

Chapter 6

Resistance to Bruton's Tyrosine Kinase Signaling Pathway Targeted Therapies



Yaya Chu, Mitchell S. Cairo, and Auke Beishuizen

Abstract Activation of B-cell receptor (BCR) signaling is an important mechanism of the development and growth of B-cell lymphomas. Bruton's tyrosine kinase (BTK) is a key component of BCR signaling and functions as an important regulator of cell proliferation and cell survival in various B-cell lymphomas. BTK inhibitors, especially ibrutinib, have shown promising anti-tumor activity in preclinical and clinical studies. High response rates of ibrutinib were reported in patients with a variety of B-cell non-Hodgkin lymphoma (B-NHL) such as chronic lymphocytic leukemia (CLL) and mantle cell lymphoma (MCL). However, clinical evidence shows primary and acquired resistance to BTK inhibitors in patients. Understanding the molecular mechanisms underlying BTK inhibitors' resistance is of paramount importance. In this review, we highlight the potential resistant mechanisms, which include mutational resistance in BTK, mutational resistance in other proteins than in BTK, chromosomal abnormalities, activation of prosurvival pathways, B-cell lymphoma 2 (BCL-2) family members mediated resistance, and tumor microenvironment mediated resistance. We also discuss the strategies that are utilized to overcome BTK inhibitors' resistance: non-covalent inhibitors of BTK, alternate kinase inhibitors, combination therapies with other oncogenic inhibitors, BCL-2 inhibitors, anti-CD20 antibodies, anti-CD19 chimeric antigen receptor (CAR) T cells, CD19/CD3 bispecific antibody, or with inhibitors targeting other cellular processes.

Y. Chu (✉)

Department of Pediatrics, New York Medical College, Valhalla, NY, USA
e-mail: yaya_chu@nymc.edu

M. S. Cairo

Departments of Pediatrics, Medicine, Pathology, Microbiology and Immunology,
Cell Biology and Anatomy, New York Medical College, Valhalla, NY, USA

A. Beishuizen

Erasmus MC-Sophia Children's Hospital, University Medical Center Rotterdam and Princess
Maxima Center for Pediatric Oncology, Utrecht, Netherlands

Keywords Targeted therapies · Bruton's tyrosine kinase · Ibrutinib · Drug resistance · B-cell lymphoma · Activation of B-cell receptor

Abbreviations

ABC-DLBCL	Activated B-Cell- Diffuse Large B-cell Lymphoma
AKT	Protein Kinase B
AS-PCR	Allele-Specific Polymerase Chain Reaction
BCR	Activation of B Cell Receptor
BCL-2	B-Cell Lymphoma 2
BL	Burkitt Lymphoma
B-NHL	B cell Non-Hodgkin Lymphoma
BLNK	B-cell Linker Protein
BTK	Bruton's Tyrosine Kinase
CAR	Chimeric Antigen Receptor
CCND1	Cell Cycle Regulator Cyclin D1
CLL	Chronic Lymphocytic Leukemia
CARD11	Caspase Recruitment Domain Family, Member 11
CDK4	Cyclin-Dependent Kinase 4
CR	Complete Response
CRM1/XPO1	Chromosome Region Maintenance1/Exportin-1 Protein
CXCR4	C-X-C Chemokine Receptor type 4
DPPYs	Diphenylpyrimidine Derivatives
DLBCL	Diffuse Large B-cell Lymphoma
DLT	Dose-Limited Toxicities
EFS	Event Free Survival
EGFR	Epidermal Growth Factor Receptor
EIF2A	Eukaryotic Translation Initiation Factor 2A
ERK	Extracellular Signal-Regulated Kinase
FDA	Food and Drug Administration
FL	Follicular Lymphoma
FLIPI	Follicular Lymphoma International Prognostic Index
GBC	Germinal Center B cell
HCL	Hairy cell Lymphoma
HDAC	Histone Deacetylase
HL	Hodgkin Lymphoma
IC50	Half Maximal Inhibitory Concentration
IκB	Inhibitor of Kappa B
IKKb	Inhibitor of Kappa Light Polypeptide Gene Enhancer in B-cells
ITAM	Immunoreceptor Tyrosine-Based Activation Motifs
Itk	Interleukin-2-Inducible T-Cell Kinase
LCK	Lymphocyte-Specific Protein Tyrosine Kinase

LNA	Locked Nucleic Acid
MALT1	Mucosa Associated Lymphoid Tissue Lymphoma Translocation Protein 1
MAPK	Mitogen-Activated Protein Kinase
MCL	Mantle Cell Lymphoma
MLL2	Mixed Lineage Leukemia 2
MOMP	Mitochondrial Outer Membrane Permeability
MPFS	Median Progression-Free Survival
MRD	Minimal Residual Disease
mTOR	Mechanistic Target of Rapamycin
MYD88	Myeloid Differentiation Primary Response Gene (88)
MZL	Marginal zone Lymphoma
NHL	Non-Hodgkin's Lymphoma
NF- κ B	Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B cells
NGS	Next-Generation Sequencing
NIK	NF-Kappa-B-Inducing Kinase
NSG	NOD.Cg-PrkdcscidIl2rgtm1Wjl/SzJ
OS	Overall Survival
ORR	Overall Response Rate
P	Phosphorylation
PARP-1	Poly [ADP-ribose] Polymerase 1
PFS	Progression-Free Survival
PH	Pleckstrin Homology
PI3K	Phosphoinositide 3-Kinase
PIM1	Serine/threonine Kinase pim-1
PIP3	Phosphatidylinositol (3,4,5)-Trisphosphate
PLC γ 2	1-phosphatidylinositol-4,5-Bisphosphate Phosphodiesterase Gamma-2
PMBCL	Primary Mediastinal B-cell Lymphoma
PR	Partial Response
RPS15	40S Ribosomal Protein S15
R/R	Relapsed/Refractory
scFv	Single Chain Fragment of Variable Region
SFK	Src Family Tyrosine Kinases
SH2	Src Homology 2
SH3	Src Homology 3
SNPs	Single Nucleotide Polymorphisms
SLL	Small Lymphocytic Lymphoma
SYK	Spleen Tyrosine Kinase
Tec	Tyrosine Kinase Expressed in Hepatocellular Carcinoma
TLR	Toll-Like Receptor
TME	Tumor Microenvironment
TRAIL	Tumor Necrosis Factor Related Apoptosis Inducing Ligand

TRAIL-R	Tumor Necrosis Factor Related Apoptosis Inducing Ligand Receptors
Txk	Tyrosine-Protein Kinase TXK
WES	Whole-Exome Sequencing
WM	Waldenström's Macroglobulinemia
XLA	X-Linked Agammaglobulinemia
2p+	Gain of the Short Arm of Chromosome 2

Introduction

B-cell lymphoma represents a heterogeneous group of B-cell malignancies with distinct pathological characteristics, clinical features and prognoses [1]. The most common types of B-cell lymphoma include chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL), diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), mantle cell lymphoma (MCL), Waldenström's macroglobulinemia (WM), and Burkitt lymphoma (BL). In children, the vast majority of B-cell lymphomas are BL and DLBCL, rarely primary mediastinal B-cell lymphoma (PMBCL) and FL are found. Cairo et al. previously demonstrated that short but intensive chemotherapy is associated with an 80% 5-year event free survival (EFS) in patients with advanced mature B-cell non-Hodgkin lymphoma (B-NHL) [2–4]. Further, an international multi-cooperative group study showed a 90% 5-year overall survival (OS) in patients with newly diagnosed mature B-NHL [5–8]. Unfortunately, the outcome is dismal in patients with aggressive B-NHL, who relapse or progress due to chemoradiotherapy resistance [5, 8]. Therefore, facilitating the development of alternative novel therapeutic strategies is required to improve the outcome in patients with relapsed/refractory (R/R) B-cell lymphoma.

Activation of the BCR signaling pathway (Fig. 6.1) is critical to the development and maturation of B cells [9, 10] and the viability of a variety of B-cell lymphomas such as DLBCL [11], marginal zone lymphoma (MZL) [12], MCL, FL [13] and BL [14]. The BCR consists of the antigen-binding immunoglobulin heavy (IgH) and light (IgL) chains coupled to the heterodimeric CD79a and CD79b proteins, which contain tyrosine-based activation motifs [15]. Crosslinking of BCR by antigen triggers the phosphorylation of tyrosines within the immunoreceptor tyrosine-based activation motifs (ITAMs) of CD79A and CD79B by Src family tyrosine kinases (SFKs) [15]. The phosphorylated ITAMs serve as a scaffolding platform for engaging and activating Src homology 2 (SH2) domains containing kinases, including spleen tyrosine kinase (SYK). Activated SYK phosphorylates the B-cell linker protein (BLNK) to further recruit both Bruton's Tyrosine Kinase (BTK) and phospholipase C- γ 2 (PLC- γ 2) through their SH2 domains. BTK is then phosphorylated and activated by SYK to drive the activation of downstream signaling pathways such as phosphatidylinositol-3-kinase (PI3K)/protein kinase B (Akt) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) [16]. The complex of

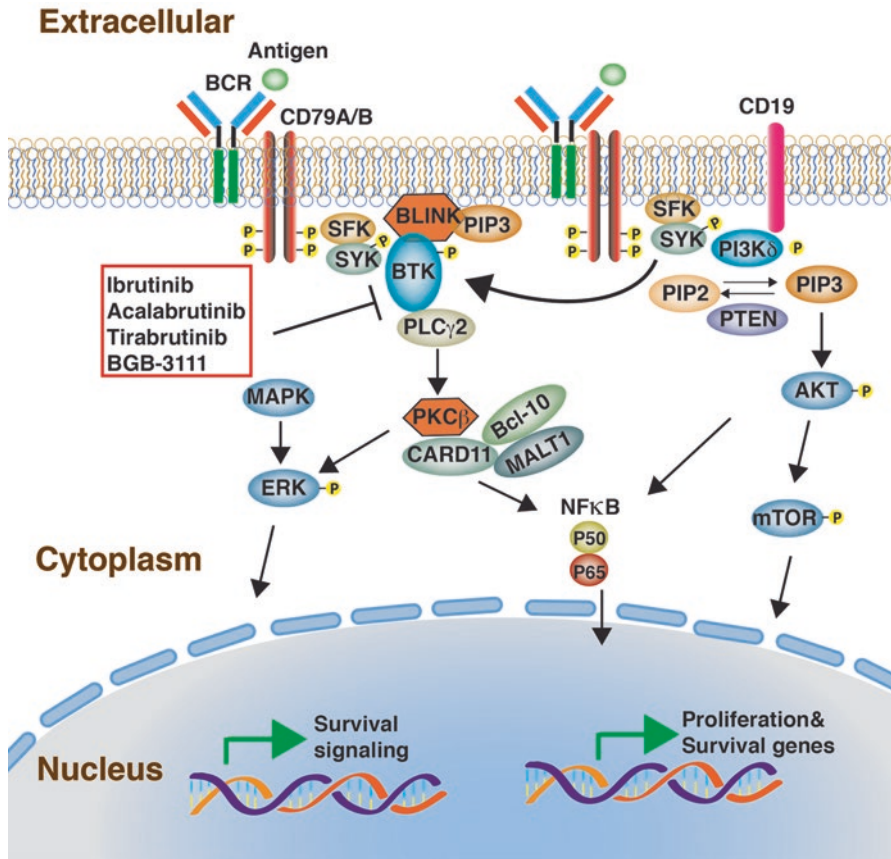


Fig. 6.1 Simplified B-cell receptor signaling. The BCR consists of the antigen-binding immunoglobulin heavy (IgH) and light (IgL) chains coupled to the heterodimeric CD79a and CD79b proteins. Antigen binding triggers the phosphorylation of tyrosines within the ITAMs of CD79A and CD79B by SFKs. And the phosphorylated ITAMs recruit SYK, which is followed by the activation of BLNK, BTK and PLC γ 2. BTK is then phosphorylated and activated by Syk to drive the activation of PKC β , PI3K/Akt/mTOR and NF- κ B. PKC β phosphorylates and activates ERK and NF- κ B transcription factors. Ibrutinib, acalabrutinib, tirabrutinib, spebrutinib, and BGB-3111 inhibit BTK activities. The complex of CARD11, MALT1, and BCL10 is an important part of the pathway activating NF- κ B. Additionally, the BCR co-receptor CD19 phosphorylation is also involved in BTK recruitment and activation by recruiting PI3K to generate PIP3 and activate the PI3K-AKT pathway. The activated ERK, PI3K/Akt/mTOR and NF- κ B pathways upregulate the genes that are involved in cell proliferation and survival in B cell lymphoma

caspace recruitment domain family member 11 (CARD11), mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1), and B-cell lymphoma/leukemia 10 (BCL10) is an important part of the pathway activating NF- κ B. Additionally, the BCR co-receptor CD19 phosphorylation is also involved in BTK recruitment and activation by recruiting PI3K to generate phosphoinositide

phosphatidylinositol-3, 4, 5-trisphosphate (PIP3) [17]. The BCR signaling pathway offers a wealth of therapeutic targets such as SYK, BTK and PI3K, and drugs targeting these kinases are in development and clinical trials to evaluate their efficacy against a variety of B-cell lymphomas.

