Ibrutinib combinations in MCL

The combination of ibrutinib and rituximab was investigated in patients with R/R MCL in a phase-2 study[47]. In this trial, ibrutinib was given until progression, together with rituximab for up to 2 years. In 50 patients, the ORR was 87% (with a CR of 38%), but median PFS had

not yet been obtained. The response rate was lower in patients with Ki-67 ≥ 50% (with an ORR of 50%, in comparison to 100% in patients with Ki-67 < 50%). Although these patients were less heavily pretreated than in the phase-2 trial with single-agent ibrutinib, the results indicate a higher efficacy of the combination, in spite of the in vitro antagonism of rituximab-induced ADCC[6]. As in CLL, a synergism has been shown in vitro between ibrutinib and the BCL2 inhibitor venetoclax[48], and promising preliminary results from a phase-2 trial with this combination in R/R MCL have recently been reported, showing CR in 5 out of 8 patients[49]. Other combinations are also being investigated, combining ibrutinib with other CD20 antibodies, lenalidomide, and CDK4/6 inhibitors.

In MCL, trials are also under way to explore the use of ibrutinib in the front-line setting. A randomized phase-3 study, the SHINE trial, is ongoing, examining the addition of ibrutinib to R-bendamustine in previously untreated patients ≥ 65 years of age with MCL. A randomized phase-2 trial, ENRICH, has recently been initiated for the same patient population, comparing a chemotherapy-free approach, rituximab + ibrutinib, with standard chemoimmunotherapy.

For younger patients who are eligible for autologous stem cell transplant (ASCT), the European MCL Network TRIANGLE trial has recently been initiated, where patients aged ≤ 65 years are randomized to one of 3 arms: (1) R-CHOP/R-DHAP induction followed by ASCT, (2) R-CHOP/R-DHAP with ibrutinib followed by ASCT and 2 years of ibrutinib maintenance, or (3) R-CHOP/R-DHAP with ibrutinib and 2 years of ibrutinib maintenance, and no ASCT. In most other trials, ibrutinib is given until progression, but this trial will be the first to examine

planned discontinuation of ibrutinib after 2 years. When introducing ibrutinib in the first-line setting, it will be critical to examine biomarkers for efficacy (such as the presence of *TP53* mutations) to be able to select populations that gain the most from the addition of this agent.

## Other BTK inhibitors in MCL

In a phase-1 trial, ONO/GS-4059 has shown very promising activity in MCL, with 11 of 12 patients (92%) responding[33]. BGB-3111 has shown similar activity, with an ORR in MCL of 80% (8 of 10 patients)[34], but data on PFS and response duration are as yet unavailable. A phase-2 trial with acalabrutinib has also been concluded, but no results have been presented yet.

## Other BCR inhibitors in MCL

In general, results with other BCR inhibitors have been less convincing in MCL, especially in terms of response duration. In the phase-1 trial with idelalisib, 40 patients with R/R MCL were included, heavily pretreated with a median of 4 previous therapies[35]. In this population, the ORR was 40% (CR 5%), but with a median duration of response of only 2.7 months, and a median PFS of 3.7 months—significantly shorter than the 13 months observed with ibrutinib. A possible explanation for secondary resistance to idelalisib may be the fact that it is a selective PI3Kδ inhibitor, and in addition to PI3Kδ, PI3Kα expression has been shown in vitro to be upregulated in relapsed MCL[50]. In line with this, the dual PI3Kα/δ inhibitor pictisilib was found to be more active than idelalisib in vitro. Preliminary results of a phase-1 study of idelalisib in combination with everolimus, bortezomib, or BR in

22 patients with relapsed/refractory MCL, revealed an ORR of 49% and a CR rate of 12% for all patients. For the subset treated with idelalisib plus BR, the ORR and CR rates were 100% and 50% respectively. The median PFS for all patients was 8 months[51].

Theoretically, a broader-spectrum PI3K inhibitor may be more clinically active in MCL. There are some data to support this. In a phase-2 trial with the PI3K$\alpha\delta$ inhibitor copanlisib, 11 patients with R/R MCL were included; previously treated with a median number of 3 lines of treatment[52]. The ORR was 64% (2 CRu and 5 PRs), with a median duration of response of 5 months, but the median PFS was 3.7 months, i. e. identical to that with idelalisib. The experience with SYK inhibitors in MCL is very limited: in the phase-1/2 trial with fostamatinib, 1 of 9 patients with MCL had a PR[2]. For PKC inhibitors, the efficacy data are modest. In a study of enzastaurin in R/R MCL, no objective tumor responses occurred, but 22 out of 60 patients (37%) were free from progression (FFP) for ≥3 cycles (12 weeks)[53].

