Review Series

SMALL-MOLECULE TARGETED THERAPIES FOR LYMPHOID MALIGNANCIES

Resistance to Bruton tyrosine kinase inhibitors: the Achilles heel of their success story in lymphoid malignancies

Deborah M. Stephens[1] and John C. Byrd[2]

[1]Division of Hematology and Hematologic Malignancies, Huntsman Cancer Institute, University of Utah, Salt Lake City, UT; and [2]Department of Internal Medicine, University of Cincinnati College of Medicine, Cincinnati, OH

Bruton tyrosine kinase inhibitors (BTKi) have significantly changed the treatment landscape for patients with B-cell malignancies, including chronic lymphocytic leukemia, Waldenstrom macroglobulinemia, mantle cell lymphoma, and marginal zone lymphoma. Unfortunately, patients with BTKi-resistant disease have shortened survival. Clinical and molecular risk factors, such as number of prior therapies and presence of *TP53* mutations, can be used to predict patients at the highest risk of developing BTKi resistance. Many mechanisms of BTKi resistance have been reported with mutations in BTK and phospholipase C γ2 supported with the most data. The introduction of venetoclax has lengthened the survival of patients with BTKi-resistant disease. Ongoing clinical trials with promising treatment modalities, such as next-generation BTKi and chimeric antigen receptor T-cell therapy, have reported promising efficacy in patients with BTKi-resistant disease. Continued research focusing on resistance mechanisms and methods of how to circumvent resistance is needed to further prolong the survival of patients with BTKi-resistant B-cell malignancies.

Introduction

Bruton tyrosine kinase inhibitors (BTKi's), such as ibrutinib, acalabrutinib, and zanubrutinib, have changed the standard of care for patients with B-cell lymphoid malignancies. BTKi's provide an oral, targeted, efficacious, and tolerable option for patients with chronic lymphocytic leukemia (CLL), Waldenstrom macroglobulinemia (WM), mantle cell lymphoma (MCL), and marginal zone lymphoma (MZL) where IV, nontargeted, and toxic chemotherapies predominated for years. BTK is a crucial component of the B-cell receptor (BCR) signaling pathway (Figure 1) and also activates integrin and toll-like receptor (TLR) signaling.[1,2] In contrast to other key components of the BCR signaling pathway, such as spleen tyrosine kinase or phosphoinositide 3-kinase (PI3K), BTK knockout mouse models and human disease have a viable phenotype, making it a relevant kinase target to disrupt BCR signaling.[3-5] When the BCR pathway is stimulated, it promotes B-cell survival, growth, differentiation, and proliferation.[6-10] The BCR signaling pathway is activated via either autonomous signaling or antigen stimulation by the microenvironment in patients with B-cell malignancies and is key to the survival of the malignant cells.[11-13] Therefore, disruption of BCR signaling via BTK blockade is a rational and attractive therapeutic opportunity. In addition, BTK is also involved in TLR9 signaling and integrin signaling and chemokine-mediated migration and adhesion.[1,2,14] These functions offer more advantages for targeting BTK in lymphoid and other hematopoietic malignancies where BTK is expressed.

Ibrutinib, an oral covalent BTKi, was the first generation of its class to be approved by the Food and Drug Administration (FDA) for marketing in the treatment of B-cell malignancies, including CLL, MCL, MZL, and WM. Ibrutinib prolongs progression-free (PFS) and/or overall survival (OS) when compared with chemotherapy, anti-CD20 monoclonal antibodies, or chemoimmunotherapy, for patients with B-cell malignancies in multiple phase 3 clinical trials.[15-20] Subsequently, ibrutinib has been quickly incorporated into the standard of care. Ibrutinib also has activity in diffuse large B-cell lymphoma (DLBCL) and other B-cell malignancies.[21] Acalabrutinib and zanubrutinib are oral, covalent, second-generation BTKi's that more selectively inhibit BTK with minimization of off-target activity. Acalabrutinib improved PFS when compared with chemoimmunotherapy in phase 3 studies and subsequently was approved for marketing by the FDA for patients with CLL and patients with MCL.[22,23] Zanubrutinib was approved for marketing in patients with MCL after demonstration of substantial efficacy and tolerability in phase 2 study.[24] Zanubrutinib was compared head to head with ibrutinib in a randomized phase 3 study in the treatment of patients with WM.[25] Response rates were similar, but the incidence and severity of most BTKi-associated toxicity, most notably atrial fibrillation, were less in zanubrutinib-treated patients.[25] The irreversible activity of both first- and second-generation BTKi's depends on a covalent bond to cysteine in the 481 moiety on the kinase domain of BTK (C481; Figure 2).[26-28] Once bound to BTK, these irreversible inhibitors prevent signaling until new protein is synthesized by the tumor cell.[26,29]

Despite the substantial efficacy of BTKi's in multiple B-cell malignancies, the Achilles heel of this drug class is either primary or acquired resistance. Primary and acquired BTKi resistance has been studied most extensively in patients with CLL receiving ibrutinib, and most of this review focuses predominately on this group. For patients with CLL, primary resistance to ibrutinib therapy is extremely rare and often suggests an alternative diagnosis than CLL or a transformation of CLL into a more aggressive lymphoma (Richter transformation). Acquired ibrutinib resistance has been reported in 11% to 38% of patients with CLL described in large series.[30-32] Acquired ibrutinib resistance can manifest both as progressive CLL (typically a later event, after 2 years on therapy) or as transformation to more aggressive entity (including DLBCL, Hodgkin lymphoma, or prolymphocytic leukemia; typically an early event, within the first 2 years).[30] Acquired resistance to acalabrutinib and zanubrutinib has been reported in 12% to 15%, although has not been well studied, and the clinical trials have shorter follow-up periods than that of ibrutinib studies.[28,33] Initial subsets of patients who developed resistance had a short expected OS (<18 months in patients with progressive CLL and <4 months in patients with Richter transformation).[30] Introduction of new therapies such as venetoclax has extended the expected OS for this group, although documented median PFS in these patients is still short (~22 months), as discussed in detail later in this paper.[34] Because of the poor clinical outcomes of this population, investigation into the mechanism of resistance and alternative treatment strategies to circumvent resistance is crucial to prolong the survival of these patients.

BTKi resistance in patients with CLL
Clinical presentation

A 67-year-old man with CLL previously treated with fludarabine, cyclophosphamide, and rituximab and receiving ibrutinib for the last 3 years presents to clinic. Review of the patient's blood counts over the last year shows a slow, but steady increase in the absolute lymphocyte count. Clinical examination reveals a marginal increase in lymphadenopathy. He feels well. This case represents a classical presentation of a patient with CLL developing resistance to ibrutinib therapy. It is essential that a treatment plan be established for this patient and implemented prior to discontinuing ibrutinib to avoid rapid onset tumor flare, which in some cases can clinically and pathologically mimic Richter's transformation.[35]