BTK is a member of the tyrosine kinase expressed in hepatocellular carcinoma (Tec) family of the non-receptor tyrosine kinases and was discovered during cloning the genes that were associated with X-linked agammaglobulinemia (XLA) in 1993 [18, 19]. The gene encodes a 659 amino acid protein that consists of several putative domains: an N-terminal Pleckstrin homology (PH) domain that binds membrane PIP3, is followed by SH2, Src homology 3 (SH3), and proline rich domains that regulate binding to other cellular signaling molecules [20]. Activation of BTK correlates with an increase in the phosphorylation of two regulatory BTK tyrosine residues: Y551 and Y223 [21]. Y551 within the Src kinase domain is transphosphorylated by the kinases Syk or Lyn during BCR signaling and promotes the catalytic activity of BTK. Y223 is an autophosphorylation site within the BTK SH3 domain and the phosphorylation of this site has little discernible influence on BTK catalytic activity *in-vitro* or *in vivo* but may be a mechanism to modify protein–protein interactions [21]. BTK has been widely characterized as a critical mediator in signaling through BCR and the Fc γ receptor (Fc γ R) and is important for B cell development, differentiation, proliferation and survival [22, 23]. Mutations in BTK gene lead to inactivating the BTK gene through an in-frame insertion of a lacZ reporter in mouse embryonic stem cells resulting in defects of B cell development from pre-B cells to immature B cells in the bone marrow and B-cell differentiation arrest during the maturation from IgD(low)IgM(high) to IgD(high)IgM(low) stages in the periphery [24]. In humans a wide spectrum of BTK loss-of-function mutations such as a PH domain mutation in the BTK gene lead to an almost complete absence of peripheral B cells and antibodies in XLA [25, 26].

In this review, we summarize the clinical results of BTK inhibitors, discuss the resistant mechanisms of BTK inhibitors, especially ibrutinib, based on the clinical and preclinical studies in B-cell lymphoma. In the end, we describe current and future novel therapeutic strategies to overcome the resistance.

Overview of BTK Inhibitors and Clinical Response

BTK is a regulator of normal B-cell development and is activated upon BCR stimulation. Activation of the BCR signaling pathway has now emerged as a central oncogenic pathway that promotes growth and survival in both normal and malignant B-cells. Antigenic activation of the dimeric membrane immunoglobulin B-cell receptor, which induces phosphorylation of BTK and PLC γ 2, results in the activation of a number of signaling pathways including mitogen-activated protein kinase (MAPK), NF- κ B and Akt [27] (Fig. 6.1). Selective and covalent BTK inhibitors such as ibrutinib can inhibit BTK activation to further block chronic active BCR signaling [28, 29].

Ibrutinib

Ibrutinib (PCI-32765, Imbruvica®) is the first-in-class, selective and irreversible small molecule inhibitor of BTK and covalently binds to cysteine residue 481 on the BTK kinase domain, thereby inhibiting the autophosphorylation of tyrosine 223 on exon 8 and resulting in irreversible inhibition of BTK enzymatic activity [30]. Ibrutinib has been demonstrated to be an active agent in activated B-cell-like diffuse large B cell lymphoma (ABC-DLBCL), a NHL subtype that is characterized by constitutively activated NF- κ B signaling [31]. Preclinical studies of ibrutinib in CLL and MCL suggested that ibrutinib inhibits cell proliferation *in-vitro* in the range of 1.0–25.0 μ M [32, 33].

Ibrutinib's unique biochemistry and *in vivo* activities in mice and dogs paved the way for not only human clinical phase 1 trials but also phase 2 and 3 (Table 6.1) trials in patients with mature B-cell lymphomas [30]. In a phase 1 study, ibrutinib was well tolerated in 50 evaluable adults with R/R B-cell lymphomas including MCL, FL, DLBCL, MZL and CLL [34]. It was associated with an overall response rate (ORR) of 60%, including complete response (CR) 16% [34]. The safety and efficacy of ibrutinib in MCL and CLL patients who had received at least one prior therapy were evaluated in single-arm, open-label, multicenter trials (NCT01236391, NCT01105247) in 2013 [35, 36]. The drug demonstrated substantial improvement on a clinically significant endpoint over available therapies. In the MCL trial, a response rate of 68% (75 patients) was observed, with a CR of 21% and a partial response rate (PR) of 47% [35]. In the CLL trial, the ORR was 71% and the PR ranges from 15–20% based on the doses [36]. Based on the highly effective treatment of refractory and relapsed adult patients with CLL and MCL, ibrutinib was granted breakthrough therapy designation and has been approved for the treatment in patients with R/R CLL or MCL with at least one prior therapy in 2013 [35–38].

To evaluate the efficacy and tolerability of ibrutinib in relapsed or refractory WM, and to examine the impact of myeloid differentiation primary response 88 (MYD88) (L265P) and WHIM-like C-X-C chemokine receptor type 4 (CXCR4) mutations on ibrutinib response, 63 patients with R/R WM were enrolled for a phase 2 study with an ORR of 90.5% and a major response rate (PR or better) of 73% with a median time to response of 4 weeks [39]. Patients with MYD88 mutation and wild type CXCR4 had better response to ibrutinib treatment than those with MYD88 wild type or WHIM-like CXCR4 mutations: 100% OR for patients with MYD88L265PCXCR4WT vs. 85.7% OR MYD88L265PCXCR4WHIM vs 71.4% OR MYD88WTCXCR4WT. The estimated 2-year progression-free and overall survival rates among all patients were 69.1% and 95.2%, respectively. Grade > 2 treatment related toxicities were observed. Based on the promising results, the Food and Drug Administration (FDA) approved ibrutinib for the treatment of patients with WM in 2015.

BTK expression was detected in approximately 20% of patients with classic Hodgkin lymphoma (HL) [40]. A single-agent ibrutinib at a dose of 560 mg was investigated in 2 primary refractory classic HL patients [41]. Two months after the

Table 6.1 Summary of phase 2 and 3 clinical trials of Ibrutinib in B cell lymphomas

Phase	Drug	N	Tumor subtypes	Response	Toxicity	Dose	Clinical trial NCT#	Year	Ref.
1b/2	Ibrutinib	85	Relapsed CLL	71% ORR; 15–20% PR; 75% PFS; 83% OS	Predominantly grade 1 or 2; Transient diarrhea, fatigue, and upper respiratory tract infection	420 mg 840 mg	NCT 01105247	2013	[36]
2	Ibrutinib	111	Relapsed/refractory MCL	68% ORR; 21% CR; 47% PR; 13.9 months of MPFS; 58% OS at 18 months	Most grade 1 or 2: mild or moderate diarrhea, fatigue, and nausea; Grade 3 or grade 4: infrequent, neutropenia (in 16% of patients), thrombocytopenia (in 11%), and anemia (in 10%)	560 mg	NCT 01236391	2013	[35]
2	Ibrutinib	63	Relapsed/refractory WM	57.1% PR; 77% ORR for patients with wild-type CXCR4 vs 30% with WHIM-like CXCR4 mutations	Grade > 2 treatment related toxicities include: thrombocytopenia; neutropenia; stomatitis; atrial fibrillation; diarrhea; hematoma; hypertension and epistaxis	420 mg	NCT 01614821	2015	[39]
2	Ibrutinib	40	Relapsed/refractory FL	37.5% ORR; 12.5% CR; 25% PR; 52.6% ORR for rituximab-sensitive vs 16.7% for rituximab-refractory (P = .04); a lower ORR for chemotherapy-refractory; 50% ORR for low- or intermediate-risk FL/PI; 14 months of MPFS; 20.4% 2 yr PFS; 79.0% 2 yr OS	42.5% of patients experienced at least 1 grade 3–4 AE	560 mg	NCT 01849263	2018	[44]

2	Ibrutinib	110	Chemo-refractory FL	20.9% ORR; 10.9% CR; 4.6 months of MPFS; 63% 1 yr OS	Grade 1/2: Diarrhea, fatigue, and cough. 48.2% serious AEs; Grade 1/2: Diarrhea, fatigue, and cough; 48.2% serious AEs. 3.6% hemorrhage; 9.1% atrial fibrillation; 6.4% discontinued; 1 pt dose reduction	560 mg	NCT 01779791	2016	[45]
2	Ibrutinib	70	Relapsed/refractory DLBCL	40% ORR in ABC vs 5.3% in GCB; 8% CR in ABC vs 0% in GCB; 32% PR in ABC vs 5.3% in GCB 2.5 months PFS in ABC vs 1.28 in GCB	N/A	560 mg	N/A	2012	[49]
3	Ibrutinib vs Ofatumumab	195 vs 196	Relapsed/refractory CLL or SLL	Improved PFS; 90% vs 81% OS; 42.6% vs 4.1%;	57% of the patients had at least one adverse event of grade 3 or higher.	420 mg	NCT 01578707	2014	[50]
3	Ibrutinib vs chlorambucil	136 vs 133	previously untreated CLL	Significantly longer PFS; 98% vs 85% OS at 24 months; 84% lower risk death; 86% vs. 35% ORR; 4% vs 2% CR; improved hematologic variables	Grade 3 diarrhea (4%), grade 3 hypertension (4%), atrial fibrillation (6%) and grade 3/4 hemorrhage (4%)	420 mg	NCT 01722487	2015	[51]
3	Ibrutinib vs Tensirolimus	139 vs 141	Relapsed/refractory MCL	57% reduction in the risk of disease progression or death; 14.6 vs 6.2 months MPFS; 41% versus 7% PFS at 2 yrs; 72–77% vs 40–42% ORR; 19% CR vs 1% CR	Diarrhea (29%), cough (22%), and fatigue (22%), atrial fibrillation (4% vs 1%), major bleeding (10% vs 6%)	560 mg	NCT 01646021	2016	[55]

CLL: Chronic lymphocytic leukemia; MCL: Mantle cell lymphoma; WM: Waldenström's macroglobulinemia; FL: Follicular lymphoma; DLBCL: Diffuse large B-cell lymphoma; ABC: Activated B-cell; GCB: Germinal center B cell; SLL: small lymphocytic lymphoma; ORR: Overall response rate; CR: Complete response; PFS: Progression-free survival; MPFS: Median progression-free survival; PR: Partial response; R/R: Relapsed/refractory; Ref.: References.

initiation of ibrutinib, positron emission tomography-computed tomography (PET-CT) showed near-complete regression of disease in one patient with subsequent disease progression. Another patient had a CR, which was still ongoing more than 6 months later. The activity of ibrutinib in patients with classic HL warrants prospective assessment. A phase 2 multicenter trial to evaluate the efficacy and safety of ibrutinib in patients with R/R classical HL is ongoing (NCT02824029).

Primary FL cells have been found to maintain enhanced BCR pathway signaling when compared to normal B cells [42]. Sixteen patients with FL were treated with ibrutinib in the phase 1 study [43]. Of the cohort of 16 patients, 11 patients were treated at doses where full occupancy of BTK was achieved by ibrutinib. The ORR was 55% and the median duration of response was 12.3 months and the median progression free survival (PFS) 13.4 months. Based upon drug occupancy and clinical responses, a phase 2 Consortium Trial of ibrutinib in R/R FL was conducted [44]. ORR was 37.5% with a complete response rate of 12.5%, median progression-free survival (PFS) of 14 months, and 2-year PFS of 20.4%. Response rates were higher among patients with rituximab-sensitive disease (52.6%) compared with those who had rituximab-refractory disease (16.7%; $P = .04$). Chemotherapy-refractory patients also had a lower ORR than chemotherapy-sensitive patients. Patients with low- or intermediate-risk FLIPI (the Follicular Lymphoma International Prognostic Index) had a trend toward a higher response rate compared with high-risk FLIPI (50% vs 25%; $p = .19$). The median PFS was 14.0 months and the 2-year PFS and OS were 20.4% and 79.0%, respectively. Similar results were found in a phase 2 study of ibrutinib in patients with chemoimmunotherapy-refractory FL (the DAWN study), which showed a significantly lower response rate of 20.9% in chemotherapy-refractory FL with a median PFS of 4.6 months, 10.9% CR and 63% 1 year OS [45].

Due to activating mutations in CD79B, MYD88, and CARD11, the BCR signaling, the toll-like receptor (TLR) and the NF- κ B pathways are often constitutively activated in ABC-DLBCL compared with germinal center B-cell (GCB) type DLBCL [46–48]. A phase 2 multicenter study was performed to determine if ibrutinib would be more efficacious in ABC-DLBCL compared with GCB-DLBCL [49]. The ORR in patients with ABC type was 40%, whereas overall response rate in the GCB type was only 5%. The CR is 8% in ABC-DLBCL vs. 0% in GCB-DLBCL; PR is 32% in ABC-DLBCL vs. 5.3% in GCB-DLBCL; PFS is 2.5 months in ABC vs. 1.28 in GCB [49]. Furthermore, ibrutinib had activity in patients with and without CD79b mutations, suggesting an alternative mechanism of BCR pathway dependence. This study indicates that further study of ibrutinib should be aimed at the ABC type of DLBCL with attention to the different somatic mutations [49].