## Diffuse large B-cell lymphoma

By gene expression profiling, 2 biologically distinct forms of DLBCL, the germinal center B-cell (GCB) and the activated B-cell (ABC) subtypes, can be distinguished[54]. A prominent feature of the ABC subtype is its dependence on BCR signaling[55]. In ABC DLBCL, 10% exhibit mutations in *CARD11*[56], which result in constitutive downstream activation of NF-kB. More frequently, in 20% of ABC DLBCLs, mutations in the ITAMs of *CD79A* and *CD79B* are present[56], resulting in downstream activation of SYK, BTK, PI3K, and PKCβ. Perhaps even more importantly, 30% of ABC DLBCLs harbor *MYD88* mutations that directly activate the

NF-κB pathway. In contrast to ABC DLBCL, survival of GCB DLBCL cells is mainly dependent on PI3K/AKT activity rather than on NF-κB activation[55].

## Ibrutinib in DLBCL

Based on these biological differences, it might be expected that ABC DLBCL would be more sensitive to BCR pathway inhibition, whereas the PI3K/AKT pathway may be the Achilles' heel of GCB DLBCL. Although the number of patients was small, this hypothesis was largely confirmed in a phase-2 trial of ibrutinib in patients with relapsed DLBCL[57]. The ORR in ABC DLBCL was 40% (10 of 29 patients), as compared to 5% (1 of 20 patients) in GCB DLBCL. Interestingly, the rate of response was dependent on the presence of specific mutations in the BCR pathway in ABC DLBCL. 3 of 5 patients with *CD79B* mutations responded, 4 of 4 patients with *CD79B* and *MYD88* mutations responded, 0 of 4 patients with isolated *MYD88* mutations responded, and 0 of 3 patients with a *CARD11* mutation responded. The median PFS in patients who responded was rather short: 5.5 months. As a result of the apparent selective activity of ibrutinib in ABC DLBCL, a randomized phase-3 trial, PHOENIX, is evaluating the addition of ibrutinib to R-CHOP as a front-line therapy for patients with ABC DLBCL.

A number of extranodal variants of DLBCL are particularly associated with genetic aberrations of the BCR pathway: primary CNS lymphoma (PCNSL), primary testicular lymphoma (PTL), and primary cutaneous B-cell lymphoma, leg-type (PCBCL-LT). In one series of PCNSL, 86% (12 of 14) of the samples analyzed showed *MYD88* mutations, 9 of 14 showed mutations of *CD79B*, and 29% (4 of 14) showed mutations of *CARD11*[58]. In this series, all

*CD79B* mutations occurred concurrently with *MYD88* mutations. The spectrum of mutations is similar in PTL. Based on the findings above, and in light of its blood-brain barrier penetrability [59], ibrutinib is now undergoing trials in PCNSL—both as part of induction and as maintenance therapy. PCLBCL-LT is also associated with a high frequency of *MYD88* mutations (60%), and it has even been proposed as a diagnostic criterion for this disorder[60]. It remains to be seen whether this very aggressive lymphoma subtype responds to BCR inhibition.

## Other BTK inhibitors in DLBCL

For BTK inhibitors other than ibrutinib, 13 patients with relapsed non-GCB DLBCL have been treated with ONO/GS-4059, and 6 of 8 patients who could be evaluated achieved a PR[33]. Even more limited data are available for BGB-3111; 1 of 4 evaluable patients had a PR, but there were no data on cell of origin[34]. Other BTK inhibitors—including acalabrutinib and sepbrutinib (CC-292)—are also undergoing evaluation in DLBCL.

## SYK and PKCβ inhibitors in DLBCL

Both SYK and PKCβ are potential therapeutic targets in DLBCL. In the phase-1/2 study of the SYK inhibitor fostamatinib, 23 patients with DLBCL were treated. There was an ORR of 22% (5 of 23 patients), but no data on cell of origin were available[2]. In a follow-up phase-2 trial of 68 patients with R/R DLBCL, disappointingly, ORR was only 3% and no responses occurred in ABC DLBCL[61].