Resistance to ibrutinib monotherapy in patients with CLL is most often acquired and has been associated with high-risk genomic features, such as complex karyotype, TP53 mutation, del(17)(p13.1), and those who are heavily pretreated. In multivariable analysis of pretreatment characteristics of patients with CLL with ibrutinib resistance, the hazard ratios for CLL progression for patients with complex karyotype and del(17)(p13.1) were 2.81 (95% confidence interval [CI], 1.34-5.88; P = .006) and 2.14 (95% CI, 1.15-3.96; P = .016), respectively.[36] Follow-up of relapsed/refractory patients with CLL receiving single-agent ibrutinib on the phase 3 RESONATE study demonstrated that patients with both del(17)(p13.1) and TP53 mutation had shorter PFS than those who had either mutation individually (P = .038).[37] Long-term (up to 8 years) follow-up of treatment-naive and previously treated patients with CLL who received single-

agent ibrutinib on the pivotal phase 1/2 study again demonstrated the detrimental effect of these high-risk features on survival.[32] Median PFS for patients with del(17)(p13.1) or complex karyotype was only 26 months (95% CI, 18-37) or 31 months (95% CI, 20-40), respectively.[32] For patients who received single-agent ibrutinib as frontline vs relapsed CLL therapy, median PFS was not reached (95% CI not evaluable [NE] to NE) compared with 52 months (95% CI, 38-70). In addition, for patients with CLL who received ibrutinib after 1 to 2, 3, or ≥4 prior lines of therapy, median PFS was 66 months (95% CI, 37 to NE), 59 months (95% CI, 22 to NE), and 39 months (95% CI, 26-51), respectively.[32] In a phase 2 study, 34 previously untreated patients with CLL harboring TP53 alterations were treated with single-agent ibrutinib.[38] The median time to disease progression was 53 months, which was shorter than what was seen in all treatment-naive patients treated on the pivotal phase 1/2 study.[32,38]

In patients with CLL, the presence of complex karyotype, TP53 mutation, del(17)(p13.1), and multiple prior lines of therapy is closely associated with the other.[39-41] This may explain why complex karyotype is not always consistently associated with limited survival in ibrutinib-treated patients with CLL.[18,37] In addition, although there are limited data, treatment-naive patients with CLL with del(17)(p13.1) appear to have reasonably good outcomes when compared with previously treated patients with CLL with del(17)(p13.1) when treated with ibrutinib monotherapy.[18,32]

To help predict which patients with CLL are at highest risk for short PFS and OS during ibrutinib therapy, 1 group developed a 4-factor prognostic model.[42] A dataset containing 720 patients with CLL treated with ibrutinib monotherapy on clinical trials was evaluated with stepwise univariable and multivariable analysis. A machine-learning program consistently identified 4 risk factors associated with survival: TP53 mutation, prior CLL therapy, β-2 microglobulin ≥5 mg/L, and lactate dehydrogenase >250 U/L. Each risk factor contributed 1 point to the prognostic model. High-, intermediate-, and low-risk groups were defined as 3 to 4, 2, and 0 to 1, respectively. The 3-year rates of PFS (47%, 74%, and 87%) and OS (63%, 83%, and 93%) were significantly different for the high-, intermediate-, and the low-risk groups, respectively (P < .0001).[42]

As assays for determining karyotype and TP53 mutation status are not always available at all centers, another group sought to establish a prognostic model based on widely available pretreatment factors.[43] This group used a model-building dataset of 969 patients with relapsed/refractory CLL who were treated on phase 3 studies comparing ibrutinib with chemoimmunotherapy. Risk factors were evaluated by univariable and multivariable analysis. The results identified 4 risk factors associated with survival: β-2 microglobulin ≥5 mg/dL, lactate dehydrogenase > upper limit of normal, hemoglobin <110 g/L for women or <120 g/L for men, and time from initiation of last therapy <24 months. High-, intermediate-, and low-risk groups were defined as 4, 2 to 3, and 0 to 1, respectively. When the model was applied to an external validation set, the 2-year rates of OS (44%, 64%, and 88%) were significantly different for the high-, intermediate-, and the low-risk groups, respectively (P < .0001).[43] Of note, this model was developed on patients treated with both

ibrutinib and chemoimmunotherapy. Therefore, it may underestimate the outcomes of patients treated with ibrutinib.

Despite our knowledge of risk factors associated with ibrutinib resistance, the mechanism of leading to resistance is not clearly known in patients with each of these risk factors. A mechanistic link that may explain some of the increased risk for ibrutinib resistance in this group is that *TP53*-mutated CLL cells demonstrate a downregulation of BCR-related genes and an upregulation of prosurvival and antiapototic genes compared with *T53*–wild-type CLL cells.[44] This finding suggests that the survival of *TP53*-mutated CLL cells is less dependent on the BCR pathway, which may result in patients with *TP53*-mutated CLL being more prone to ibrutinib resistance. Multiple additional specific mechanisms of BTKi's have been described. Herein, we discuss select mechanisms of BTKi resistance (Table 1; Figures 1 and 2).

Mutations of BTK

The most common BTKi resistance mechanism described in patients with CLL is mutation of the active kinase domain on the BTK enzyme (C481; Figure 2). RNA sequencing revealed a thymidine to adenine mutation at nucleotide 1634.[45] This mutation induces a cysteine to serine missense mutation at the 481 residue (C481S).[45] Woyach et al originally performed whole exome sequencing (WES) on 6 patients with CLL with ibrutinib resistance and found the C481S mutation in 5 of these patients.[46] This mutation was not present in the pretreatment samples. However, computational models suggest that these mutations may exist pretreatment at very low levels and are selected for during therapy, indicating clonal evolution of disease during treatment.[47,48] The consequence of the C481S mutation was disruption of the covalent binding of ibrutinib to BTK, making this a reversible bond, which decreased the potency of the drug

by 25-fold.[46] Subsequently, additional mutations in C481 have been associated with ibrutinib resistance, including substitution of cysteine with alanine (C481A), arginine (C481R), phenylalanine (C481F), tyrosine (C481Y), and others.[36] These mutations have similar consequences and make the BTK-ibrutinib bond reversible and unstable. In a larger group of 40 ibrutinib-resistant patients with CLL with WES data available, 38 (95%) patients had a mutation of C481 either alone or in combination with other mutations.[36] Notably, in patients with CLL with the C481S mutation with serial WES data available, expansion of a CLL C481S mutant clone was detected in 100% (8/8) of patients prior to clinical relapse.[36] Similarly, in 16 patients with CLL who developed resistance to acalabrutinib, WES revealed that 11 (69%) patients had a mutation in C481.[33]

C481S mutations have also been detected in ~50% patients with WM who have acquired resistance to ibrutinib therapy.[49] C481S mutations have also been documented in ibrutinib-resistant patients with MCL and MZL.[50,51] However, unlike in patients with CLL and WM, these mutations are relatively infrequent.

Less commonly, mutations in the threonine 474 moiety (T474) of the BTK kinase domain have been reported in patients resistant to ibrutinib or acalabrutinib.[33,36] Mutations to T474 are termed "gatekeeper mutations" because they interfere with ibrutinib or acalabrutinib binding at *any* location on BTK.[52]

Another rare BTK mutation that has been described is a mutation in the threonine 316 moiety, T316A (alanine).[53,54] In cell lines, this mutation resulted in equal attenuation of ibrutinib's effects as seen in a BTK C481S cell line. This mutation is unique, as it is not located in the active kinase domain, but rather in the

Table 1. Select known mechanisms of BTK inhibitor resistance

Gene or chromosome region affected	Mechanism of BTK inhibitor resistance	B-cell malignancies affected
BTK	Turns covalent bond into noncovalent	CLL, WM, MCL, MZL
PLCγ2	Constitutive activation of BCR signaling pathway	CLL, MCL, MZL
Del(8p)	Downregulation of TRAIL-induced apoptosis	CLL, WM
CARD11	BTK-independent activation of BCR signaling pathway	CLL, DLBCL, FL, MCL, WM
TRAF2, TRAF3, BIRC3, MAP3K14	Constitutive activation of alternative NF-κB pathway leading to cell survival independent of BCR signaling	MCL
ARID2, SMARCA2, SMARCA4	Increased BCL-XL, an antiapoptotic protein, limiting cell death	MCL
MYD88, KLHL14	Promotes assembly of multiprotein complex that constitutively activates NF-κB pathway leading to cell survival independent of BCR signaling	DLBCL
TNFAIP3	Inactivation of negative regulator of NF-κB, which constitutively activates NF-κB pathway leading to cell survival independent of BCR signaling	DLBCL

Del(8p), deletion of chromosome 8p; FL, follicular lymphoma; XPO1, exportin-1; 2p+, gain of chromosome 2p.