Based on the early promising results of the phase 2 trial on CLL, a multicenter, open-label, randomized, phase 3 trial (RESONATE) was opened to the study of ibrutinib vs. ofatumumab in patients with relapsed or refractory CLL or SLL [50]. Ibrutinib significantly improved the PFS and the OS (90% vs. 81%; $p = .005$) and ORR (42.6% vs. 4.1%; $p < .001$). Patients with a 17p13.1 deletion also had a

markedly improved PFS with ibrutinib compared with ofatumumab. In this trial, ibrutinib was associated with a slightly increased risk of grade 3/4 (57% vs. 47%) adverse events (AE) compared with ofatumumab. Based on the superior efficacy of ibrutinib compared to ofatumumab in difficult-to-treat patients with R/R CLL or SLL, the FDA expanded the approval of ibrutinib to include treatment of CLL patients with 17p deletion. In the following phase 3 RESONATE-2 study, the efficacy and safety of ibrutinib was compared with chlorambucil in patients 65 years of age or older with previously untreated CLL [51]. Consistent with the high-risk group, ibrutinib resulted in significantly longer PFS than that with chlorambucil with 98% OS at 24 months vs. 85% with chlorambucil. The relative risk of death with ibrutinib was 84% lower than that with chlorambucil ($p = .001$). The ORR was significantly higher in the ibrutinib group than in the chlorambucil group (86% vs. 35%; $p < .001$). CR occurred in 4% of the patients in the ibrutinib group and in 2% of those in the chlorambucil group. The hematologic variables were significantly improved in the ibrutinib treated group. Grade III diarrhea (4%), grade III hypertension (4%), atrial fibrillation (6%) and grade III/IV hemorrhage (4%) were more common in the ibrutinib treated group. These results support the use of ibrutinib as a first-line agent in CLL.

Temsirolimus is an inhibitor of the mechanistic target of rapamycin (mTOR) pathway that has been used to treat patients with relapsed MCL with 22–40% ORR and a median OS of 12.8 months [52–54]. A randomized phase 3 clinical trial led by the European MCL Network compared ibrutinib with temsirolimus in patients with R/R MCL [55]. With a median follow-up of 20 months, ibrutinib treatment resulted in a 57% reduction in the risk of disease progression or death compared with temsirolimus ($p < .0001$). The median progression-free survival (MPFS) was 14.6 months for the ibrutinib group vs. 6.2 months for the temsirolimus group. At a 2 year landmark, the PFS rate is 41% versus 7% and the ORR was 72–77% vs. 40–42% with a 19% CR vs. 1% CR. Median OR was not reached for ibrutinib versus 21.3 months for temsirolimus. The reported AEs were consistent with previous studies, including diarrhea (29%), cough (22%), fatigue (22%), atrial fibrillation (4% with ibrutinib vs. 1% with temsirolimus) and major bleeding (10% with ibrutinib vs. 6% with temsirolimus).

We investigated the efficacy of ibrutinib alone and in selective adjuvant combinations against BL *in vitro* and in a human BL xenografted immune-deficient NOD.Cg-PrkdcscidII2rgtm1Wjl/SzJ (NSG) mouse model [56]. Our data demonstrated that phospho-BTK level was significantly reduced in BL cells treated with ibrutinib ($p < .001$). Moreover, we observed a significant decrease in cell proliferation as well as significant decrease in half maximal inhibitory concentration (IC_{50}) of ibrutinib in combination with dexamethasone, rituximab, obinutuzumab, carfilzomib, and doxorubicin ($p < .001$). *In vivo* studies demonstrated ibrutinib-treated mice had a significantly prolonged survival compared to vehicle controls ($p < .02$). Our findings demonstrate the significant *in vitro* and preclinical *in vivo* effects of ibrutinib in BL. Based on our preclinical results, there is an ongoing clinical trial comparing OS in children and adolescents with R/R BL treated with chemoimmunotherapy with or without ibrutinib (NCT02703272).

Second-Generation Inhibitors of BTK

Ibrutinib binds to Cys-481 of the BTK but it also binds to several other kinases [57]. These off-target effects of ibrutinib contribute to its activity and toxicity such as bleeding [50]. Therefore, second-generation BTK inhibitors such as acalabrutinib (ACP-196), Tirabrutinib (ONO/GS-4059, GS-4059), spebrutinib (CC-292, AVL-292), and BGB-3111 are being developed with more selective kinase activity profiles (Table 6.2).

Table 6.2 Summary of clinical trials of second-generation BTK inhibitors

Inhibitor name	Off-targets	Phase of trials	N	Tumor subtypes	Overall response rate	Approved indication	Ref.
Acalabrutinib	NO	1/2	61	R CLL	95% ORR	NO	[165]
					85% PR		
					10% PR with lymphocytosis		
					5% SD		
					For del(17)(p13.1): 100% ORR		
					89% PR		
					11% PR with lymphocytosis		
					With prior idelalisib therapy: 100% ORR		
					75% PR		
					25% PR with lymphocytosis		
Acalabrutinib	NO	2	124	R/R MCL	81% ORR	YES for R/R MCL	[62]
					40% CR		
					72% medians for duration of response		
					67% PFS		
					87% OR		
Tirabrutinib (ONO/GS-4059)	NO	1	90	R/R CLL; MCL; DLBCL; FL; WM; MZL; SLL	PFS in CLL, MCL, and DLBCL: 874, 341, and 54 days, respectively	NO	[66]
					CLL: 96% ORR		
					MCL: 91.7% ORR, 50% PR, and 41.7% CR		
					DLBCL: 35% ORR, 29% PR, 6.45% CR		
BGB-3111	NO	1	25	CLL, MCL, WM, DLBCL, FL, MZL, HCL	64% OR (16/25), including 1 CR and 6 SD	NO	[67]

Acalabrutinib

Acalabrutinib (ACP-196) binds covalently to BTK with greater *in vivo* potency and selectivity than ibrutinib [58]. *In-vitro* studies demonstrated that acalabrutinib and ibrutinib had similar molecular and biologic consequences in primary CLL cells but different effects on lymphocyte-specific protein tyrosine kinase (LCK) and proto-oncogene tyrosine-protein kinase Src phosphorylation in primary T-lymphocytes [59]. The IC₅₀ of acalabrutinib for the BTK protein is 5.1 nmol/L vs 1.5 nmol/L of ibrutinib, indicating a weaker BTK inhibition than ibrutinib. However, ACP-196 demonstrated higher selectivity for BTK than ibrutinib when profiled against a panel of 395 non-mutant kinases (1 μM) in a competitive binding assay [60]. Importantly, ACP-196 did not inhibit epidermal growth factor receptor (EGFR), interleukin-2-inducible T-cell kinase (Itk) or tyrosine-protein kinase TXK (Txk) [60]. The phase 1/2 ACE-CL-001 trial of acalabrutinib monotherapy in patients with relapsed CLL showed that acalabrutinib was well tolerated and no major hemorrhage or atrial fibrillation was noted [61]. The clinical activity of acalabrutinib was rapid and robust. With a median follow up of 14.3 months, the ORR was 95% with 85% PR, 10% PR with lymphocytosis and 5% stable disease. The ORR was 100% for patients with del(17)(p13.1) with 89% PR, 11% PR with lymphocytosis. In the 4 patients with prior idelalisib therapy, the response rate was 100% (PR, 75%, PR with lymphocytosis, 25%). A direct comparison of acalabrutinib with ibrutinib in a phase 3 study (NCT02477696) is active and on the way to recruit patients with high-risk CLL.

Acalabrutinib is also active in clinical trials as a single agent or in combination for the treatment of other lymphomas including MCL (NCT02213926), FL (NCT02180711), WM (NCT02180724), and DLBCL (NCT03205046). In 2015, a phase 2 trial (ACE-LY-004) was conducted on patients with R/R MCL (NCT02213926) [62]. One hundred twenty-four patients with R/R MCL were enrolled in this trial. At a median follow-up of 15.2 months, the ORR was 81% and CR was 40%. The Kaplan-Meier estimated medians for duration of response, PFS, and OR rates at 12 months were 72%, 67%, and 87%, respectively. Primarily grade 1 or 2 adverse events were the most common. Consistent with CLL trials, atrial fibrillation and worse hemorrhage events were rare. The results demonstrated that acalabrutinib treatment provided a high rate of durable responses and a favorable safety profile in patients with relapsed or refractory MCL. Based on the promising results in the ACE- LY-004 trial and other clinical data, acalabrutinib was granted Breakthrough Therapy Designation by the FDA in 2017 for patients with MCL who have received at least one prior therapy.

Tirabrutinib

Tirabrutinib (ONO/GS-4059) is an irreversible inhibitor with a greater selectivity for BTK than for LCK, proto-oncogene tyrosine-protein kinase Fyn (FYN), tyrosine-protein kinase LynA (LYNA), and Itk [63]. *In-vitro* studies showed that IC₅₀ of ONO/

GS-4059 to BTK was 2 nmol/L and it induced apoptosis at nanomolar concentrations in the activated DLBCL cell lines [64]. ONO/GS-4059 treatment resulted in inhibition of tumor growth in an ABC-DLBCL xenograft model [65]. These promising preclinical data prompted clinical evaluation of ONO/GS-4059. In a multicenter phase 1 dose escalation study, 90 patients with R/R B cell malignancies including CLL/SLL, MCL, DLBCL, FL, MZL, and WM, were enrolled in a 3 + 3 dose-escalation study [66]. The overall estimated mean PFS in CLL, MCL, and DLBCL were 874, 341, and 54 days, respectively. CLL patients had a 96% ORR. Of these patients, 13 had loss of TP53 and 21 had unmutated immunoglobulin heavy-chain variable region (IGHV) gene segments. All 12 patients with TP53/17p deletion or TP53 mutations had a response, with 9 remaining on study. MCL patients had 91.7% ORR, 50% PR, and 41.7% CR. DLBCL patients had 35% ORR, 29% PR, and 6.45% CR. A striking feature of this study was that ONO/GS-4059 across all disease subsets showed a low incidence of associated toxicities. ONO/GS-4059 may have significant advantages over other selective kinase inhibition in terms of reduced toxicities.

BGB-3111

BCB-3111 is another more selective, irreversible BTK inhibitor with higher BTK specificity than ibrutinib [67, 68]. In biochemical and cellular assays, BGB-3111 demonstrated nanomolar BTK inhibition activity and showed less off-target kinase inhibition against a panel of kinases [68]. Both in the MCL and ABC-DLBCL tumor cells xenografted models, BGB-3111 demonstrated dose-dependent antitumor effects and prolonged the overall survival of xenografts [68]. Additionally, BGB-3111 demonstrated at least ten-fold weaker than ibrutinib in inhibiting rituximab induced antibody-dependent cellular cytotoxicity (ADCC), supporting the combination therapy with anti-CD20 antibodies in lymphoma [68]. In the phase 1 trial of BGB-3111, 24 patients with advanced lymphoma (CLL, MCL, WM, DLBCL, FL, MZL) and 1 with Hairy cell lymphoma (HCL) were enrolled [67]. Sixty-four percent (16/25) of patients had objective responses, including 1 CR and 6 SD. Drug-related AEs and dose-limited toxicities (DLT) were not reported. These preliminary phase 1 results suggest that BGB-3111 is safe and highly clinically active but clinical efficacy remains to be further determined.

Molecular Mechanisms of BTK Inhibitors' Resistance

Despite the promising clinical responses of BTK inhibitors especially ibrutinib in a variety of B-cell lymphomas, cases of primary and secondary resistance were recognized [69]. Clinically, ibrutinib resistance presents in two forms: primary resistance in which patients demonstrate lack of response at initial therapy due to disease transformation (Richter transformation, an aggressively ibrutinib-resistant disease), and secondary resistance which is characterized by an initial disease response but it

is subsequently lost due to the cell's ability to bypass the target via alternative pathways or acquired mutations in the target or its pathway [15, 69, 70]. Understanding the molecular mechanisms (Fig. 6.2) underlying BTK inhibitors' resistance is of paramount importance. The reported resistant mechanisms of BTK inhibitors, especially ibrutinib resistance are summarized in Table 6.3.

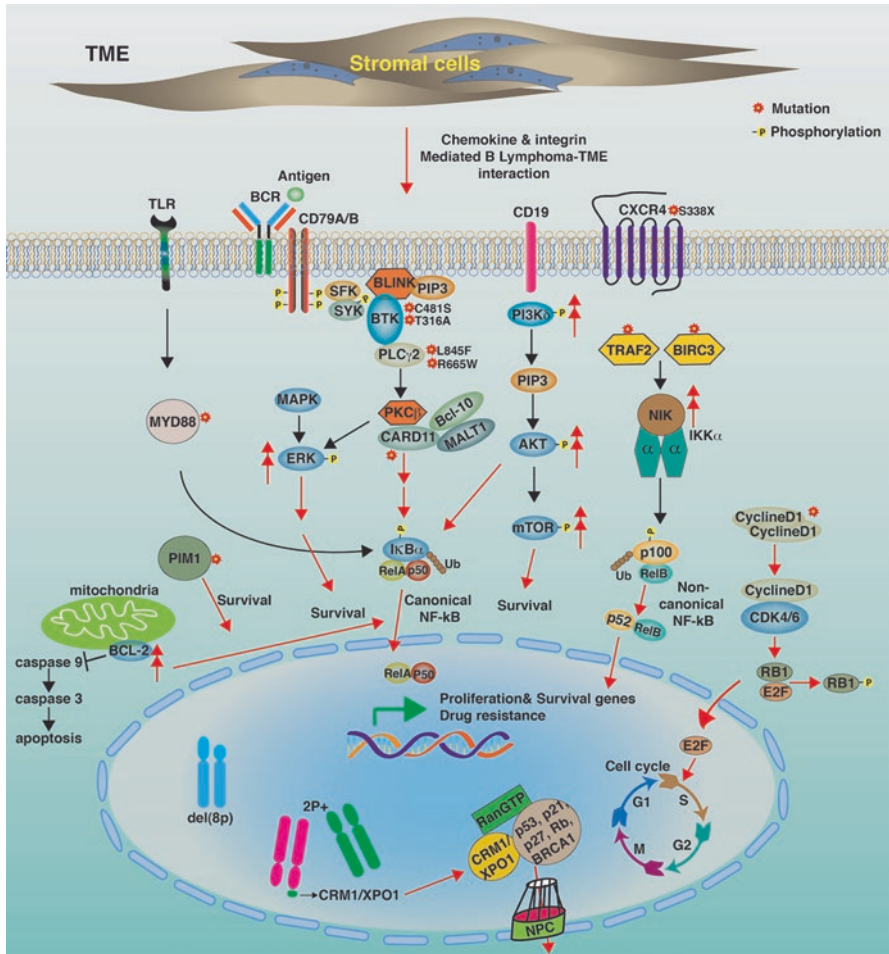


Fig. 6.2 Mechanisms of resistance of BTK inhibitors in B-cell lymphomas. Primary resistance of BTK inhibitors may be caused by sustained activation of other oncogenic pathways such as PI3K-AKT/mTOR, MAPK/ERK independent of BTK. Acquired resistances include mutations in BTK, PLC γ 2, CARD11 and the activation of alternative NF- κ B or PI3K/mTOR pathways. MYD88 and CXCR4 mutations in WM patients trigger pro-survival NF- κ B signaling, activate AKT and ERK and promote resistance to ibrutinib. Chromosomal abnormality such as del(8p) and 2p+ has been documented in acquired ibrutinib resistance. Overexpression of CRM1/XPO1 is involved in nuclear export of a number of tumor suppressor proteins such as p53 and BRCA1, which is associated with drug resistance. Mutations in CCND1 stabilize cyclin D1 and subsequently activate cyclin-dependent kinase (CDK) 4 to phosphorylate and inactivate retinoblastoma (Rb) protein. This event leads to G1/S cell cycle progression, cell proliferation and ibrutinib resistance. Last, TME-lymphoma interactions activate integrin b1-integrin-linked kinase (ILK)/PI3K-AKT-mTOR to mediate ibrutinib resistance