Enzastaurin is a potent inhibitor of PKCβ in vitro, and in a phase-2 study in R/R DLBCL, 15% of the patients remained progression-free after ≥ 4 cycles, which was considered a positive signal[62]. In a placebo-controlled phase-3 trial (PRELUDE), patients with DLBCL and International Prognostic Index ≥ 3 in CR after 6–8 cycles of R-CHOP were randomized to receive enzastaurin or placebo for 3 years. In 758 patients, 504 received enzastaurin and 254 received placebo. After a median follow-up time of 48 months, the DFS hazard ratio for enzastaurin versus placebo was 0.92 (95% CI 0.689–1.216). Interestingly, and independently of treatment, no significant associations were observed between PKCβ protein expression or cell of origin and DFS[18]. Another PKCβ inhibitor, sotrastaurin, has shown activity in vitro, which is apparently selective for tumors with a *CD79A/B* mutation, in an ABC DLBCL model[63], and it is currently being tested in a phase-1 clinical trial specifically for patients with *CD79*-mutant DLBCL.

## PI3K inhibitors in DLBCL

Although functional and gene expression data suggest that the PI3K pathway may be a relevant target in GCB DLBCL, there are surprisingly few data available on the efficacy of PI3K inhibitors in DLBCL, although a phase-2 trial has recently been initiated within the Nordic Lymphoma Group network (ILIAD). In a phase-1 trial involving duvelisib, 2 of 6 patients with DLBCL or transformed B-cell lymphoma showed a PR, and both responders had previously had failure with ibrutinib[64]. In a phase-2 study with the pan-PI3K inhibitor buparlisib, 26 patients with DLBCL were included but only 3 responded (with an ORR of 12%)[65].

# Follicular lymphoma

## PI3K inhibitors in FL

In the phase-2 trial of idelalisib in double-refractory indolent B-cell lymphoma, 72 patients with FL were included, and in this group ORR was 54% with CR in 14%[66]. In all 125 patients the ORR was 57%, with CR in 6%, a median duration of response of 12.5 months, and a median PFS of 11.0 months. The response was consistent across all subgroups, regardless of disease histology, number of prior regimens, refractoriness to bendamustine or tumor bulk. In FL patients PFS was approximately doubled compared with the most recent regimen before study entry. Grade-3 or higher toxicities included neutropenia (27%), diarrhea (13%), transaminitis (13%), pneumonia (7%), and thrombocytopenia (6%). The result of this trial idelalisib granted accelerated approval by FDA for patients with follicular lymphoma and small lumphocytic lymphoma (SLL) with at least two previous treatments.

The dual PI3K-δ/γ inhibitor duvelisib is also under evaluation in FL. In a phase-2 study involving 129 patients with indolent B-cell lymphomas, 83 patients with FL were enrolled. The ORR in this population was 41%, i.e. seemingly not significantly superior to idelalisib. The results have not been published yet, but only reported in a company press release.

Common toxicities with idelalisib and other PI3K inhibitors have included transaminitis, diarrhea, and neutropenia, as above. However, in addition, serious toxicities including late-onset colitis and pneumonitis have been reported. In May 2016, 6 trials with idelalisib in combination with rituximab and BR were stopped amid an increased rate of adverse events,

specifically deaths related to *Pneumocystis jiroveci* pneumonia and reactivation of cytomegalovirus infection. The current US Food and Drug Administration and European Medicines Agency recommendation is to keep the approved indications and to continue to use idelalisib in patients who are benefiting, but to closely monitor them for signs of infection. In addition, all patients should receive prophylaxis for *P jiroveci* pneumonia during idelalisib treatment.

The US Alliance consortium has conducted 2 phase-1 trials, studying the triplet combination of idelalisib, lenalidomide, and rituximab in patients with relapsed MCL and FL[67]. Unexpectedly, both of these studies had to be terminated early because of sepsis-like, uncontrollable reactions. Serious life-threatening toxicities were observed in 4 of 8 patients, consisting of grade-3/4 transaminitis, fevers, chills, hypotension, and rash on days 11–22 of cycle 1, requiring intensive care unit support for 3 patients. This indicates that unexpected toxicities including life-threatening cytokine storm, and severe rashes, can occur when BCR-signaling inhibitors are combined with other agents. The risk may be particularly high with immunosensitizing agents such as lenalidomide. This indicates that very careful dose escalation and toxicity evaluation should be performed for every new combination regimen studied.