Figure 1. Overview of the BCR pathway with highlighted mechanisms of BTKi resistance. *Location of known BTKi resistance mutation. ↑ Protein is upregulated as a mechanism of BTKi resistance. ↓ Protein is upregulated as a mechanism of BTKi resistance. BCL10, B-cell leukemia/lymphoma 10; BLNK, B-cell linker protein; CDK, cyclin dependent kinase; ERK 1/2, extracellular signal-regulated kinase 1/2; MALT1, mucosa-associated lymphoid tissue lymphoma translocation protein 1; PKC, protein kinase C; RB1, retinoblastoma 1; SMARCA, SWI/SNF related, matrix-associated, actin-dependent regulator of chromatin, subfamily a, member; SYK, spleen tyrosine kinase; XPO1, exportin-1.

Src-homology 2 (SH2) domain. Therefore, it is not expected to cause BTKi resistance by preventing the drug from binding to BTK. The normal function of the SH2 domain is interaction with phosphotyrosine-containing peptide substrates. B-cell linker protein normally binds to BTK at the SH2 domain, leading to downstream activation of phospholipase C γ 2 (PLCγ2; Figures 1 and 2). A mutation at T316 might be expected to limit the B-cell linker protein–BTK interaction and prevent activation of PLCγ2. However, downstream PLCγ2 activity remained intact in the T316A-mutated cell model.[53,54] It was hypothesized that this

mutation could possibly cause a change in the protein structure that prevents BTKi binding at the kinase domain, but further research is needed to define the exact mechanism of this mutation.

Mutations of PLCγ2

The next most common BTKi resistance mechanism described in patients with CLL is the mutation of PLCγ2. Multiple mutations have been described, including mutations of arginine to

Figure 2. Schematic representation of the functional domains of BTK. Kinase: this domain is responsible for activation of the BTK protein. This region hosts the binding sites for BTK inhibitors as depicted in this graphic.[89] PH, pleckstrin homology domain. This domain binds to phosphatidylinositol lipids which aids recruitment of proteins to the cellular membrane; SH, SRC homology domains. SH2 and SH3 are involved in protein-protein interactions and bind to phosphorylated tyrosines and proline regions, respectively. TH, TEC homology domain. This domain contains a proline rich region and is involved in protein-protein interactions.

tryptophan at the 665 residue (R665W), leucine to phenylalanine at the 845 residue (L845F), serine to tyrosine at the 707 residue (S707Y), and others.[46] These gain-of-function mutations result in prolonged BCR signaling when activated.[46] In a group of 40 ibrutinib-resistant patients with CLL with WES data available, 7 (17%) patients had a mutation of PLCγ2 either alone or in combination with other PLCγ2 or BTK mutations.[36] PLCγ2 mutations have also been described in acalabrutinib-resistant patients with CLL and rarely in ibrutinib-resistant patients with MCL and MZL.[33,50,51]

Deletion of chromosome 8p [del(8p)]

Deletion of the short arm of chromosome 8 (del(8p)) has been associated with ibrutinib resistance.[55] Del(8p) resulted in the insufficiency of TRAIL-R protein via downregulation of *TRAIL-R1* and *TRAIL-R2* genes. When TRAIL-R joins with TRAIL, apoptosis is induced.[56] Therefore, CLL cells harboring del(8p) were insensitive to TRAIL-induced apoptosis, allowing for continuous cell growth. This insensitivity was hypothesized to be a source of ibrutinib resistance in patients with CLL.[55] Del(8p) has also been associated with ibrutinib resistance in patients with WM.[57]

Mutations in CARD11

CARD11 is part of the CARD11-BCL10-MALT1 complex that activates downstream NF-κB. Mutations in CARD11 allow for BTK-independent activation of NF-κB. Mutations in CARD11 have been documented in ibrutinib-resistant patients with CLL, DLBCL, follicular lymphoma, MCL, and WM.[21,49,58-60]

BTKi resistance mutations in patients with WM

As noted previously, BTKi resistance in patients with WM has some common features with BTKi resistance in patients with CLL. BTK C481S mutations are detected in ∼50% patients with WM who have acquired resistance to ibrutinib therapy.[49] Interestingly, BTKi resistance is clearly apparent, although the allele frequency of BTK C481S mutations is quite low.[49] Therefore, 1 group hypothesized that there may be a mechanism by which the BTK C481S mutant cells may be able to confer resistance to the BTK C481 wild-type malignant cells.[61] They found that in patients with WM, the BTK C481S mutation restored BCR signaling via extracellular signal-regulated kinase 1/2.[61] This signaling is accompanied by the release of prosurvival and inflammatory cytokines, including interleukin-6 (IL-6) and IL-10.[61] This cytokine release rescues WM cells from ibrutinib-induced cell death, supporting a paracrine mechanism of BTKi resistance in patients with WM with a BTK C481S mutation.[61] Of note, this paracrine mechanism of resistance has not been well studied in patients with CLL, although similarly low-allele frequencies of the BTK C481S clearly lead to resistance in this disease as well.[36,62] The reactivation of extracellular signal-regulated kinase 1/2 has been reported in a patient with CLL after development of a BTK C481S mutation.[63] Chemokines may also be involved in BTKi resistance. For example, the chemokines CCL3 and CCL4 (inflammatory chemokines secreted in response to BCR activation) were reported to decrease in patients with CLL responding to ibrutinib and then increased at the time of development of ibrutinib resistance, indicating reactivation of BCR signaling in the ibrutinib-resistant cells.[48] In contrast to what was seen in patients with CLL, decreases in CCL4 (but not CCL3) were

reported in patients with WM responding to ibrutinib, but these changes were not as prominent as the changes seen in IL-6 and IL-10.[61,64] These data indicate that paracrine resistance mechanisms may differ by disease subtype.

In >90% of patients with WM, mutations in myeloid differentiation primary response gene 88 (MYD88) are present.[65] MYD88 mutation leads to constitutive activation of BTK and the BCR signaling pathway through nuclear factor κ-light-chain–enhancer of activated B cells (NF-κB). Approximately one-third of patients with WM also have a mutation in CXCR4, which regulates chemotaxis of lymphocytes. BTK C481S mutations were associated with CXCR4 mutations in patients with ibrutinib-resistant WM.[49,65]

As seen in patients with CLL, del(8p) and mutations in CARD11 have been documented in ibrutinib-resistant patients with WM.[49,57]

BTKi resistance mutations in patients with MCL

Ibrutinib resistance in patients with MCL is complex, and primary resistance to therapy is more commonly demonstrated. Mutations that activate downstream kinases in the BCR signaling pathway have been implicated as 1 cause of primary ibrutinib resistance in patients with MCL. Overactivation of PI3K or protein kinase B (or ATK) can enhance cell survival via the BCR pathway independently of BTK and is associated with ibrutinib resistance.[50,66] In addition to mutations, the tumor microenvironment likely has complex interplay with development of resistance. Supporting this, activation of integrin β1-integrin–linked kinase in the tumor microenvironment has been linked to overactivation of the PI3K kinase pathway, leading to both primary and acquired BTKi resistance.[67]