Table 6.3 Summary of the reported mechanisms of BTK inhibitors' resistance

BTK inhibitor	Diseases	Resistance mechanism	Report year	Ref.
Ibrutinib	CLL	Mutations: BTKC481S, PLC γ 2 (R665W, L845F, S707Y)	2014	[72]
Ibrutinib	CLL	Mutations: PLC γ 2 (S707F, M1141R, M1141K and D993H)	2016	[89]
Ibrutinib	CLL	Mutation: BTKT316A	2016	[76]
Ibrutinib	CLL	Mutations: EIF2A, RPS15, EP300, MLL2	2016	[89]
Ibrutinib	CLL	Del(8p) TRAIL receptor haploinsufficiency	2016	[89]
Ibrutinib	CLL	2p+ XPO1 overexpression	2017	[166]
Ibrutinib	Mouse E μ -myc B cells	Myc amplification	2017	[92]
Ibrutinib	MCL	Activation of the alternative NF-kB pathway	2013	[100]
Ibrutinib	MCL	Mutations in TRAF2, BIRC3, Activation of alternative NK-kB pathway	2014	[100]
Ibrutinib	MCL	Mutations in CARD11	2016	[87]
Ibrutinib	MCL	Mutations in primary resistance: A20, BIRC2, epigenetic modifiers, EGFR family; Mutations in acquired resistance: PLC γ 2, CARD11, epigenetic modifiers, NF-kB and PI3K/mTOR pathways	2016	[78]
Ibrutinib	MCL	PI3K-AKT activation	2014	[102]
Ibrutinib	MCL	ERK1/2 and AKT activation	2014	[103]
Ibrutinib	MCL	Upregulating the c-Myc and mTOR signaling pathways and metabolic pathways	2017	[88]
Ibrutinib	ABC-DLBCL	Mutations in PIM1	2016	[80]
Ibrutinib and ONO/GS-4059	ABC-DLBCL	NF-kB pathway activation	2017	[129]
Ibrutinib	DLBCL	Upregulation of IAP, survivin, cIAP2, BCL2 and BCL6, PI3K α and PI3K β ; Downregulation of PTEN	2017	[105]
Ibrutinib	WM	CXCR4S338X mutation leads to activation of both AKT and ERK	2015	[85]
Ibrutinib	WM	Mutations: BTKC481S, CXCR4, CARD11, PLC γ 2	2017	[83]
		Upregulation of Bcl-2 and AKT	2017	[106]

Mutational Resistance in BTK

The development of mutations within the drug target that alter drug sensitivity is an important mechanism of acquired resistance to ibrutinib. Whole-exome sequencing (WES), Sanger sequencing, and Ion Torrent deep sequencing of pre-treatment and

relapse samples from six CLL patients confirmed a cysteine-to-serine mutation at BTK position 481 (C481S) in five of the six patients [71, 72]. No patient at baseline had evidence of mutations in either BTK on the basis of WES and Ion Torrent sequencing. This finding was further confirmed by another study using peripheral blood cells and cell free DNA samples from ibrutinib naïve and treated CLL patients with custom DNA or locked nucleic acid (LNA) oligos in a wild-type blocking polymerase chain reaction, followed by Sanger sequencing and Next-generation sequencing (NGS) methods [73]. Functional characterization demonstrated that mutant BTK has significantly lowered affinity to ibrutinib than nonmutant BTK [72]. When transfected to cells with mutant BTK, ibrutinib was significantly less effective at blocking BTK auto-phosphorylation and downstream signaling than nonmutant BTK [72]. The data from the mutational analyses, signal transduction and gene expression profiling strongly suggest C481S mutation confers resistance to ibrutinib leading to increased BCR signaling at patient's relapse [74]. These functional studies suggest that the C481S mutation in BTK confers resistance to ibrutinib by preventing irreversible drug binding [72, 75]. Another mutation in BTK that is associated with ibrutinib resistance was identified at the center of the positively-charged binding pocket in the SH2 domain with a threonine to alanine change at BTKT316 site [76]. Unlike the C481, T474 and L528 mutations in the kinase domain to either directly attenuate or hinder ibrutinib binding, structure analysis revealed that T316A does not directly interfere with ibrutinib binding. *In vitro* cellular and molecular studies demonstrated that ibrutinib did not inhibit the cell proliferation of the transfected lymphoma cells with BTKT316A mutation and the degree of phosphorylation inhibition in p-BTK (Y223), p-PLC γ 2, p-AKT and p-ERK following ibrutinib treatment was significantly less in C481S and T316A mutant cells than in wild type cells [76]. This data firmly established that the BTKT316A mutant is as capable as BTKC481S to confer ibrutinib resistance. The resistant BTK mutations were not detectable at the baseline before ibrutinib exposure [72, 75]. It might be limited by the sensitivity of the detection methods that may not identify small numbers of BTK mutant CLL cells in the presence of large numbers of nonmutant CLL cells. To investigate this possibility, Fam'a, R. et al. used an allele-specific polymerase chain reaction (AS-PCR) which is highly sensitive and can detect 1 mutant allele per 1000 wild-type alleles, to assess the occurrence of small subclones harboring the C481S codon mutations in ibrutinib-naïve CLL patient samples [77]. Among CLLs that have not been exposed to ibrutinib, the BTK C481S variant was not detected, indicating the ibrutinib resistance in CLL is not mutation driven resistance [77].

Mutational Resistance in Other Proteins than in BTK

Mutations in the prosurvival pathways to bypass BTK appear to be another common mechanism of resistance. The samples from relapsed CLL patients were detected to have gain-of-function mutations targeting PLC γ 2, a direct downstream target of

BTK phosphorylation [72]. When transfected with PLC γ 2 with the L845F mutation, or with the R665W mutation into human embryonic kidney (HEK) 293 T cells and DT40 cells, which lack endogenous PLC γ 2 expression, upon activating BCR signaling, phosphorylation of extracellular signal-regulated kinases (ERK) and AKT was less inhibited by ibrutinib than nonmutant cells [72]. In the phase 3 MCL 3001 (RAY) trial, mutations were identified in NF- κ B signaling pathways, both canonical (e.g., A20) and noncanonical (e.g., BIRC2); in epigenetic modifiers; and in the epidermal growth factor receptor (EGFR) family in primary resistance to ibrutinib [78]. Mutations were found in PLC γ 2, CARD11, epigenetic modifiers and alternate NF- κ B or PI3K/mTOR pathways in the MCL patients with acquired resistance after a short treatment duration [78]. Serine/threonine kinase (PIM1) encodes a serine/threonine kinase that is a critical regulator of tumorigenesis in a number of hematologic malignancies [79]. Interestingly, in 48 DLBCL patient samples with available genomic profiling, PIM1 mutations appeared more frequently in patients with ABC-DLBCL than those with GCB-DLBCL [80]. PIM1 mutations were also identified in patients with poor response to ibrutinib, indicating PIM1 mutations are associated with intrinsic ibrutinib resistance in ABC-DLBCL. *In vitro* studies demonstrated that introducing one of these mutations into an ABC-DLBCL cell line is sufficient to induce ibrutinib resistance through stabilizing the protein and enhancing NF- κ B signaling [80]. The combination of pan-PIM inhibitors such as AZD-1208 with ibrutinib results in greater efficacy than ibrutinib as a single agent and can circumvent resistance. Activating somatic mutations in MYD88 and CXCR4 are present in 90–95% and 30–40% of WM patients, respectively [81–83]. MYD88 mutations trigger pro-survival NF- κ B signaling through BTK [84]. The WHIM-like CXCR4 (S338X) somatic mutation activates AKT and ERK and promotes resistance to ibrutinib [85]. In a multicenter study that administered ibrutinib to rituximab refractory WM patients, patients with CXCR4 mutations showed delayed responses, and the 1 patient with WT MYD88 showed no response to ibrutinib [86]. Xu et al. utilized Sanger sequencing, highly sensitive AS-PCR assays and targeted NGS to identify mutations associated with clinical progression in WM patients treated with ibrutinib [83]. Their study revealed that 5.1% patients on ibrutinib without clinical progression had BTKC481S mutation. And BTKC481 mutations are associated with mutated CXCR4 [83]. Akin as in CLL, BTKC481 mutations were not detected in baseline samples or ibrutinib-naïve WM patients. Additional mutations in ibrutinib resistant WM samples were identified in CARD11 and PLC γ 2 [83]. CARD11 is a scaffold protein required for BCR induced NF- κ B activation. Its mutation may result in a constitutive activation of B-cell receptor (BCR)/NF- κ B signaling and render the mutant cells resistant or sensitive to some of the BCR/NF- κ B inhibitors [47]. CARD11 mutations were observed in 5.5% of MCL samples [87]. When over-expressed *in vitro*, CARD11 mutants conferred resistance to ibrutinib, providing new insights for ibrutinib resistance in MCL and continuous activation of NF- κ B pathway.

In addition to acquisition of these mutations, other mechanisms of resistance, such as upregulation of potentially druggable survival pathways [88], clonal evolution of genetic alterations [89, 90], presence of BCL-6 abnormalities [91], complex

karyotype [91], TP53 abnormality [90, 91], MYC amplification [90–92] and baseline del(17p) [90, 91], are associated with an increased risk of acquired resistance to ibrutinib.

Chromosomal Abnormality

Deletions in chromosomes have been documented in acquired ibrutinib resistance such as large deletions in the short arm of chromosome 8 [89]. Deletions of chromosome 8p were reported as a recurrent event in B-NHL and tumor necrosis factor related apoptosis inducing ligand receptors (TRAIL-R) were identified as dosage-dependent tumor suppressor genes in this region whose monoallelic deletion can impair TRAIL-induced apoptosis in B-cell lymphoma [93]. In ibrutinib resistant CLL patients, del(8p) was not present at baseline, but was detected at the time of progression on ibrutinib, indicating ibrutinib therapy favors the selection and expansion of CLL subclones carrying del(8p) [89]. The region of del(8p) was confirmed to encompass TRAIL-R [89]. Treatment with TRAIL decreased cell viability in a greater proportion of non-del(8p) CLL samples compared to the del(8p) CLL samples (16% vs. 5%), indicating monoallelic deletion of chromosome 8p was sufficient to abrogate the positive or negative effects of TRAIL on cell viability *in vitro*. The expected sensitivity to TRAIL in the pre-treatment samples and resistance in the relapse samples further confirmed the role of del(8p) in protection from TRAIL-induced apoptosis [89]. Some ibrutinib resistant CLL patients acquired additional putative driver mutations in eukaryotic translation initiation factor 2A (EIF2A), 40S ribosomal protein S15 (RPS15), the histone acetyltransferase EP300 (Y1397F) and the chromatin regulator MLL2 (the mixed lineage leukemia 2) without detectable mutations in BTK and PLC γ 2 genes, which likely confer proliferative advantage and bypass the BTK pathway [89].

The gain of the short arm of chromosome 2 (2p+) was reported as a frequent chromosomal abnormality in CLL [94, 95]. Using single nucleotide polymorphisms array and fluorescence in situ hybridization approaches, chromosome region maintenance1/Exportin-1 gene (CRM1/XPO1) was identified to be overexpressed in the tested 2p + CLL samples [95]. CRM1/XPO1 is a ubiquitous nuclear export receptor protein that regulates intracellular nuclear export of many substrates, including both proteins and ribonucleic acid (RNA) [96]. CRM1/XPO1 is often overexpressed in cancer cells and its overexpression is involved in nuclear export of a number of tumor suppressor proteins such as p53, BRCA1, retinoblastoma, forkhead box O (FOXO), cell cycle inhibitors (p21, p27) and other drug targets [96]. CRM1/XPO1 overexpression and its mediated export has been associated with poor prognosis and resistance to therapy in various cancers [96]. Relapsed 2p+/CLL patients after treatment showed a similar or increasing percentage of cells carrying a XPO1 gain compared with the patients at the time of diagnosis, indicating the potential relevance of XPO1 in CLL drug resistance [95]. *In vitro* ibrutinib induced significantly lower programmed cell death in the 2p+/CLL cells compared with the 2p-/CLL control

cells, indicating XPO1 overexpression associated with 2p+ is associated with ibrutinib resistance in the 2p+/CLL cells [95]. Further studies are needed to investigate if the combination of selinexor, a selective inhibitor of XPO1 currently in Phase 1/2 clinical trials, with ibrutinib can enhance cell death in the 2p+/CLL cells.