## BTK, SYK, and PKCβ inhibitors in FL

BTK inhibitors, including ibrutinib, are currently undergoing evaluation in FL. In the phase-1 study of ibrutinib, 6 of 16 patients (38%) with FL responded, 3 with CR[41]. In a phase-2 study with ibrutinib in patients with R/R FL, the ORR in 40 patients was 30%, with only 1 CR

at a median follow-up of 6.5 months[68]. In a phase-1/1b study of BR + ibrutinib [69], 12 patients with relapsed FL were included, with an ORR of 90% (50% had CR). It remains to be seen, however, whether this may surpass the ORR seen with BR alone.

Recently, results from a trial with a triplet combination of rituximab, lenalidomide, and ibrutinib in 22 previously untreated FL patients were reported, the aim being to improve on the promising activity with rituximab and lenalidomide (R2) in FL, where ORR values of 90–96% have been reported[70]. Unexpectedly, there was a high incidence of rash (all grades, 82%; grade 3, 36%). The ORR for the entire cohort was 95%, and the 12-month progression-free survival was 80% (95% CI 57–92%). As the efficacy was not clearly superior to that of R2, and in light of excessive cutaneous toxicity, this regimen was eliminated from further study[71].

In a study of the selective SYK inhibitor entospletinib[72], 7 of 41 patients (17%) achieved a PR, and 1 of 41 patients (2%) achieved a CR, with an ORR of 20% in FL. The median PFS was 5.7 months in that study. There are also limited results with other BCR inhibitors, such as fostamatinib and enzastaurin, indicating that the activity of these agents in FL is modest.

## Waldenström's macroglobulinemia

The central genetic aberration in Waldenström's macroglobulinemia (WM) is the *MYD88 L265P* mutation, which is present in about 90% of patients and causes activation of BTK and the BCR pathway, making WM particularly sensitive to BCR inhibition[73]. In a multicenter

phase-2 trial of ibrutinib in 63 patients with R/R WM, the ORR was 90%, and a major response was achieved in 73%[74]. The median duration of treatment was 19.1 months, and the estimated 2-year PFS was 69.1%. The second most common mutation in WM is found in *CXCR4*, and in the trial above, the response rate was shown to be dependent on the presence of *MYD88* and *CXCR4* mutations. The ORR was 100%, 85.7%, and 71.4% in patients with *MYD88+,CXCR4-, MYD88+,CXCR4+,* and *MYD88-,CXCR4-,* respectively, indicating that *CXCR4* mutation may be associated with relative resistance to ibrutinib.

Idelalisib also has significant activity in WM, although currently there is little accumulated experience. In the phase-2 trial of idelalisib in indolent B-cell lymphoma, 8 of 10 patients with WM responded, with a median PFS of 22 months[66].

## Marginal zone lymphoma

In the initial phase-1 trial with ibrutinib, 1 of 4 patients with marginal zone lymphoma (MZL) responded[41]. This led to a phase-2 single-arm trial, which included 63 patients with splenic MZL, nodal MZL, or extranodal MZL. The trial has recently been concluded and shows an ORR of 51%, with an additional 38% with stable disease (SD). The median PFS was 18 months[75].

For PI3K inhibitors, there is limited data on efficacy in MZL. In the phase-2 trial with idelalisib, 15 patients with MZL previously treated with alkylators and rituximab were included, and 7 responded (with an ORR of 47%)[66]. Treatment with single-agent duvelisib

(IPI-145) showed similar efficacy in a phase-2 study, where 6 out of 18 patients with MZL responded to this agent.

## Future prospects

Needless to say, the introduction of BCR inhibitors is a breakthrough in the treatment of B-cell malignancies, especially for CLL, MCL, and WM. In these disorders, it is likely that BCR inhibitors will maintain a role as the basis of therapeutic combinations in first-line therapy also, possibly free from the blunt tools of chemotherapy. We have learned that BCR inhibition is very effective in CLL and MCL, but the reasons for these disorders being particularly sensitive are less well understood. When the mechanisms behind sensitivity and resistance have been elucidated, even more rational treatment combinations may be designed.