Mutations that enhance cell survival are another key factor in the development of ibrutinib resistance in patients with MCL. Signaling via NF-κB is a crucial component of the BCR signaling pathway located downstream of BTK, which promotes cell survival. This mechanism of activation is referred to as the classical NF-κB pathway. There is an alternate pathway for NF-κB signaling, which stimulates cell survival without the need for BTK activation. MCL cell lines utilizing the alternative pathway of NF-κB signaling are primarily resistant to ibrutinib therapy. Key components and negative regulators of the alternative NF-κB pathway include tumor necrosis factor receptor–associated factor-2 (TRAF2), TRAF3, and baculoviral inhibitor of apoptosis proteins repeat-containing 3 (BIRC3). These proteins are negative regulators of the alternative NF-κB pathway, and loss of their function stimulates cell survival. These proteins also serve as negative regulators of mitogen-activated protein kinase kinase kinase 14 (MAP3K14), the central activating kinase of the alternative NF-κB pathway.[68] Loss of TRAF2, TRAF3, or BIRC3 function results in constitutive activation of MAP3K14 and subsequent cell survival via the alternative NF-κB pathway.[69] As such, mutations in TRAF2, TRAF3, BIRC3, and MAP3K14 have been described in patients with MCL with primary ibrutinib resistance.[68]

Another mechanism of primary BTKi resistance in patients with MCL treated with a combination of ibrutinib and venetoclax is

mutations that prohibit cancer cell death. The switch/sucrose nonfermentable (SWI/SNF) complex acts as a tumor suppressor in MCL and other malignancies. This complex remodels chromatin and packages DNA. The complex is composed of many subunits, including but not limited to, AT-rich interactive domain 2 (ARID2); SMARCA2; and SMARCA4. Mutations of these complex subunits are associated with increased B-cell lymphoma-extralarge (BCL-XL, an antiapoptotic protein), which inhibits cell death.[70] Recurrent mutations of ARID2, SMARCA2, and SMARCA4 have been associated with primary ibrutinib resistance in patients with MCL.[70]

As described in prior sections, acquired resistance to ibrutinib is poorly characterized in patients with MCL, but has been associated with BTK, PLCγ2, or CARD11 mutations.[50,60] As ibrutinib resistance is complex with multiple etiologies, continued research is necessary to unravel all mechanisms of ibrutinib resistance in patients with MCL.

BTKi resistance mutations in patients with DLBCL

DLBCL is a heterogeneous lymphoma with distinctive subtypes. Here, ibrutinib resistance is complex and most commonly primary. One major subtype of DLBCL, termed "activated B-cell-like (ABC)," defines a group that has acquired mutations in the BCR pathway to enhance lymphoma survival via chronic activation of this pathway.[71] Thus, it was hypothesized that this subgroup would have enriched response to ibrutinib compared with other DLBCL subtypes. A phase 2 study using ibrutinib to treat DLBCL confirmed that ABC patients with DLBCL had better responses than other DLBCL subtypes. However, responses were modest with an overall response rate of only 37%, indicating a high prevalence of primary resistance to therapy.[21] Ibrutinib-responsive DLBCL cell lines often have mutations in both MYD88 and CD79B, a subunit of the BCR.[21] Evidence to explain this observation may be related to the discovery of a multiprotein supercomplex formed in these cells.[72] This supercomplex includes MYD88, the BCR, and TLR9. The supercomplex colocalizes with mammalian target of rapamycin on endolysosomes and drives survival signals through both NF-κB and mammalian target of rapamycin signaling.[73] Blockade of the BCR signaling pathway with ibrutinib successfully inhibits tumor growth in these cells. One mechanism of primary ibrutinib resistance occurs in the presence of an inactivating mutation of KLHL14, a negative regulator of the BCR signaling pathway.[73] Loss of KLHL14 promotes the assembly of the supercomplex, which leads to overactivation of the NF-κB pathway, and subsequently, survival of the malignant cell. DLBCL cells with loss of KLHL14 are primarily resistant to ibrutinib.[73] Another DLBCL group that demonstrates primary resistance to ibrutinib is patients who have MYD88 mutations and wild-type CD79B.[21] These cells do not form the supercomplex, and it is thought that these tumors are able to activate the NF-κB survival pathway independently of the BCR pathways.[21] Mutations of tumor necrosis factor-α–induced protein 3 (TNFAIP), a negative regulator of the NF-κB pathway, also promote primary ibrutinib resistance.[21] Inactivation of TNFAIP3 leads to overactivation of the NF-κB pathway and enhances survival of the lymphoma cell. None of the patients with TNFAIP3 mutation responded to ibrutinib therapy in the phase 2 study.[21] Finally, as mentioned previously, mutations in CARD11 or BCL10 in the CBM complex leading to activation of NF-κB have also been documented in patients with DLBCL with primary ibrutinib resistance.[21,75] In summary, multiple known mutations focus on enhancement of cell survival via activation of the NF-κB pathway and lead to primary resistance in patients with DLBCL.

Clinical case: CLL therapy after BTKi resistance is detected

The patient's blood was sequenced for common ibrutinib mutations with an institutional next-generation sequencing panel, which revealed a BTK C481S and a PLCγ2 L845F mutation. As the patient was feeling clinically well, he continued ibrutinib therapy with careful clinical observation. After 6 months, he developed symptomatic splenomegaly and progressive cytopenias.

As mentioned previously, the survival of patients with CLL after developing BTKi resistance is quite short. It is hoped that novel therapeutic agents and continued research in this area will prolong the survival of this patient population (Table 2).

Venetoclax

Venetoclax is an oral inhibitor of the antiapoptotic protein BCL2. This drug is currently approved for marketing by the FDA for all patients with CLL to be used as monotherapy or in combination with an anti-CD20 monoclonal antibody. In a prospective phase 2 study (n = 127), single-agent venetoclax was administered to patients with CLL who had previously been treated with BTKi.[34] Of these patients, 21 had samples that underwent screening for mutations in BTK, and PLCγ2 and mutations were found in 17 patients. Of the 17 patients with known BTK and PLCγ2, 12 mutations (71%) had a response to venetoclax therapy (one was a complete response). Median PFS of patients with known BTK and PLCγ2 mutations was 21.9 months (95% CI, 4.4 to NR).[34] In a large retrospective study (n = 683), patients with CLL who received targeted kinase inhibitors were reviewed. In patients who initially received a BCR inhibitor (most commonly ibrutinib or idelalisib), 74% had a response to venetoclax-based therapy, and median PFS was not reached.[75] Unfortunately, resistance mutation status was unknown in these patients. These data support the use of venetoclax following detection of BTKi resistance.

PI3K inhibitors (PI3Ki's)

Idelalisib and duvelisib are oral PI3Ki that are currently approved for marketing by the FDA in patients with relapsed CLL. PI3Ki showed some evidence of efficacy in CLL cell lines with the BTK C481S mutation.[76] Of note, in a CLL cell line that harbors a PLCγ2 mutation at R665W, neither idelalisib or duvelisib could block the activation of the BCR signaling pathway.[77] In the previously mentioned large retrospective study, patients with CLL who received idelalisib therapy following ibrutinib therapy had an overall response rate of 47% with a short median PFS of 9 months.[75] Several prospective studies have attempted to show efficacy of PI3Ki in BTKi-resistant patients, but there are minimal available published data to support the use of PI3Ki's in this setting.[78]

STEPHENS and BYRD

Table 2. Therapies for patients with CLL with available data on patients with BTKi resistance mutations

Treatment	Study description	No. of patients with documented BTKi resistance mutations	No. of ORR in patients with BTKi resistance mutations (%)	No. of median PFS in patients with BTKi resistance mutations (95% CI)	Clinical trial reference
Venetoclax	Prospective phase 2	17	12 (71)	21.9 (4.4-NR)	34
Duvelisib	Prospective phase 1	3	0 (0)	NA	79
ARQ-531	Prospective phase 1	9*	8 (89)	NA	80
LOXO-305	Prospective phase 1	24†	17 (70)	NA	.81

NA, not available; NR, not reached; ORR, overall response rate.