Activation of Prosurvival Pathways

Canonical and Non-canonical NF- κ B Signaling Pathways

NF- κ B signaling is an integral important part of the BCR signaling pathway in B cell lymphoma [97]. In canonical NF- κ B pathway, NF- κ B activation relies on inducible degradation of inhibitor of kappa B (I κ Bs), leading to nuclear translocation of various NF- κ B complexes, predominantly the p50/RelA dimer [98]. While, in a non-canonical (alternative) NF- κ B pathway, the RelB/p52 NF- κ B complex activation uses a mechanism that relies on the inducible processing of p100 instead of degradation of I κ B α [99]. The deregulated non-canonical NF- κ B signaling has associated with hematologic malignancies [99]. In a study of ibrutinib-resistance in MCL, Rahal et al. revealed that the resistant MCL cell lines depended on the alternative NF- κ B pathway rather than on the canonical pathway [100]. RNA sequencing and single nucleotide polymorphism arrays showed recurrent mutations in TNF receptor associated factor 2 (TRAF2) or baculoviral IAP repeat containing 3 (BIRC3) in 15% of these individuals in ibrutinib-insensitive cell lines. The BIRC3 mutations were not only less efficient at destabilizing NIK (also known as NF-kappa-B-inducing kinase, mitogen-activated protein 3 kinase 14 or MAP3K14) but also markedly impaired in their ability to suppress p52 production [100]. And these MCL cell lines with alternative NF- κ B pathway alterations are dependent on the NIK signaling both *in vitro* and *in vivo*, suggesting that NIK inhibition may offer a novel, targeted therapeutic strategy for this ibrutinib-resistant population of patients [100].

PI3K-AKT/mTOR Pathway

PI3K-AKT/mTOR activation represents a crucial downstream event of BCR/pre-BCR signaling [101]. The relapse-specific C481S mutation is often absent in patients with primary resistance or progression following transient response to ibrutinib, suggesting alternative mechanisms of resistance in MCL [102]. Chiron et al. found that primary ibrutinib resistance or transient response seems not to stem from defective ibrutinib inhibition of BTK in MCL cells but rather may involve sustained PI3K-AKT activation [102]. Ma et al. found that inhibition of ERK1/2 and AKT, but not BTK phosphorylation, correlates well with the extent of cell death to BTK inhibition in MCL cell lines as well as in primary tumors [103]. RNA-Seq and gene set enrichment analysis (GSEA) revealed the marked upregulation of

components of the c-Myc and mTOR signaling pathways in the ibrutinib-resistant MCL patient samples, indicating that the activation of the pathways may mediate ibrutinib resistance [104]. The role of PI3K-AKT pathway in ibrutinib resistance is also reported in DLBCL and WM. DLBCL Ibrutinib resistance cell lines were generated by continuous culturing of parental DLBCL cell lines in increasing concentrations of ibrutinib [105]. In the resistant cells, besides the increased expression of inhibitors of apoptosis (IAP) family members, survivin, cIAP2 (cellular inhibitor of apoptosis protein 2) and oncogenic BCL2 and BCL6, the deoxyribonucleic acid (DNA) damage repair pathway, and the checkpoint kinase 1 (CHK1), PI3K isoforms PI3K α and PI3K β were upregulated with decreased expression of PI3K δ and phosphatase and tensin homolog (PTEN) which is a PI3K negative regulator. When treating these resistant cells with the PI3K β/δ isoform targeting Drug KA2237, metabolic activity (survival) and surviving of these cells were reduced [105]. Although ibrutinib is highly effective in WM, no complete remissions in WM patients treated with ibrutinib have been reported to date, indicating the WM cell's ability to maintain their survival under ibrutinib-induced stress [106]. Paulus et al. developed ibrutinib resistant WM cell lines to identify the potential mechanisms of ibrutinib resistance in WM cells [106]. These cells exhibited decreased survival dependency on BTK-mediated signaling, but phospho-AKT level was increased in ibrutinib resistant WM cells. When the resistance cells were treated with clinical-grade allosteric pan-AKT inhibitor, MK2206, pAKT level was marked reduced and apoptosis was enhanced as indicated by poly [ADP-ribose] polymerase 1 (PARP-1) cleavage. Remarkably, when cells were treated concurrently with ibrutinib and MK2206, pBTK and pAKT levels were significantly reduced with more robust cleavage of PARP-1 and resistant tumor cell viability was synergistically reduced. This data demonstrated that drug combination strategies encompassing BTK + AKT/PI3K inhibition may potentially overcome ibrutinib resistance in WM [106].

B-Cell Lymphoma-2 (BCL-2) Family Members Mediated Resistance

BCL-2 was initially discovered as a part of the t(14;18) chromosomal translocation in patients with NHLs [107]. The dysregulation of *BCL-2* leads to high levels of Bcl-2 protein in B-cells, which alters the balance between pro-apoptotic and anti-apoptotic members of the Bcl-2 family [108]. The resulting inhibition of apoptosis is thought to lead to chemoresistance [108]. Recent studies show that Bcl-2 is involved in ibrutinib resistance. CLL patient samples treated *ex vivo* with ibrutinib or acalabrutinib and the primary samples from CLL patients on clinical trials of both drugs show enhanced mitochondrial Bcl-2 dependence without significantly altering overall mitochondrial priming [109]. The Bcl-2 family regulators profiles restored to pre-treatment levels in the samples of CLL patients that developed ibrutinib resistance [110]. Treatment of DLBCL cells with ibrutinib increased Bcl-2 expression and combination treatment with Bcl-2 inhibitors and ibrutinib

completely inhibited tumor growth in murine models of ABC-DLBCL [111]. In ibrutinib resistant WM cell lines, apoptosis regulators Bcl-2 and Mcl-1 expression were increased [106]. With Bcl-2 inhibitor, venetoclax compromised mitochondrial function in ibrutinib-resistant WM cells by increasing mitochondrial outer membrane permeability (MOMP) with induction of apoptosis [106]. These data demonstrated that drug combination strategies encompassing BTK + Bcl-2 inhibition can potentially overcome ibrutinib resistance.

Cell Cycle Deregulation

Cell cycle regulator Cyclin D1, encoded by CCND1, binds and activates cyclin-dependent kinase (CDK) 4 to phosphorylate and inactivate retinoblastoma (Rb) protein [112]. This event leads to G1/S cell cycle progression and cell proliferation [112].

High-throughput sequencing has consistently revealed CCND1 was frequently mutated in MCL [113]. Recently, Mohanty et al. found some recurrent mutations located in the N-terminus of CCND1, which interfere with T286 phosphorylation and lead deregulated CCND1 turnover and increased protein levels [114]. More importantly, these mutated CCND1-expressing MCL cells were more resistant to ibrutinib [114]. In another study, it was found that tissue-specific proliferation of ibrutinib resistant MCL cells was driven by the activation CDK4 [102]. Cyclin-dependent kinase 4 specific inhibitor palbociclib prolonged early G1 arrest and sensitized resistant MCL cells to ibrutinib killing, suggesting a strategy to override acquired ibrutinib resistance [102].

Tumor Microenvironment Mediated Resistance

Tumor microenvironment (TME) is known as a critical regulator of immune escape, progression, metastasis of cancer, and tumor resistance to various therapies [115]. The complex cell-signaling relationship between MCL cells, TME and ibrutinib resistance, is currently under investigation but it is less studied in other types of B-cell lymphoma [116]. Zhao et al. recently revealed how the TME contributes to the development of acquired ibrutinib resistance in MCL [117]. They found that co-culture of MCL cells with lymph node stromal cells or bone marrow stromal cells significantly increased pBTK, pERK and pAKT in MCL cell lines and primary MCL cells. Ibrutinib resistant MCL cells had a marked increase in adhesion to stromal cells and enhanced clonogenic growth in the presence of ibrutinib. Combining kinomics, longitudinal drug screening with *ex vivo*, *in vivo* TME, and patient-derived xenograft models, Zhao et al. identified a major kinase network involving PI3K-AKT-mTOR/integrin $\beta 1$ -integrin-linked kinase (ILK) as a central hub for TME-lymphoma interactions mediating ibrutinib resistance [117]. When PI3K inhibitor dactolisib or mTOR inhibitor AZD8055 was combined with ibrutinib, cell

survival, b1 expression, cell adhesion and clonogenic growth were substantially inhibited in all ibrutinib resistant MCL lines and in patient samples of acquired ibrutinib resistant MCL. AZD8055 in combination with ibrutinib induced remarkable inhibition of ibrutinib resistant MCL, reduction of pAKT, pS6K1, p4EBP and b1 expression levels and reduced cell adhesion to stromal cells in these xenograft tumor cells [117]. Their finding suggested that combined disruption of BCR signaling and central pathways resulting from kinome reprogramming is critical for overcoming ibrutinib resistance in MCL.

Novel Approaches to Overcome BTK Inhibitors Resistance

Overcome Ibrutinib C481 Mutation Resistance with Non-Covalent Inhibitors of BTK

Based on the improved understanding of ibrutinib resistance, several strategies have been utilized to overcome BTK inhibitor especially ibrutinib resistance. The second-generation BTK inhibitors such as acalabrutinib are covalent, target-specific and have shown improved clinical responses. However, these covalent inhibitors often lose potency against BTK C481 mutations. One strategy to treat C481-mutant based ibrutinib resistance is to develop small molecule BTK inhibitors that do not depend upon binding to the C481 site for inhibition of BTK. Non-covalent inhibitors GDC-0853, SNS-062 (Vecabrutinib®), and GNE-431 have been evaluated in preclinical and clinical studies with potency against C481 mutant BTK [118–120]. GDC-0853 is a novel non-covalent, reversible, selective, orally bioavailable, and ATP-competitive inhibitor of BTK that effectively blocks BCR signaling in the treatment of B-cell malignancies including CLL [118]. *In vitro* studies showed that GDC-0853 reduced the activations of BTK, PLC γ 2, AKT, and ERK. Unlike ibrutinib, GDC-0853 inhibited signaling of both WT and C481S mutated BTK in transfected HEK293T cell lines and preserved NK cell mediated ADCC with clinical anti-CD20 antibodies [121]. In a phase 1 trial, unlike ibrutinib, GDC-0853 was able to inhibit BTK C481S mutants in CLL and NHL patients, demonstrated by reductions in C-C motif chemokine Ligand 3 (CCL3), which is one of the biomarkers to assess systemic inhibition of BTK in B-cell lymphoma [118]. SNS-062 is another non-covalent inhibitor of BTK unaffected by the C481S mutation. Fabian et al. found that SNS-062 and ibrutinib demonstrated comparable activity in inhibiting BTK, decreasing the expression of B cell activation markers, and reducing CLL cell viability in in BTK wild type CLL cells [119]. More importantly, SNS-062 was not affected by BTK C481S mutation but the activities of ibrutinib and acalabrutinib were hindered. SNS-062 also showed 6 times more potent than ibrutinib and more than 640 times more potent than acalabrutinib against C481S BTK [119]. Finally, the investigators found that SNS-062 diminished stromal cell protection in CLL cells, suggesting the drug can reduce the protection from the TME to CLL [119]. Their findings support clinical investigation of SNS-062 in patients with acquired

resistance to covalent BTK inhibitors. A phase 1b study is currently recruiting B lymphoid cancers (NCT03037645). Non-covalent inhibitor GNE-431 also showed excellent potency against the C481S, C481R, T474I, and T474 M mutants with nanomolar potency *in-vitro*, in cells, and in whole blood [120]. These non-covalent inhibitors may provide a potentially effective treatment option to ibrutinib resistant patients, but further studies are needed to demonstrate their clinical response.

Utilize Alternate Kinase Inhibitors to Overcome Ibrutinib Resistance

Researchers have proposed and investigated several other salvage approaches to overcome BTK inhibitors' resistance, which include using alternate kinase inhibitors. Based on an *in vitro* CLL proliferation model, Cheng et al. demonstrated that the ibrutinib resistant CLL cells were sensitive to the inhibition of dasatinib (blocking multiple tyrosine kinases including LYN and BTK), and SYK inhibitors (Cerdulatinib (PRT062070) and PRT060318) and idelalisib (PI3K δ inhibitor) [74]. In a recent study, the finding of the increased incidence of PI3K α in DLBCL sheds light on the molecular basis of the intrinsic resistance of DLBCL to PI3K δ inhibition observed in the clinic [122]. Copanlisib is a predominant PI3K α/δ dual inhibitor [122]. It led to significantly reduced cell viability *in-vitro* in both ibrutinib-sensitive and -resistant ABC-DLBCL cell lines by suppression of p-AKT and blocking nuclear factor- κ B activation driven by CD79mut, CARD11mut, TNFAIP3mut, or MYD88mut [122]. Copanlisib also demonstrated potent *in vivo* anti-tumor effect in ibrutinib-resistant CARD11mutand/or MYD88mut DLBCL mice models [122]. Dasatinib was identified as the most DLBCL-specific agent in a drug screen composed of 2160 FDA-approved drugs and other targeted drugs. Notably, dasatinib overcomes ibrutinib-resistance caused by BTK C481S mutation through FYN suppression [123]. These results are consistent with the previous report.