For DLBCL, somewhat more is known about genetic lesions conferring sensitivity to BCR inhibition, especially for the ABC DLBCL subset—most notably *MYD88* and *CD79A/B* mutations. However, in DLBCL, the prospects of a chemotherapy-free treatment seem distant at this point. In the front-line setting, we are awaiting results from the PHOENIX phase-3 trial of R-CHOP against R-CHOP with ibrutinib in ABC DLBCL, which will probably be available within the next year. For GCB DLBCL, there has been less progress towards specific targeted therapy. To proceed, the single-agent activity of idelalisib and other PI3K inhibitors needs to be determined in this disorder, and investigation of PI3K inhibitors in combination with chemotherapy—and with newer SYK and PKCβ inhibitors—should be undertaken.

In FL, BTK inhibitors appear to be less effective than agents that affect the PI3K pathway. Unfortunately, progress in the development of PI3K combinations has been halted. As mentioned above, due to toxicity, all trials evaluating novel combinations with the PI3Kδ inhibitor idelalisib have been stopped, including those with R-idelalisib and BR-idelalisib, and the clinical development of duvelisib has been delayed due to lower than expected response rates when it is used as a single agent. In MZL, we currently have very limited data on the efficacy of BCR-inhibiting agents, although data on ibrutinib are promising.

There are certainly reasons to be optimistic, but in light of the experience with unexpected toxicities when combining potent biological agents, we also need to carefully evaluate the risks and benefits of novel combinations. Health economic considerations must also be integrated into this risk-benefit analysis.

# Figure legend

## Figure 1

Overview of the B-cell receptor pathway. Enzymes where pharmacological inhibitors are available are highlighted, i e Bruton tyrosine kinase (BTK), phosphatidylinositol 3-kinase (PI3K), splenic tyrosine kinase (SYK) and protein kinase Cβ (PKCβ).

Table 1

Trials with BCR inhibitors in Chronic Lymphatic Leukemia (CLL)

| Agent(s) | Population | Phase | Number of patients | ORR [2] | Median PFS[3] (months) | Reference |
|---|---|---|---|---|---|---|
| Ibrutinib | R/R[1] CLL | 1b/2 | 85 | 71% | Not reached | [19] |
| Ibrutinib | R/R CLL | 3 | 195 | 63% | Not reached | [20] |
| Ibrutinib | Untreated CLL | 3 | 136 | 86% | Not reached | [21] |
| Ibrutinib+rituximab | R/R CLL, high risk | 2 | 40 | 95% | Not reached | [23] |
| Ibrutinib+rituximab+bendamustine | R/R CLL | 3 | 289 | 83% | Not reached | [25] |
| Acalabrutinib | R/R CLL | 1-2 | 61 | 95% | Not reached | [31] |
| Spebrutinib | R/R CLL/SLL | 2 | 84 | 53% | N/A | [32] |
| ONO/GS-4059 | R/R CLL | 1 | 25 | 96% | N/A | [33] |
| BGB-3111 | R/R CLL | 1 | 14 | 93% | N/A | [34] |
| Idelalisib | R/R CLL | 1 | 54 | 39% | 16 | [35] |
| Idelalisib+rituximab | R/R CLL | 3 | 110 | 81% | Not reached | [36] |
| Idelalisib+bendamustine+rituximab | R/R CLL | 3 | 209 | 86% | 23 | [37] |
| Duvelisib+bendamustine+rituximab | R/R CLL | 1/2 | 13 | 92% | N/A | [38] |
| Fostamatinib | R/R CLL | 1 | 11 | 55% | N/A | [2] |

| | | | | | | |
|---|---|---|---|---|---|---|
| Entospletinib | R/R CLL | 2 | 8 | 50% | N/A | [39] |

[1] Relapsed/refractory; [2] Overall response rate; [3] Progression-free survival.