*No. of patients with BTKi-resistant mutations that were treated at the recommended dose of 65 mg daily.

†Twenty-four patients on study had a BTK C481S mutation, and 4 additional patients had a PLC2 mutation, but response data were not available for this population.

Third-generation reversible BTKi's

At the time of manuscript preparation, third-generation BTKi, such as ARQ-531 and LOXO-305, are only available via participation in clinical trials. These BTKi's are distinguished from the currently available BTKi's, as they bind in a noncovalent and reversible manner at the adenosine triphosphate binding region of BTK, which negates the need for C481 binding for activity.[79,80] Early phase 1 data show that when oral ARQ-531 was administered at its target dose of 65 mg daily, responses were seen in 8 of 9 patients with CLL with known BTK C481S mutations.[79] Early phase 1 data show that when oral LOXO-305 was administered to relapsed patients with CLL, responses were seen in 17 of 24 patients with CLL with known BTK mutations.[80] The durability of response with these agents is uncertain. Nonetheless, these data make the third-generation BTKi's an interesting class of drugs for further study in this setting.

Cellular therapies

Limited data support allogeneic hematopoietic stem cell transplantation (HSCT) in patients with CLL who have received prior BTKi therapy. In a small retrospective study, 65 patients with CLL who underwent allogeneic HSCT who had previously received ibrutinib, venetoclax, or idelalisib were reviewed.[81] Details about presence of resistance mutations were not available, but 82% of these patients had previously received ibrutinib and 26% had received both ibrutinib and venetoclax therapy prior to allogeneic HSCT. In this study, the 2-year PFS and OS were 63% and 81%, respectively.[81] The 2-year nonrelapse mortality was 13%, indicating patients should be carefully selected for this therapy. Accordingly, allogeneic HSCT could be considered for young and healthy patients who have progressed on both BTKi and venetoclax therapy.[82]

CART is a novel therapy where the patient's own T cells are extracted and a multidomain (chimeric), engineered molecule is inserted into the T cells prior to infusing the cells back into the patient. Early clinical trial data support the efficacy in patients with heavily pretreated CLL. In a phase 1/2 study using a defined (1:1) composition of CD4 and CD8 anti-CD19 directed CART, 24 patients with CLL previously treated with ibrutinib (19 with progression on ibrutinib) were evaluated.[83] In 19 patients that underwent response assessment, the ORR was 74% with 21% in complete remission. After a short median follow-up of 6.6 months, the median PFS was 9.8 months and not reached in patients who achieved complete response and partial response, respectively. Nine of the 19 patients with prior progression on
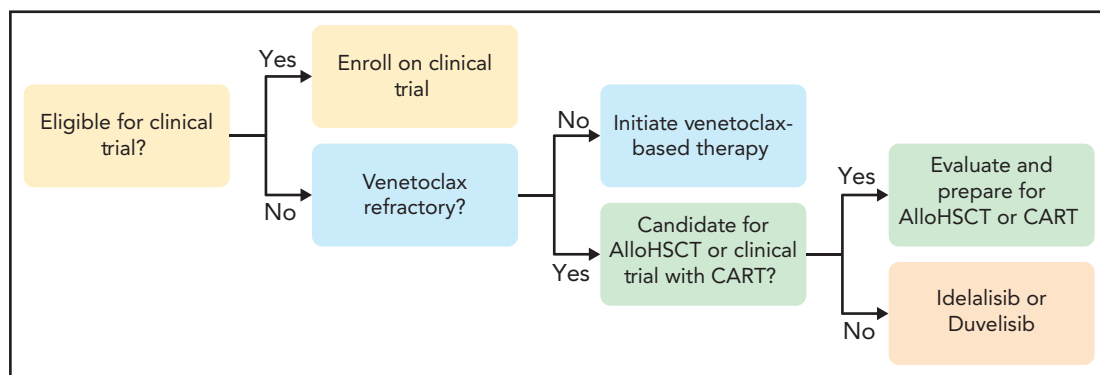

Figure 3. Suggested management for patient with CLL who develop resistance to currently approved BTKi. AlloHSCT, allogeneic hematopoietic stem cell transplantation; CART, chimeric antigen receptor T cell.

ibrutinib had known resistance mutations (BTK = 7 and PLCγ2 = 2); however, clinical outcomes were not reported separately for this subgroup.[83] In an attempt to improve the CART efficacy and reduce the toxicity of cytokine release, the same group combined the anti-CD19–directed CART with ibrutinib in 19 ibrutinib-resistant patients with CLL.[84] The ORR was 83%. In 18 evaluable patients, the 1-year PFS and OS rates were 38% (95% CI, 19-78) and 64% (95% CI, 42-98), respectively. Most patients did not have testing for resistance mutations.[84] In a phase 1 study using lisocabtagene maraleucel, a similar defined composition CD4:CD8 anti-CD19 directed CART, a subgroup of 11 patients who had previously progressed on both ibrutinib and venetoclax therapy.[85] Response was seen in 8 of 10 evaluable patients. The median PFS for the 11 patients in this group was 13 months (95% CI, 2.8–not reached). For the 8 patients who responded, median duration of response was 17 months (95% CI, 1.9 to NR). Resistance mutation status was not reported.[85] In a phase 1 study using tisagenlecleucel, another anti-CD19–directed CART, 8 of 14 heavily pretreated patients responded to therapy with 4 achieving complete remission. Only 1 patient had received prior ibrutinib, and no mutation status was noted.[86] Another phase 1 study using axicabtagene ciloleucel, a third anti-CD19–directed CART, 7 of 8 patients with CLL had response. The median event-free survival for these patients was 40.5 months. The percentage of treatments leading to a duration of response >3 years was 50% (95% CI, 16% to 84%).[87] Data regarding prior therapy and resistance mutation status were not reported. A related immunotherapy termed chimeric antigen receptor natural killer (CAR-NK) cell therapy uses a similar process of retroviral vector insertion of an anti-CD19 CAR into donor NK cells before infusion into the patient.[88] Early data from a phase 1/2 study demonstrated the anti-CD19 CAR-NK induced a complete response in 3 of the 5 heavily treated (all with prior ibrutinib) patients with CLL. One of these patients that did not achieve a CLL response also had Richter transformation and achieved complete response of the Richter transformation. Follow-up of this study was a short 13.8 months, and no details of BTK resistance mutations were available.[88] In this initial study, most patients also received alternative therapies after attaining response to the CAR-NK cell therapy, making evaluation of durability of remission problematic. A follow-up clinical trial will be required to assure the benefit of this therapy in the absence of follow-up treatments.

Clinical case: management algorithm for BTKi resistance

The patient was switched to venetoclax therapy. He had an initial response to therapy, which lasted ~18 months and then had symptomatic relapse. He was referred for a clinical trial with CART.

Figure 3 shows an algorithm with our recommended management of a patient with CLL with BTKi resistance. In general, once clinical BTKi resistance is detected, the patient should be enrolled on a clinical trial, if eligible. If the patient is not yet venetoclax refractory, the combination of venetoclax and rituximab should be considered. If the patient is

refractory to both the currently approved BTKi's and venetoclax, the patient should be considered for allogeneic HSCT or a clinical trial with CART therapy. If the patient is not a candidate for HSCT or CART therapy, idelalisib or duvelisib should be considered.