Combine BTK Inhibitors with Other Oncogenic Inhibitors

The second strategy is to utilize drug combination that targets multiple components or multiple oncogenic pathways (Table 6.4). Most of the reported combination studies are in preclinical evaluation with promising results. For example, addition of ONO/GS-4059 + entospletinib (SYK inhibitor) or idelalisib, had an additive effect on induction of apoptosis in primary CLL cells. The addition of ABT-199 to entospletinib, ONO/GS-4059, or idelalisib showed additive to synergistic effects on induction of apoptosis in primary CLL cells, and increased the maximal level of apoptosis [124]. The safety and tolerability of the combination was evaluated in a Phase 1b clinical trial [125]. ONO/GS-4059 at up to 160 mg in combination with entospletinib up to 400 mg daily was safe and well tolerated [125]. The combination

Table 6.4 Summary of combination therapies with BTK inhibitors

BTK inhibitor	Combination reagents	Role of the combination reagent	Diseases	Study stage	Year	Ref.
ONO/GS-4059	Idelalisib or Entospletinib or ABT-199	PI3K δ inhibitor, SYK inhibitor, Bcl-2 inhibitor	CLL	Preclinical	2015	[124]
Acalabrutinib	ACP-319	PI3K δ inhibition	CLL	Preclinical	2017	[126]
ONO/GS-4059	Idelalisib	PI3K δ inhibition	ABC-DLBCL	Preclinical	2017	[129]
ONO/GS-4059	Entospletinib	SYK inhibitor	CLL	Preclinical	2015	[124]
ONO/GS-4059	Entospletinib	SYK inhibitor	CLL; Non-GCB DLBCL; FL; WM; MCL; SLL; MZL	Phase 1b clinical	2017	[125]
Ibrutinib	AZD2014	mTOR1/2 inhibitor	ABC-DLBCL	Preclinical	2014	[128]
PLS-123	Everolimus	mTOR inhibitor	MCL	Preclinical	2018	[167]
Ibrutinib	Ulixertinib	ERK1/2 inhibitor	MYD88 mutated WM ABC DLBCL	Preclinical	2016	[132]
Ibrutinib	Pimasertib	MEK1/2 inhibitor	DLBCL MCL	Preclinical	2016	[168]
PLS-123	N/A	BTK/ PLC- γ 2 dual inhibitor	B cell lymphoma	Preclinical	2015	[133]
QLX138	N/A	BTK/MNK dual inhibitor	B cell lymphoma	Preclinical	2016	[134]
MDVN1003	N/A	BTK/PI3K δ dual inhibitor	B cell lymphoma	Preclinical	2017	[135]
Pyrimidine derivatives compounds	N/A	BTK/JAK dual inhibitor	B cell lymphoma	Preclinical	2018	[136]
Ibrutinib	Venetoclax (ABT-199)	Bcl-2 inhibitor	MCL, ABC-DLBCL, FL	Preclinical	2015, 2017	[138, 140]
Ibrutinib	Venetoclax	Bcl-2 inhibitor	MCL	Phase 2 clinical	2018	[139]
Acalabrutinib	Venetoclax	Bcl-2 inhibitor	CLL	Preclinical	2018	[169]
ONO/GS-4059	Venetoclax (ABT-199)	Bcl-2 inhibitor	CLL	Preclinical	2015	[124]
Ibrutinib	Rituximab	Anti-CD20 antibody	High risk CLL	Phase 2 clinical	2014	[141]
Ibrutinib	Rituximab	Anti-CD20 antibody	R/R MCL	Phase 2 clinical	2016	[142]
Ibrutinib	Rituximab	Anti-CD20 antibody	Naïve FL	Phase 2 clinical	2016	[143]

(continued)

Table 6.4 (continued)

BTK inhibitor	Combination reagents	Role of the combination reagent	Diseases	Study stage	Year	Ref.
Acalabrutinib	Rituximab	Anti-CD20 antibody	R/R FL	Phase 1/2 clinical	2015	[144]
Ibrutinib	R-CHOP	Rituximab + cyclophosphamide, doxorubicin, vincristine, and prednisone	Naïve B-NHL	Phase 1b clinical	2014	[145]
Ibrutinib	Bendamustine + rituximab	a nitrogen mustard drug + anti-CD20 antibody	Naïve or R/R NHL	Phase 1/1b clinical	2015	[146]
Ibrutinib	Bendamustine + rituximab	a nitrogen mustard drug + anti-CD20 antibody	R/R CLL	Phase 3 clinical	2016	[147]
ONO/GS-4059	Obinutuzumab	Anti-CD20 antibody	ABC-DLBCL	Preclinical	2017	[149]
Ibrutinib	CTL019	Anti-CD19 CAR T	MCL	Preclinical	2016	[152]
Ibrutinib	CD19/CD3-scFv-Fc	Bispecific antibody	CLL	Preclinical	2018	[155]
Ibrutinib	ACY1215	HDAC6 inhibitor	MCL	Preclinical	2012	[156]
Ibrutinib	Panobinostat	HDAC inhibitor	ABC-DLBCL	Preclinical	2017	[157]
Ibrutinib	Palbociclib	CDK4 inhibitor	MCL	Preclinical	2014	[102]
Ibrutinib	PF-00477736	Chk1 inhibitor	MCL	Preclinical	2018	[158]
Ibrutinib	Bortezomib	Proteasome inhibitor	Bortezomib-sensitive or – resistant DLBCL MCL	Preclinical	2013	[160]
Ibrutinib	Carfilzomib	Proteasome inhibitor	MCL	Preclinical	2014	[161]
Ibrutinib	VS-6063	Focal adhesion kinase inhibitor	MCL	Preclinical	2018	[164]

of acalabrutinib with the PI3K-delta inhibitor ACP-319 significantly reduced CLL tumor proliferation and tumor burden in the peripheral blood and spleen with reduced NF- κ B signaling and enhanced expression of BCL-xL and MCL-1 than single-agent therapy [126]. Vistusertib (AZD2014) is an ATP-competitive mTOR inhibitor, which can block the activity of both the mTORC1 (rapamycin-sensitive) and mTORC2 (rapamycin-insensitive) complexes and is highly selective against PI3K superfamily kinases [127]. The combination of ibrutinib and AZD2014 was shown to strongly induce apoptosis in ABC-DLBCL by regulation of 4EBP1 and cap-dependent translation (CDT) as well as Janus kinase (JAK) 3 / signal transducer and activator of transcription (STAT) 3, NF- κ B, STAT3, and mTOR pathways [128]. The combination treatment of another mTOR inhibitor everolimus and the second-generation BTK inhibitor PLS-123 significantly induced cell apoptosis, blocked cell cycle

progression and synergistically downregulates activation of BCR, AKT/mTOR, JAK2/STAT3 and MAPK signaling in MCL cell lines *in vitro* and effectively inhibited MCL tumor growth *in vivo* in severe combined immunodeficiency SCID mice. These combinations promise to be attractive therapeutic approaches in patients. However, further investigations are needed on ibrutinib resistant tumor cells.

The combinations of BTK inhibitors with PI3K inhibitors, MEK1/2 inhibitor, ERK1/2 inhibitor, or PIM1 inhibitor have been investigated in BTK inhibitor resistant B lymphoma cells with promising results. The combination of ONO/GS-4059 and idelalisib was investigated in ibrutinib resistant DLBCL cells. The acquired ibrutinib resistant DLBCL cells, which had loss of A20 and BTK C481F mutation, were insensitive to both idelalisib and ONO/GS-4059 as single agents but were significantly inhibited with the combination of both agents [129]. The decrease in p-I κ B α by the combination suggested that inhibition of MAPK and NF- κ B pathways might be the mechanism that leads to the decreased cell viability seen with combination treatment in this resistant cell line [129]. A clinical trial is currently underway to evaluate the combination of idelalisib and ONO/GS-4059 (NCT02457598) [129]. Activation of the ERK pathway is a very frequent observation in mature B-cell lymphoid tumors [130] and is implicated in the resistance to ibrutinib [85]. Pimasertib is a highly selective and ATP non-competitive MEK1/2 inhibitor and currently being tested in clinical phase 1/2 trials [131]. The combination of pimasertib and ibrutinib induced apoptosis with an increase of cleaved poly ADP ribose polymerase (PARP) and is active in ABC-DLBCL xenografts. MYD88 mutated WM and ABC DLBCL cells with BTK C481S mutation showed persistent activation of PLC γ 2-ERK1/2 signaling [132]. Ulixertinib (BVD-523, VRT752271) is a highly selective ERK1/2 inhibitor that is currently under clinical investigation. The combination of ulixertinib with ibrutinib produced higher levels of tumor cell killing than either agent alone, and significantly reduced interleukin 6 (IL-6) and interleukin 10 (IL-10) secretions which are associated with prosurvival signaling pathways [132]. The findings provide rationale for the investigation of ERK1/2 inhibitors in ibrutinib resistant MYD88 driven WM and ABC-DLBCL disease mediated by BTK mutations. As described in the early section, PIM1 inhibitor AZD-1208 may be a good choice to combine with ibrutinib to suppress ibrutinib resistance in ABC-DLBCL cells with mutations in PIM1 through NF- κ B pathway [80].

Dual inhibitors have emerged as an attractive strategy by inhibiting the catalytic activity of BTK and other kinases such as PLC- γ 2 kinase, Mitogen-Activated Protein Kinase interacting kinase (MNK kinase), PI3K δ kinase and JAK3. PLS-123 displayed impressive potency against BTK Tyr551 and PLC- γ 2 Tyr1217 phosphorylation [133]. It significantly reduced the phosphorylation of the BCR downstream signal pathways such as AKT/mTOR and MAPK [133]. Gene expression profile analysis further suggested that the different selectivity profile of PLS-123 led to significant downregulation of oncogenic gene tyrosine-protein phosphatase non-receptor type 11 (PTPN11) expression [133]. In addition, PLS-123 mediated TME to attenuate lymphoma cell adhesion and migration [133]. MNK kinase is one of the key downstream regulators in the RAF/MEK/ERK signaling pathway and controls protein synthesis via regulating the activity of

eukaryotic translation initiation factor 4E (eIF4E) [134]. Through a structure-based drug design approach, a potent BTK/MNK dual kinase inhibitor (QLX138) was discovered with covalent binding to BTK and non-covalent binding to MNK ability [134]. QLX138 enhanced the antiproliferative and apoptosis efficacies in-vitro against a variety of B-cell lymphoma cells, which respond moderately to BTK inhibitor in-vitro [134]. MDVN1003 is a first-in-class dual inhibitor of BTK and PI3K δ kinases [135]. MDVN1003 induced cell death of a B-cell lymphoma cell line and it reduced tumor growth in a B-cell lymphoma xenograft model more effectively than either ibrutinib or idelalisib [135]. JAK3 plays an important role in survival of B cells by regulating the activity of STAT3 (the antiapoptotic transcription factors signal transducer and activator of transcription 3), STAT5 and the antiapoptotic PI3K-AKT pathway and its downstream targets [136].

Using diphenylpyrimidine derivatives (DPPYs) as scaffolds, Ge et al. synthesized a new class of DPPY derivatives bearing a variety of the flexible C-2 aniline side chains [136]. Some of the pyrimidine derivatives showed high inhibitory potency of BTK and JAK3. Flow cytometric analysis, and a xenograft model for *in vivo* evaluation indicated the efficacy and low toxicity of 2 derivatives in the treatment of B-cell lymphoma [136]. These primary studies indicate that simultaneous inhibition of BTK and other kinases' activity might be a new therapeutic strategy for B-cell lymphoma and may overcome BTK inhibitor resistance.

Combine BTK Inhibitors With BCL-2 Inhibitors

The BH3-only mimetic Venetoclax® (ABT-199) selectively inactivates BCL-2 and is a promising drug for treatment of BCL2-dependent cancers [137]. The preclinical study shows that the combination of ibrutinib and Venetoclax® displayed strongly synergistic effects in MCL cell lines and primary cells from recurrent MCL patients, mechanistically, by perturbation of p-BTK and p-AKT mediated survival signals and of BCL2 family proteins [138]. A single-group, phase 2 study of the combination was conducted in R/R MCL patients compared with historical controls [139]. The CR rate at week 16 was 42%, which was higher than the historical result of 9% at this time point with ibrutinib monotherapy ($p < .001$) [139]. Seventy-eight percent of the patients with a response were estimated to have an ongoing response at 15 months [139]. The estimated rates of PFS were 75% at 12 months and 57% at 18 months [139]. Minimal residual disease (MRD) clearance was confirmed by flow cytometry and allele-specific oligonucleotide polymerase chain reaction (ASO-PCR), 67% and 38% respectively [139]. The side effects were generally low grade [139]. Additionally, the combination of ibrutinib and venetoclax also synergistically suppressed cell growth in ibrutinib-resistant ABC-DLBCL and FL cells that overexpressed BCL-2 in a preclinical study [140].