Table 2

Trials with BCR inhibitors in Mantle cell lymphoma (MCL)

| Agent(s) | Population | Phase | Number of patients | ORR[2] | Median PFS[3] (months) | Reference |
|---|---|---|---|---|---|---|
| Ibrutinib | R/R[1] MCL | 1 | 9 | 78% | N/A | [41] |
| Ibrutinib | R/R MCL | 2 | 111 | 68% | 14 | [42] |
| Ibrutinib | R/R MCL | 3 | 139 | 72% | 15 | [43] |
| Ibrutinib+rituximab | R/R MCL | 2 | 50 | 88% | Not reached | [47] |
| Ibrutinib+venetoclax | R/R MCL | 2 | 8 | 88% | N/A | [49] |
| ONO/GS-4059 | R/R MCL | 1 | 12 | 92% | N/A | [33] |
| BGB-3111 | R/R MCL | 1 | 10 | 80% | N/A | [34] |
| Idelalisib | R/R MCL | 1 | 40 | 40% | 3.7 | [35] |
| Idelalisib+bendamustine+rituximab | R/R MCL | 1 | 22 | 100% | N/A | [51] |
| Copanlisib | R/R MCL | 2 | 11 | 64% | 3.7 | [52] |
| Fostamatinib | R/R MCL | 1-2 | 9 | 11% | N/A | [2] |

[1] Relapsed/refractory; [2] Overall response rate; [3] Progression-free survival.

Table 3

Trials with BCR inhibitors in Diffuse large B-cell lymphoma (DLBCL)

| Agent(s) | Population | Phase | Number of patients | ORR[2] | Median PFS[3] (months) | Reference |
|---|---|---|---|---|---|---|
| Ibrutinib | R/R[1] ABC-DLBCL[4] | 2 | 29 | 40% | N/A | [57] |
| Ibrutinib | R/R GCB-DLBCL[5] | 2 | 20 | 5% | N/A | [57] |
| ONO/GS-4059 | R/R Non-GCB DLBCL | 1 | 13 | 75 | N/A | [33] |
| BGB-3111 | R/R DLBCL | 1 | 4 | 25 | N/A | [34] |
| Fostamatinib | R/R DLBCL | 1-2 | 23 | 22 | N/A | [2] |
| Enzastaurin | R/R DLBCL | 2 | 55 | 5 | N/A | [62] |

[1] Relapsed/refractory; [2] Overall response rate; [3] Progression-free survival; [4] Activated B-cell; [5] Germinal center B-cell

Table 4

Trials with BCR inhibitors in Follicular lymphoma (FL)

| Agent(s) | Population | Phase | Number of patients | ORR[2] | Median PFS[3] (months) | Reference |
|---|---|---|---|---|---|---|
| Idelalisib | R/R[1] FL | 2 | 72 | 54% | 11 | [66] |
| Duvelisib | R/R FL | 2 | 83 | 41% | N/A | Press release |
| Ibrutinib | R/R FL | 1 | 16 | 38% | N/A | [41] |
| Ibrutinib | R/R FL | 2 | 40 | 30% | N/A | [68] |
| Ibrutinib-bendamustine-rituximab | R/R FL | 1 | 12 | 90% | N/A | [69] |
| Ibrutinib-lenalidomide-rituximab | R/R FL | 1 | 22 | 95% | N/A | [71] |
| Entospletinib | R/R MCL | 1 | 41 | 20% | 5.7 | [72] |

[1] Relapsed/refractory; [2] Overall response rate; [3] Progression-free survival.

Table 5

Trials with BCR inhibitors in Waldenström's macroglobulinemia

| Agent(s) | Population | Phase | Number of patients | ORR[2] | Median PFS[3] (months) | Reference |
|---|---|---|---|---|---|---|
| Ibrutinib | R/R[1] WM | 2 | 63 | 90% | N/A | [74] |
| Idelalisib | R/R WM | 2 | 10 | 80% | 22 | [66] |

[1] Relapsed/refractory; [2] Overall response rate; [3] Progression-free survival.

Table 6

Trials with BCR inhibitors in Marginal zone lymphoma (MZL)

| Agent(s) | Population | Phase | Number of patients | ORR[2] | Median PFS[3] (months) | Reference |
|---|---|---|---|---|---|---|
| Ibrutinib | R/R[1] MZL | 1 | 4 | 25% | N/A | [41] |
| Ibrutinib | R/R MZL | 2 | 63 | 51% | 18 | [75] |
| Idelalisib | R/R MZL | 2 | 15 | 47% | N/A | [66] |
| Duvelisib | R/R MZL | 2 | 18 | 33% | N/A | Press release |

[1] Relapsed/refractory; [2] Overall response rate; [3] Progression-free survival.

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