Summary

Although BTKi's have significantly changed the treatment landscape for patients with B-cell malignancies, BTKi resistance remains a clinical problem. A major consequence of BTKi resistance is shortened survival. BTKi resistance has been associated with several genetic and clinical risk factors, which have been incorporated into predictive models for clinical use.[42,43] Mutations in BTK and PLCγ2 are the most common and most robustly researched mechanism leading to BTKi resistance. Many other potential mechanisms have been described, and ongoing research will clarify the relevance of these mechanisms. The introduction of venetoclax has improved the survival for patients with BTKi-resistant disease. Ongoing clinical trials of third-generation noncovalent BTKi's and cellular therapies, such as CART, provide much hope for these patients. In summary, continued additional research is needed to further prolong the survival of patients with BTKi-resistant B-cell malignancies.

Acknowledgments

Authorship

Contribution: D.M.S. and J.C.B. collected and analyzed data, drafted the original manuscript, revised the manuscript, and approved the final draft.

Conflict-of-interest disclosure: D.M.S. has received research funding from Acerta, Gilead, Karyopharm, Verastem, JUNO, Arqule, Mingsight. She has served on advisory boards for Innate, Jannsen/Pharmacyclics, Epizyme, Karyopharm, Beigene, Adaptive, and TG Therapeutics. J.C.B. has received research funding from AstraZeneca, Gilead, Karyopharm, Celgene, Pharmacyclics/Abvie, Kartos, Telios, and Newave. He has consulted for Jannsen, Pharmacyclics, AstraZeneca, Novartis, Trillium, and Syndax. He is a founder and major stock owner in Vincera Pharmaceutical, Inc.

ORCID profile: D.M.S., 0000-0001-9188-5008.

Correspondence: John C. Byrd, 231 Albert Sabin Way, ML 0551, Room 6065, Cincinnati, OH 45267-0551; e-mail: byrd2jc@ucmail.uc.edu; and Deborah M. Stephens, 2000 Circle of Hope, Research South 5509, Huntsman Cancer Institute, University of Utah, Salt Lake City, UT 84112; e-mail: deborah.stephens@hci.utah.edu.

Footnotes

REFERENCES

1. Doyle SL, Jefferies CA, Feighery C, O'Neill LA. Signaling by Toll-like receptors 8 and 9 requires Bruton's tyrosine kinase. *J Biol Chem.* 2007;282(51):36953-36960.

2. Spaargaren M, Beuling EA, Rurup ML, et al. The B cell antigen receptor controls integrin activity through Btk and PLCgamma2. *J Exp Med.* 2003;198(10):1539-1550.

3. Turner M, Mee PJ, Costello PS, et al. Perinatal lethality and blocked B-cell development in mice lacking the tyrosine kinase Syk. *Nature.* 1995;378(6554):298-302.

4. Harris DP, Vogel P, Wims M, et al. Requirement for class II phosphoinositide 3-kinase C2alpha in maintenance of glomerular structure and function. *Mol Cell Biol.* 2011;31(1):63-80.

5. Khan WN, Alt FW, Gerstein RM, et al. Defective B cell development and function in Btk-deficient mice. *Immunity.* 1995;3(3):283-299.

6. Stevenson FK, Krysov S, Davies AJ, Steele AJ, Packham G. B-cell receptor signaling in chronic lymphocytic leukemia. *Blood.* 2011;118(16):4313-4320.

7. Stevenson FK, Forconi F, Packham G. The meaning and relevance of B-cell receptor structure and function in chronic lymphocytic leukemia. *Semin Hematol.* 2014;51(3):158-167.

8. Paterson A, Mockridge CI, Adams JE, et al. Mechanisms and clinical significance of BIM phosphorylation in chronic lymphocytic leukemia. *Blood.* 2012;119(7):1726-1736.

9. Krysov S, Dias S, Paterson A, et al. Surface IgM stimulation induces MEK1/2-dependent MYC expression in chronic lymphocytic leukemia cells. *Blood.* 2012;119(1):170-179.

10. Bond DA, Woyach JA. Targeting BTK in CLL: beyond ibrutinib. *Curr Hematol Malig Rep.* 2019;14(3):197-205.

11. Ferrer G, Montserrat E. Critical molecular pathways in CLL therapy. *Mol Med.* 2018;24(1):9-18.

12. Burger JA, Chiorazzi N. B cell receptor signaling in chronic lymphocytic leukemia. *Trends Immunol.* 2013;34(12):592-601.

13. Dühren-von Minden M, Übelhart R, Schneider D, et al. Chronic lymphocytic leukaemia is driven by antigen-independent cell-autonomous signalling. *Nature.* 2012;489(7415):309-312.

14. Chen SS, Chang BY, Chang S, et al. BTK inhibition results in impaired CXCR4 chemokine receptor surface expression, signaling and function in chronic lymphocytic leukemia. *Leukemia.* 2016;30(4):833-843.

15. Byrd JC, Brown JR, O'Brien S, et al; RESONATE Investigators. Ibrutinib versus ofatumumab in previously treated chronic lymphoid leukemia. *N Engl J Med.* 2014;371(3):213-223.

16. Burger JA, Tedeschi A, Barr PM, et al; RESONATE-2 Investigators. Ibrutinib as initial therapy for patients with chronic lymphocytic leukemia. *N Engl J Med.* 2015;373(25):2425-2437.

17. Shanafelt TD, Wang XV, Kay NE, et al. Ibrutinib-rituximab or chemoimmunotherapy for chronic lymphocytic leukemia. *N Engl J Med.* 2019;381(5):432-443.

18. Woyach JA, Ruppert AS, Heerema NA, et al. Ibrutinib regimens versus chemoimmunotherapy in older patients with untreated CLL. *N Engl J Med.* 2018;379(26):2517-2528.

19. Dreyling M, Jurczak W, Jerkeman M, et al. Ibrutinib versus temsirolimus in patients with relapsed or refractory mantle-cell lymphoma: an international, randomised, open-label, phase 3 study. *Lancet.* 2016;387(10020):770-778.

20. Dimopoulos MA, Tedeschi A, Trotman J, et al; iNNOVATE Study Group and the European Consortium for Waldenström's Macroglobulinemia. Phase 3 trial of ibrutinib plus rituximab in Waldenström's macroglobulinemia. *N Engl J Med.* 2018;378(25):2399-2410.

21. Wilson WH, Young RM, Schmitz R, et al. Targeting B cell receptor signaling with ibrutinib in diffuse large B cell lymphoma. *Nat Med.* 2015;21(8):922-926.

22. Sharman JP, Egyed M, Jurczak W, et al. Acalabrutinib with or without obinutuzumab versus chlorambucil and obinutuzmab for treatment-naive chronic lymphocytic leukaemia (ELEVATE TN): a randomised, controlled, phase 3 trial. *Lancet.* 2020;395(10232):1278-1291.

23. Ghia P, Pluta A, Wach M, et al. ASCEND: phase III, randomized trial of acalabrutinib versus idelalisib plus rituximab or bendamustine plus rituximab in relapsed or refractory chronic lymphocytic leukemia. *J Clin Oncol.* 2020;38(25):2849-2861.

24. Song Y, Zhou K, Zou D, et al. Treatment of patients with relapsed or refractory mantle-cell lymphoma with zanubrutinib, a selective inhibitor of Bruton's tyrosine kinase. *Clin Cancer Res.* 2020;26(16):4216-4224.

25. Tam CS, Opat S, D'Sa S, et al. A randomized phase 3 trial of zanubrutinib vs ibrutinib in symptomatic Waldenström macroglobulinemia: the ASPEN study. *Blood.* 2020;136(18):2038-2050.