Combine BTK Inhibitors With Immunotherapies (Anti-CD20 Antibodies, Anti-CD19 CAR T Cells, CD19/CD3 Bispecific Antibody)

The activity and safety of adding ibrutinib or acalabrutinib to rituximab based therapies (alone, with rituximab-cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP), or with bendamustine) have been evaluated in early phase trials in patients with high-risk CLL, R/R MCL, naïve FL, R/R FL or R/R B-NHL [141–147] (Table 6.4). The encouraging results merit further investigation of the combinations in phase 3 trials in BTK resistant patients. ABC-DLBCL often has low response rate to BTK inhibitors ibrutinib or ONO/GS-4059 [66, 148]. The combination of ibrutinib combined with rituximab did not result in improved efficacy compared with respective monotherapy [149]. To overcome BTK inhibitor's resistance in ABC-DLBCL, the combination of ONO/GS-4059 with obinutuzumab (glycoengineered Type II CD20 antibody) or rituximab was evaluated for ABC-DLBCL in a preclinical study [149]. The combination was significantly better than the respective monotherapy with tumor growth inhibition of 90% for the obinutuzumab combination and 86% for the rituximab combination [149]. This result indicates that the combination of the second-generation inhibitor with rituximab and particularly obinutuzumab may be an effective treatment for resistant B-cell lymphoma.

Besides the combination with the antibody-based therapy, chimeric antigen receptor engineered T cells may be another good choice for the combination. Infusion of anti-CD19 CAR⁺ autologous T-cells (CART19, CTL019) into patients with B-cell lymphomas such as CLL and DLBCL leads to dramatic clinical responses [150, 151]. Taking the advantage of the vastly different mechanisms of action of CTL019 and ibrutinib, in a preclinical study, the combination of ibrutinib with CTL019 augmented the antitumor effect compared to single agent and led to prolonged remissions in MCL xenografts [152]. Strikingly, a recent study found that anti-CD19 modified CAR-T cells induced 71% ORR in CLL patients after ibrutinib failure [153]. Since ibrutinib has been shown to improve T-cell function in CLL [154], a novel CD19/CD3-scFv-Fc bispecific antibody was developed to work as an adjunct with ibrutinib to target ibrutinib-resistant disease [155]. CD19/CD3-scFv-Fc was shown to have the ability to eliminate ibrutinib resistant CLL cells *in vitro* and *in vivo* [155].

Combine BTK Inhibitors with Inhibitors Targeting Other Cellular Processes

The inhibitors involved in histone deacetylation, cell cycle regulation, protein degradation, cell stress and TME also show promising anti-tumor effects when combined with ibrutinib for resistant lymphoma diseases (Table 6.4). Treatment with ibrutinib plus ACY1215, a selective histone deacetylase 6 (HDAC6) inhibitor,

resulted in a three-fold increase in apoptosis induction in MCL tumor cell lines, pointing to a synergistic effect of BTK and HDAC6 inhibition in MCL [156]. Panobinostat, a non-selective histone deacetylase inhibitor, inhibited MyD88-driven NF- κ B activation, and enhanced ibrutinib efficacy in MyD88 mutant ABC-DLBCL [157]. The unrestrained proliferation of relapsed lymphoma cells after ibrutinib treatment suggests that simultaneous targeting of cell cycle regulators may override some mechanisms of resistance [102, 158]. Cyclin-dependent kinase 4 (CDK4) specific inhibitor palbociclib has been shown to prolong early G1 arrest and sensitized resistant MCL cells to ibrutinib killing [102]. Checkpoint kinase 1 (Chk1) inhibitor PF-00477736 also showed a synergistic anti-tumor effect with ibrutinib in-vitro in MCL cell lines that are sensitive or resistant to ibrutinib [158]. The ubiquitin-proteasome system degrades a variety of intracellular proteins, and plays an important role in maintenance of the balance between pro and anti-apoptotic proteins, and signal transduction regulation [159]. Synergistic interactions between ibrutinib and proteasome inhibitor bortezomib (the first approved therapeutic proteasome inhibitor) or carfilzomib (a selective proteasome inhibitor of the 20S proteasome) have been observed in a variety of DLBCL and MCL cells [160, 161]. However, further evaluations are needed for ibrutinib resistant cells. Additionally, heat shock proteins as molecular chaperones are exploited by tumor cells to buffer malignancy-associated cellular stress and facilitate the maturation, activation, and stabilization of many oncoproteins [162]. It was reported that heat shock protein 90 (HSP90) inhibitor AUY922 overcame nonclassical NF- κ B signaling and BTK C481S in MCL. Focal adhesion kinase (FAK) functions downstream of integrins and mediate signals from the extracellular matrix to tumor cells to enhance tumor cell proliferation, survival and migration in response to stromal interaction [163]. A recent study shows the role of FAK in bone marrow stroma-mediated enhancement of MCL proliferation and survival and the combined treatment of ibrutinib and defactinib (VS-6063), a FAK inhibitor in ibrutinib resistant MCL cells, was highly synergistic, and overcame the resistance by abrogation of the NF- κ B signaling pathway [164].

Conclusion

The BCR signaling pathway plays a crucial role in the development of B-cell lymphomas, providing a rationale to therapeutically target this pathway (Fig. 6.1). Several inhibitors targeting the members of this pathway have been developed and evaluated. Among these agents, BTK inhibitor ibrutinib with impressive clinical response and tolerability was the first to receive FDA approval for the treatment of patients with relapsed MCL and CLL. Ibrutinib has also shown promising activity in WM, FL and ABC-type DLBCL (Table 6.1). Ibrutinib has less kinase targeting specificity. It binds to BTK but also several other kinases. To improve the therapeutic effect, second-generation BTK inhibitors with more selective kinase activity profiles are developed and evaluated in early clinical trials (Table 6.2). With the promising clinical response and safer profile, acalabrutinib was granted Breakthrough

Therapy Designation by the FDA for patients with MCL who have received at least one prior therapy. Since many B-cell lymphoma depends on BCR signaling, the potential utility of BTK inhibitors will be tremendous. However, some patients show PRs or no response to BTK inhibitors at initial treatment and others developed disease progression and drug resistance during ibrutinib treatment. A better understanding of the resistant mechanism will allow accurate molecular classification of patients and assist in designing or choosing targeted therapies unique to that resistant mechanism. Advances in molecular genomics such as RNA-seq and whole genome sequencing have been instrumental in uncovering the ibrutinib resistant mechanisms. These mechanisms include mutational resistance in BTK and in other proteins, chromosomal abnormality, activation of prosurvival pathways, BCL-2 family members mediated resistance, and tumor microenvironment mediated resistance and potential other mechanisms that are beyond our discussion in this review (Fig. 6.2 and Table 6.3). The resistant mechanisms of the second-generation BTK inhibitors are less studied and further investigation is needed to compare with ibrutinib resistance. Non-covalent inhibitors of BTK have been developed to bypass C481 mutation in ibrutinib. Extensive preclinical studies of utilization of the inhibitors of alternate kinases other than BTK in the BCR pathway, and the combination therapies of BTK inhibitors with other oncogenic inhibitors, or with inhibitors involved in histone deacetylation, cell cycle regulation, protein degradation, cell stress and TME are encouraging to move on to clinical trials to overcome ibrutinib resistance. Furthermore, the combinations of BTK inhibitors with the novel agents of immunotherapies such as anti-CD20 antibodies, anti-CD19 CAR T cells, CD19/CD3 bispecific antibodies hold great promise for eradicating resistance and achieving better clinical outcomes in patients with B-cell lymphoma.

Acknowledgements This work was supported by the grants from Pediatric Cancer Research Foundation (MSC) and New York Medical College Translational Science Institute, Children Health Translational Research Grant (YC). YC reviewed the literatures, developed the design of the paper and wrote the manuscript. MSC and AB critically revised the manuscript and have approved the final version for publication. The authors would like to thank Erin Morris, RN, and Virginia Davenport, RN for their excellent assistance with the preparation of this manuscript.

Disclosure of Conflict of Interest No potential conflicts of interest were disclosed.

References

1. Campo E, Swerdlow SH, Harris NL, Pileri S, Stein H, Jaffe ES. The 2008 WHO classification of lymphoid neoplasms and beyond: evolving concepts and practical applications. *Blood*. 2011;117(19):5019–32.
2. Cairo MS, Krailo MD, Morse M, Hutchinson RJ, Harris RE, Kjeldsberg CR, Kadin ME, Radel E, Steinherz LJ, Morris E, Finlay JL, Meadows AT. Long-term follow-up of short intensive multiagent chemotherapy without high-dose methotrexate ('Orange') in children with advanced non-lymphoblastic non-Hodgkin's lymphoma: a children's cancer group report. *Leukemia*. 2002;16(4):594–600.

3. Cairo MS, Sposto R, Perkins SL, Meadows AT, Hoover-Regan ML, Anderson JR, Siegel SE, Lones MA, Tedeschi-Blok N, Kadin ME, Kjeldsberg CR, Wilson JF, Sanger W, Morris E, Krailo MD, Finlay JL. Burkitt's and Burkitt-like lymphoma in children and adolescents: a review of the Children's Cancer group experience. *Br J Haematol.* 2003;120(4):660–70.
4. Miles RR, Arnold S, Cairo MS. Risk factors and treatment of childhood and adolescent Burkitt lymphoma/leukaemia. *Br J Haematol.* 2012;156(6):730–43.
5. Cairo MS, Gerrard M, Sposto R, Auperin A, Pinkerton CR, Michon J, Weston C, Perkins SL, Raphael M, McCarthy K, Patte C, Committee FLIS. Results of a randomized international study of high-risk central nervous system B non-Hodgkin lymphoma and B acute lymphoblastic leukemia in children and adolescents. *Blood.* 2007;109(7):2736–43.
6. Gerrard M, Cairo MS, Weston C, Auperin A, Pinkerton R, Lambilliotte A, Sposto R, McCarthy K, Lacombe MJ, Perkins SL, Patte C. Excellent survival following two courses of COPAD chemotherapy in children and adolescents with resected localized B-cell non-Hodgkin's lymphoma: results of the FAB/LMB 96 international study. *Br J Haematol.* 2008;141(6):840–7.
7. Patte C, Auperin A, Gerrard M, Michon J, Pinkerton R, Sposto R, Weston C, Raphael M, Perkins SL, McCarthy K, Cairo MS, Committee FLIS. Results of the randomized international FAB/LMB96 trial for intermediate risk B-cell non-Hodgkin lymphoma in children and adolescents: it is possible to reduce treatment for the early responding patients. *Blood.* 2007;109(7):2773–80.
8. Cairo MS, Sposto R, Gerrard M, Auperin A, Goldman SC, Harrison L, Pinkerton R, Raphael M, McCarthy K, Perkins SL, Patte C. Advanced stage, increased lactate dehydrogenase, and primary site, but not adolescent age (≥ 15 years), are associated with an increased risk of treatment failure in children and adolescents with mature B-cell non-Hodgkin's lymphoma: results of the FAB LMB 96 study. *J Clin Oncol.* 2012;30(4):387–93.
9. Rickert RC. New insights into pre-BCR and BCR signalling with relevance to B cell malignancies. *Nat Rev Immunol.* 2013;13(8):578–91.
10. Kraus M, Alimzhanov MB, Rajewsky N, Rajewsky K. Survival of resting mature B lymphocytes depends on BCR signaling via the Igalpha/beta heterodimer. *Cell.* 2004;117(6):787–800.
11. Havranek O, Xu J, Kohrer S, Wang Z, Becker L, Comer JM, Henderson J, Ma W, Man Chun Ma J, Westin JR, Ghosh D, Shinnars N, Sun L, Yi AF, Karri AR, Burger JA, Zal T, Davis RE. Tonic B-cell receptor signaling in diffuse large B-cell lymphoma. *Blood.* 2017;130(8):995–1006.
12. Martinez N, Almaraz C, Vaque JP, Varela I, Derdak S, Beltran S, Mollejo M, Campos-Martin Y, Agueda L, Rinaldi A, Kwee I, Gut M, Blanc J, Oscier D, Strefford JC, Martinez-Lopez J, Salar A, Sole F, Rodriguez-Peralto JL, Diez-Tascon C, Garcia JF, Fraga M, Sebastian E, Alves J, Menarguez J, Gonzalez-Carrero J, Casado LF, Bayes M, Bertoni F, Gut I, Piris MA. Whole-exome sequencing in splenic marginal zone lymphoma reveals mutations in genes involved in marginal zone differentiation. *Leukemia.* 2014;28(6):1334–40.
13. Krysiak K, Gomez F, White BS, Matlock M, Miller CA, Trani L, Fronick CC, Fulton RS, Kreisel F, Cashen AF, Carson KR, Berrien-Elliott MM, Bartlett NL, Griffith M, Griffith OL, Fehniger TA. Recurrent somatic mutations affecting B-cell receptor signaling pathway genes in follicular lymphoma. *Blood.* 2017;129(4):473–83.
14. Corso J, Pan KT, Walter R, Doebele C, Mohr S, Bohnenberger H, Strobel P, Lenz C, Slabicki M, Hullein J, Comoglio F, Rieger MA, Zenz T, Wienands J, Engelke M, Serve H, Urlaub H, Oellerich T. Elucidation of tonic and activated B-cell receptor signaling in Burkitt's lymphoma provides insights into regulation of cell survival. *Proc Natl Acad Sci USA.* 2016;113(20):5688–93.
15. Burger JA, Wiestner A. Targeting B cell receptor signalling in cancer: preclinical and clinical advances. *Nat Rev Cancer.* 2018;18(3):148–67.
16. Weber ANR, Bittner Z, Liu X, Dang TM, Radsak MP, Brunner C. Bruton's tyrosine kinase: an emerging key player in innate immunity. *Front Immunol.* 2017;8:1454.
17. Buhl AM, Cambier JC. Phosphorylation of CD19 Y484 and Y515, and linked activation of phosphatidylinositol 3-kinase, are required for B cell antigen receptor-mediated activation of Bruton's tyrosine kinase. *J Immunol.* 1999;162(8):4438–46.