26. Herman SE, Gordon AL, Hertlein E, et al. Bruton tyrosine kinase represents a promising therapeutic target for treatment of chronic lymphocytic leukemia and is effectively targeted by PCI-32765. *Blood.* 2011;117(23):6287-6296.

27. Byrd JC, Harrington B, O'Brien S, et al. Acalabrutinib (ACP-196) in relapsed chronic lymphocytic leukemia. *N Engl J Med.* 2016;374(4):323-332.

28. Tam CS, Trotman J, Opat S, et al. Phase 1 study of the selective BTK inhibitor zanubrutinib in B-cell malignancies and safety and efficacy evaluation in CLL. *Blood.* 2019;134(11):851-859.

29. Herman SEM, Montraveta A, Niemann CU, et al. The Bruton tyrosine kinase (BTK) inhibitor acalabrutinib demonstrates potent on-target effects and efficacy in two mouse models of chronic lymphocytic leukemia. *Clin Cancer Res.* 2017;23(11):2831-2841.

30. Maddocks KJ, Ruppert AS, Lozanski G, et al. Etiology of ibrutinib therapy discontinuation and outcomes in patients with chronic lymphocytic leukemia. *JAMA Oncol.* 2015;1(1):80-87.

31. Jain P, Keating M, Wierda W, et al. Outcomes of patients with chronic lymphocytic leukemia after discontinuing ibrutinib. *Blood.* 2015;125(13):2062-2067.

32. Byrd JC, Furman RR, Coutre SE, et al. Ibrutinib treatment for first-line and relapsed/refractory chronic lymphocytic leukemia: final analysis of the pivotal phase Ib/II PCYC-1102 study. *Clin Cancer Res.* 2020;26(15):3918-3927.

33. Woyach J, Huang Y, Rogers K, et al. Resistance to acalabrutinib in CLL is mediated primarily by BTK mutations [abstract]. *Blood.* 2019;134(suppl_1). Abstract 504.

34. Jones JA, Mato AR, Wierda WG, et al. Venetoclax for chronic lymphocytic leukaemia progressing after ibrutinib: an interim analysis of a multicentre, open-label, phase 2 trial. *Lancet Oncol.* 2018;19(1):65-75.

35. Hampel PJ, Ding W, Call TG, et al. Rapid disease progression following discontinuation of ibrutinib in patients with chronic lymphocytic leukemia treated in routine clinical practice. *Leuk Lymphoma.* 2019;60(11):2712-2719.

36. Woyach JA, Ruppert AS, Guinn D, et al. BTK^{C481S}-mediated resistance to ibrutinib in chronic lymphocytic leukemia. *J Clin Oncol.* 2017;35(13):1437-1443.

37. Brown JR, Hillmen P, O'Brien S, et al. Extended follow-up and impact of high-risk prognostic factors from the phase 3 RESONATE study in patients with previously treated CLL/SLL. *Leukemia.* 2018;32(1):83-91.

38. Ahn IE, Tian X, Wiestner A. Ibrutinib for chronic lymphocytic leukemia with *TP53* alterations. *N Engl J Med.* 2020;383(5):498-500.

39. Haferlach C, Dicker F, Schnittger S, Kern W, Haferlach T. Comprehensive genetic characterization of CLL: a study on 506 cases analysed with chromosome banding analysis, interphase FISH, IgV(H) status and immunophenotyping. *Leukemia.* 2007;21(12):2442-2451.

40. Malcikova J, Stano-Kozubik K, Tichy B, et al. Detailed analysis of therapy-driven clonal evolution of TP53 mutations in chronic lymphocytic leukemia. *Leukemia.* 2015;29(4):877-885.

41. Brejcha M, Stoklasová M, Brychtová Y, et al. Clonal evolution in chronic lymphocytic leukemia detected by fluorescence in situ hybridization and conventional cytogenetics after stimulation with CpG oligonucleotides and interleukin-2: a prospective analysis. *Leuk Res.* 2014;38(2): 170-175.

42. Ahn IE, Tian X, Ipe D, et al. Prediction of outcome in patients with chronic lymphocytic leukemia treated with ibrutinib: development and validation of a four-factor prognostic model. *J Clin Oncol.* 2021;39(6): 576-585.

43. Soumerai JD, Ni A, Darif M, et al. Prognostic risk score for patients with relapsed or refractory chronic lymphocytic leukaemia treated with targeted therapies or chemoimmunotherapy: a retrospective, pooled cohort study with external validations. *Lancet Haematol.* 2019;6(7): e366-e374.

44. Guarini A, Peragine N, Messina M, et al. Unravelling the suboptimal response of TP53-mutated chronic lymphocytic leukaemia to ibrutinib. *Br J Haematol.* 2019;184(3): 392-396.

45. Furman RR, Cheng S, Lu P, et al. Ibrutinib resistance in chronic lymphocytic leukemia [published correction appears in *N Engl J Med.* 2014;370(26):2547]. *N Engl J Med.* 2014;370(24):2352-2354.

46. Woyach JA, Furman RR, Liu TM, et al. Resistance mechanisms for the Bruton's tyrosine kinase inhibitor ibrutinib. *N Engl J Med.* 2014;370(24): 2286-2294.

47. Komarova NL, Burger JA, Wodarz D. Evolution of ibrutinib resistance in chronic lymphocytic leukemia (CLL). *Proc Natl Acad Sci USA.* 2014;111(38):13906-13911.

48. Ahn IE, Underbayev C, Albitar A, et al. Clonal evolution leading to ibrutinib resistance in chronic lymphocytic leukemia. *Blood.* 2017;129(11):1469-1479.

49. Xu L, Tsakmaklis N, Yang G, et al. Acquired mutations associated with ibrutinib resistance in Waldenström macroglobulinemia. *Blood.* 2017;129(18): 2519-2525.

50. Chiron D, Di Liberto M, Martin P, et al. Cell-cycle reprogramming for PI3K inhibition overrides a relapse-specific C481S BTK mutation revealed by longitudinal functional genomics in mantle cell lymphoma. *Cancer Discov.* 2014;4(9):1022-1035.

51. Epperla N, Shana'ah AY, Jones D, et al. Resistance mechanism for ibrutinib in marginal zone lymphoma. *Blood Adv.* 2019; 3(4):500-502.

52. Johnson AR, Kohli PB, Katewa A, et al. Battling Btk mutants with noncovalent inhibitors that overcome Cys481 and Thr474 mutations. *ACS Chem Biol.* 2016;11(10): 2897-2907.

53. Sharma S, Galanina N, Guo A, et al. Identification of a structurally novel BTK mutation that drives ibrutinib resistance in CLL. *Oncotarget.* 2016;7(42): 68833-68841.

54. Kadri S, Lee J, Fitzpatrick C, et al. Clonal evolution underlying leukemia progression and Richter transformation in patients with ibrutinib-relapsed CLL. *Blood Adv.* 2017; 1(12):715-727.

55. Burger JA, Landau DA, Taylor-Weiner A, et al. Clonal evolution in patients with chronic lymphocytic leukaemia developing resistance to BTK inhibition. *Nat Commun.* 2016;7(1):11589-11602.

56. Rubio-Moscardo F, Blesa D, Mestre C, et al. Characterization of 8p21.3 chromosomal deletions in B-cell lymphoma: TRAIL-R1 and TRAIL-R2 as candidate dosage-dependent tumor suppressor genes. *Blood.* 2005; 106(9):3214-3222.

57. Jiménez C, Chan GG, Xu L, et al. Genomic evolution of ibrutinib-resistant clones in Waldenström macroglobulinaemia. *Br J Haematol.* 2020;189(6):1165-1170.

58. Kanagal-Shamanna R, Jain P, Patel KP, et al. Targeted multigene deep sequencing of Bruton tyrosine kinase inhibitor-resistant chronic lymphocytic leukemia with disease progression and Richter transformation. *Cancer.* 2019;125(4):559-574.

59. Bartlett NL, Costello BA, LaPlant BR, et al. Single-agent ibrutinib in relapsed or refractory follicular lymphoma: a phase 2 consortium trial. *Blood.* 2018;131(2): 182-190.

60. Wu C, de Miranda NF, Chen L, et al. Genetic heterogeneity in primary and relapsed mantle cell lymphomas: impact of recurrent CARD11 mutations. *Oncotarget.* 2016;7(25):38180-38190.

61. Chen JG, Liu X, Munshi M, et al. BTK^Cys481Ser drives ibrutinib resistance via ERK1/2 and protects BTK^wild-type MYD88-mutated cells by a paracrine mechanism. *Blood.* 2018;131(18):2047-2059.

62. Burger J, Landau D, Hoellenriegel J, et al. Clonal evolution in patients with chronic lymphocytic leukemia (CLL) developing resistance to BTK inhibition [abstract]. *Blood.* 2013;122(21). Abstract 866.

63. Cheng S, Guo A, Lu P, Ma J, Coleman M, Wang YL. Functional characterization of BTK(C481S) mutation that confers ibrutinib resistance: exploration of alternative kinase inhibitors. *Leukemia.* 2015;29(4): 895-900.

64. Vos JM, Tsakmaklis N, Patterson CJ, et al. CXCL13 levels are elevated in patients with Waldenström macroglobulinemia, and are predictive of major response to ibrutinib. *Haematologica.* 2017;102(11):e452-e455.

65. Treon SP, Tripsas CK, Meid K, et al. Ibrutinib in previously treated Waldenström's macroglobulinemia. *N Engl J Med.* 2015; 372(15):1430-1440.

66. Ma J, Lu P, Guo A, et al. Characterization of ibrutinib-sensitive and -resistant mantle lymphoma cells. *Br J Haematol.* 2014;166(6): 849-861.

67. Zhao X, Lwin T, Silva A, et al. Unification of de novo and acquired ibrutinib resistance in mantle cell lymphoma. *Nat Commun.* 2017; 8(1):14920-14935.

68. Rahal R, Frick M, Romero R, et al. Pharmacological and genomic profiling identifies NF-κB-targeted treatment strategies for mantle cell lymphoma. *Nat Med.* 2014;20(1):87-92.

69. Diop F, Moia R, Favini C, et al. Biological and clinical implications of *BIRC3* mutations in chronic lymphocytic leukemia. *Haematologica.* 2020;105(2):448-456.

70. Agarwal R, Chan YC, Tam CS, et al. Dynamic molecular monitoring reveals that SWI-SNF mutations mediate resistance to ibrutinib plus venetoclax in mantle cell lymphoma. *Nat Med.* 2019;25(1):119-129.

71. Alizadeh AA, Eisen MB, Davis RE, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature.* 2000;403(6769):503-511.

72. Phelan JD, Young RM, Webster DE, et al. A multiprotein supercomplex controlling oncogenic signalling in lymphoma. *Nature.* 2018;560(7718):387-391.

73. Choi J, Phelan JD, Wright GW, et al. Regulation of B cell receptor-dependent NF-κB signaling by the tumor suppressor KLHL14. *Proc Natl Acad Sci USA.* 2020; 117(11):6092-6102.

74. Xia M, David L, Teater M, et al. BCL10 gain-of-function mutations aberrantly induce canonical and non-canonical NF-Kb activation and resistance to ibrutinib in ABC-DLBCL [abstract 2-3]. *Blood.* 2020; 136(suppl 1). Abstract 2-3.

75. Mato AR, Hill BT, Lamanna N, et al. Optimal sequencing of ibrutinib, idelalisib, and venetoclax in chronic lymphocytic leukemia: results from a multicenter study of 683 patients. *Ann Oncol.* 2017;28(5):1050-1056.

76. Dong S, Guinn D, Dubovsky JA, et al. IPI-145 antagonizes intrinsic and extrinsic survival signals in chronic lymphocytic leukemia cells. *Blood.* 2014;124(24): 3583-3586.

77. Liu TM, Woyach JA, Zhong Y, et al. Hypermorphic mutation of phospholipase C, γ2 acquired in ibrutinib-resistant CLL confers BTK independency upon B-cell receptor activation. *Blood.* 2015;126(1): 61-68.

78. Porcu P, Flinn I, Kahl BS, et al. Clinical activity of duvelisib (IPI-145), a phosphoinositide-3-kinase-δ,γ inhibitor, in patients previously treated with ibrutinib [abstract]. *Blood.* 2014;124(21). Abstract 3335.

79. Woyach J, Stephens DM, Flinn IW, et al. Final results of phase 1, dose escalation study evaluating ARQ 531 in patients with relapsed or refractory B-cell lymphoid malignancies [abstract]. *Blood.* 2019;134(suppl_1). Abstract 4298.

80. Mato AR, Pagel JM, Coombs CC, et al. LOXO-305, a next generation, highly

STEPHENS and BYRD

selective, non-covalent BTK inhibitor in previously treated CLL/SLL: results from the phase 1/2 BRUIN Study [abstract 35-37]. *Blood*. 2020;136(suppl 1). Abstract 35-37.

81. Roeker LE, Dreger P, Brown JR, et al. Allogeneic stem cell transplantation for chronic lymphocytic leukemia in the era of novel agents. *Blood Adv*. 2020;4(16): 3977-3989.

82. Kharfan-Dabaja MA, Kumar A, Hamadani M, et al. Clinical practice recommendations for use of allogeneic hematopoietic cell transplantation in chronic lymphocytic leukemia on behalf of the guidelines committee of the American Society for Blood and Marrow Transplantation. *Biol Blood Marrow Transplant*. 2016;22(12):2117-2125.

83. Turtle CJ, Hay KA, Hanafi LA, et al. Durable molecular remissions in chronic lymphocytic leukemia treated with CD19-specific chimeric antigen receptor-modified T cells after failure of ibrutinib. *J Clin Oncol*. 2017;35(26):3010-3020.

84. Gauthier J, Hirayama AV, Purushe J, et al. Feasibility and efficacy of CD19-targeted CAR T cells with concurrent ibrutinib for CLL after ibrutinib failure. *Blood*. 2020;135(19): 1650-1660.

85. Siddiqi T, Soumerai JD, Dorritie KA, et al. Updated follow-up of patients with relapsed/refractory chronic lymphocytic leukemia/small lymphocytic lymphoma treated with lisocabtagene maraleucel in the phase 1 monotherapy cohort of transcend CLL 004, including high-risk and ibrutinib-treated patients [abstract]. *Blood*. 2020;136(suppl 1). Abstract 40-41.

86. Porter DL, Hwang WT, Frey NV, et al. Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia. *Sci Transl Med*. 2015;7(303): 303ra139.

87. Cappell KM, Sherry RM, Yang JC, et al. Long-term follow-up of anti-CD19 chimeric antigen receptor T-cell therapy. *J Clin Oncol*. 2020;38(32):3805-3815.

88. Liu E, Marin D, Banerjee P, et al. Use of CAR-transduced natural killer cells in CD19-positive lymphoid tumors. *N Engl J Med*. 2020;382(6):545-553.

89. Hendriks RW, Yuvaraj S, Kil LP. Targeting Bruton's tyrosine kinase in B cell malignancies. *Nat Rev Cancer*. 2014;14(4): 219-232.