18. Vetrie D, Vorechovsky I, Sideras P, Holland J, Davies A, Flinter F, Hammarstrom L, Kinnon C, Levinsky R, Bobrow M, et al. The gene involved in X-linked agammaglobulinaemia is a member of the src family of protein-tyrosine kinases. *Nature*. 1993;361(6409):226–33.
19. Tsukada S, Saffran DC, Rawlings DJ, Parolini O, Allen RC, Klisak I, Sparkes RS, Kubagawa H, Mohandas T, Quan S, et al. Deficient expression of a B cell cytoplasmic tyrosine kinase in human X-linked agammaglobulinemia. *Cell*. 1993;72(2):279–90.
20. Mohamed AJ, Nore BF, Christensson B, Smith CI. Signalling of Bruton's tyrosine kinase, Btk. *Scand J Immunol*. 1999;49(2):113–8.
21. Wahl MI, Fluckiger AC, Kato RM, Park H, Witte ON, Rawlings DJ. Phosphorylation of two regulatory tyrosine residues in the activation of Bruton's tyrosine kinase via alternative receptors. *Proc Natl Acad Sci USA*. 1997;94(21):11526–33.
22. Mohamed AJ, Yu L, Backesjo CM, Vargas L, Faryal R, Aints A, Christensson B, Berglof A, Vihinen M, Nore BF, Smith CI. Bruton's tyrosine kinase (Btk): function, regulation, and transformation with special emphasis on the PH domain. *Immunol Rev*. 2009;228(1):58–73.
23. Di Paolo JA, Huang T, Balazs M, Barbosa J, Barck KH, Bravo BJ, Carano RA, Darrow J, Davies DR, DeForge LE, Diehl L, Ferrando R, Gallion SL, Giannetti AM, Gribling P, Hurez V, Hymowitz SG, Jones R, Kropf JE, Lee WP, Maciejewski PM, Mitchell SA, Rong H, Staker BL, Whitney JA, Yeh S, Young WB, Yu C, Zhang J, Reif K, Currie KS. Specific Btk inhibition suppresses B cell- and myeloid cell-mediated arthritis. *Nat Chem Biol*. 2011;7(1):41–50.
24. Hendriks RW, de Bruijn MF, Maas A, Dingjan GM, Karis A, Grosveld F. Inactivation of Btk by insertion of lacZ reveals defects in B cell development only past the pre-B cell stage. *EMBO J*. 1996;15(18):4862–72.
25. Vihinen M, Brandau O, Branden LJ, Kwan SP, Lappalainen I, Lester T, Noordzij JG, Ochs HD, Ollila J, Pienaar SM, Riikonen P, Saha BK, Smith CI. BTKbase, mutation database for X-linked agammaglobulinemia (XLA). *Nucleic Acids Res*. 1998;26(1):242–7.
26. Thomas JD, Sideras P, Smith CI, Vorechovsky I, Chapman V, Paul WE. Colocalization of X-linked agammaglobulinemia and X-linked immunodeficiency genes. *Science*. 1993;261(5119):355–8.
27. Qiu Y, Kung HJ. Signaling network of the Btk family kinases. *Oncogene*. 2000;19(49):5651–61.
28. Davis RE, Ngo VN, Lenz G, Tolar P, Young RM, Romesser PB, Kohlhammer H, Lamy L, Zhao H, Yang Y, Xu W, Shaffer AL, Wright G, Xiao W, Powell J, Jiang JK, Thomas CJ, Rosenwald A, Ott G, Muller-Hermelink HK, Gascoyne RD, Connors JM, Johnson NA, Rimsza LM, Campo E, Jaffe ES, Wilson WH, Delabie J, Smeland EB, Fisher RI, Braziel RM, Tubbs RR, Cook JR, Weisenburger DD, Chan WC, Pierce SK, Staudt LM. Chronic active B-cell-receptor signalling in diffuse large B-cell lymphoma. *Nature*. 2010;463(7277):88–92.
29. Young RM, Staudt LM. Targeting pathological B cell receptor signalling in lymphoid malignancies. *Nat Rev Drug Discov*. 2013;12(3):229–43.
30. Honigberg LA, Smith AM, Sirisawad M, Verner E, Louny D, Chang B, Li S, Pan Z, Thamm DH, Miller RA, Buggy JJ. The Bruton tyrosine kinase inhibitor PCI-32765 blocks B-cell activation and is efficacious in models of autoimmune disease and B-cell malignancy. *Proc Natl Acad Sci USA*. 2010;107(29):13075–80.
31. Mathews Griner LA, Guha R, Shinn P, Young RM, Keller JM, Liu D, Goldlust IS, Yasgar A, McKnight C, Boxer MB, Duveau DY, Jiang JK, Michael S, Mierzwa T, Huang W, Walsh MJ, Mott BT, Patel P, Leister W, Maloney DJ, Leclair CA, Rai G, Jadhav A, Peyser BD, Austin CP, Martin SE, Simeonov A, Ferrer M, Staudt LM, Thomas CJ. High-throughput combinatorial screening identifies drugs that cooperate with ibrutinib to kill activated B-cell-like diffuse large B-cell lymphoma cells. *Proc Natl Acad Sci USA*. 2014;111(6):2349–54.
32. Herman SE, Gordon AL, Hertlein E, Ramanunni A, Zhang X, Jaglowski S, Flynn J, Jones J, Blum KA, Buggy JJ, Hamdy A, Johnson AJ, Byrd JC. 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):6287–96.
33. Cinar M, Hamedani F, Mo Z, Cinar B, Amin HM, Alkan S. Bruton tyrosine kinase is commonly overexpressed in mantle cell lymphoma and its attenuation by Ibrutinib induces apoptosis. *Leuk Res*. 2013;37(10):1271–7.

34. Advani RH, Buggy JJ, Sharman JP, Smith SM, Boyd TE, Grant B, Kolibaba KS, Furman RR, Rodriguez S, Chang BY, Sukbuntherng J, Izumi R, Hamdy A, Hedrick E, Fowler NH. Bruton tyrosine kinase inhibitor ibrutinib (PCI-32765) has significant activity in patients with relapsed/refractory B-cell malignancies. *J Clin Oncol.* 2013;31(1):88–94.
35. Wang ML, Rule S, Martin P, Goy A, Auer R, Kahl BS, Jurczak W, Advani RH, Romaguera JE, Williams ME, Barrientos JC, Chmielowska E, Radford J, Stilgenbauer S, Dreyling M, Jędrzejczak WW, Johnson P, Spurgeon SE, Li L, Zhang L, Newberry K, Ou Z, Cheng N, Fang B, McGreivy J, Clow F, Buggy JJ, Chang BY, Beaupre DM, Kunkel LA, Blum KA. Targeting BTK with ibrutinib in relapsed or refractory mantle-cell lymphoma. *N Engl J Med.* 2013;369(6):507–16.
36. Byrd JC, Furman RR, Coutre SE, Flinn IW, Burger JA, Blum KA, Grant B, Sharman JP, Coleman M, Wierda WG, Jones JA, Zhao W, Heerema NA, Johnson AJ, Sukbuntherng J, Chang BY, Clow F, Hedrick E, Buggy JJ, James DF, O'Brien S. Targeting BTK with ibrutinib in relapsed chronic lymphocytic leukemia. *N Engl J Med.* 2013;369(1):32–42.
37. Aalipour A, Advani RH. Bruton tyrosine kinase inhibitors: a promising novel targeted treatment for B cell lymphomas. *Br J Haematol.* 2013;163(4):436–43.
38. Advani RH, Buggy JJ, Sharman JP, Smith SM, Boyd TE, Grant B, Kolibaba KS, Furman RR, Rodriguez S, Chang BY, Sukbuntherng J, Izumi R, Hamdy A, Hedrick E, Fowler NH. Bruton tyrosine kinase inhibitor ibrutinib (PCI-32765) has significant activity in patients with relapsed/refractory B-cell malignancies. *J Clin Oncol.* 2013;31(1):88–94.
39. Treon SP, Tripsas CK, Meid K, Warren D, Varma G, Green R, Argyropoulos KV, Yang G, Cao Y, Xu L, Patterson CJ, Rodig S, Zehnder JL, Aster JC, Harris NL, Kanan S, Ghobrial I, Castillo JJ, Laubach JP, Hunter ZR, Salman Z, Li J, Cheng M, Clow F, Graef T, Palomba ML, Advani RH. Ibrutinib in previously treated Waldenström's macroglobulinemia. *N Engl J Med.* 2015;372(15):1430–40.
40. Fernandez-Vega I, Quiros LM, Santos-Juanes J, Pane-Foix M, Marafioti T. Bruton's tyrosine kinase (Btk) is a useful marker for Hodgkin and B cell non-Hodgkin lymphoma. *Virchows Arch.* 2015;466(2):229–35.
41. Hamadani M, Balasubramanian S, Hari PN. Ibrutinib in refractory classic Hodgkin's lymphoma. *N Engl J Med.* 2015;373(14):1381–2.
42. Sachen KL, Strohmman MJ, Singletary J, Alizadeh AA, Kattah NH, Lossos C, Mellins ED, Levy S, Levy R. Self-antigen recognition by follicular lymphoma B-cell receptors. *Blood.* 2012;120(20):4182–90.
43. Fowler HN, Advani HR, Sharman J, Smith MS, McGreivy J, Kunkel L, Troung V, Zhou C, Boyd TE. The Bruton's tyrosine kinase inhibitor ibrutinib (PCI-32765) is active and tolerated in relapsed follicular lymphoma. *Blood.* 2012;120:156.
44. Bartlett NL, Costello BA, LaPlant BR, Ansell SM, Kuruvilla JG, Reeder CB, Thye LS, Anderson DM, Krysiak K, Ramirez C, Qi J, Siegel BA, Griffith M, Griffith OL, Gomez F, Fehniger TA. Single-agent ibrutinib in relapsed or refractory follicular lymphoma: a phase 2 consortium trial. *Blood.* 2018;131(2):182–90.
45. Gopal AK, Schuster SJ, Fowler N, Trotman J, Hess G, Hou JZ, Yacoub A, Lill M, Martin P, Vitolo U, Jurczak W, Morton J, Osmanov D, Gartenberg GJ, Vermeulen J, Balasubramanian S, Wang SS, Deshpande S, Salles GA. Ibrutinib as treatment for Chemoimmunotherapy-resistant Patients with follicular Lymphoma: first results from the open-label, multicenter, phase 2 DAWN study. *Blood.* 2016;128:1217.
46. Ngo VN, Young RM, Schmitz R, Jhavar S, Xiao W, Lim KH, Kohlhammer H, Xu W, Yang Y, Zhao H, Shaffer AL, Romesser P, Wright G, Powell J, Rosenwald A, Muller-Hermelink HK, Ott G, Gascoyne RD, Connors JM, Rimsza LM, Campo E, Jaffe ES, Delabie J, Smeland EB, Fisher RI, Braziel RM, Tubbs RR, Cook JR, Weisenburger DD, Chan WC, Staudt LM. Oncogenically active MYD88 mutations in human lymphoma. *Nature.* 2011;470(7332):115–9.
47. Lenz G, Davis RE, Ngo VN, Lam L, George TC, Wright GW, Dave SS, Zhao H, Xu W, Rosenwald A, Ott G, Muller-Hermelink HK, Gascoyne RD, Connors JM, Rimsza LM, Campo

- E, Jaffe ES, Delabie J, Smeland EB, Fisher RI, Chan WC, Staudt LM. Oncogenic CARD11 mutations in human diffuse large B cell lymphoma. *Science*. 2008;319(5870):1676–9.
48. Kraan W, Horlings HM, van Keimpema M, Schilder-Tol EJ, Oud ME, Scheepstra C, Kluin PM, Kersten MJ, Spaargaren M, Pals ST. High prevalence of oncogenic MYD88 and CD79B mutations in diffuse large B-cell lymphomas presenting at immune-privileged sites. *Blood Cancer J*. 2013;3:e139.
49. Wilson WH, Gerecitano JF, Goy A, de Vos S, Kenkre VP, Barr PM, Blum KA, Shustov AR, Advani RH, Lih J, Williams M, Schmitz R, Yang Y, Pittaluga S, Wright G, Kunkel LA, McCreivy J, Balasubramanian S, Cheng M, Moussa D, Buggy J, Staudt LM. The bruton's tyrosine kinase (BTK) inhibitor, ibrutinib (PCI-32765), has preferential activity in the ABC subtype of relapsed/refractory De Novo Diffuse Large B-Cell Lymphoma (DLBCL): interim results of a multicenter, open-label, phase 2 study. *Blood*. 2012;120:686.
50. Byrd JC, Brown JR, O'Brien S, Barrientos JC, Kay NE, Reddy NM, Coutre S, Tam CS, Mulligan SP, Jaeger U, Devereux S, Barr PM, Furman RR, Kipps TJ, Cymbalista F, Pocock C, Thornton P, Caligaris-Cappio F, Robak T, Delgado J, Schuster SJ, Montillo M, Schuh A, de Vos S, Gill D, Bloor A, Dearden C, Moreno C, Jones JJ, Chu AD, Fardis M, McCreivy J, Clow F, James DF, Hillmen P. Ibrutinib versus ofatumumab in previously treated chronic lymphoid leukemia. *N Engl J Med*. 2014;371(3):213–23.
51. Burger JA, Tedeschi A, Barr PM, Robak T, Owen C, Ghia P, Bairey O, Hillmen P, Bartlett NL, Li J, Simpson D, Grosicki S, Devereux S, McCarthy H, Coutre S, Quach H, Gaidano G, Maslyak Z, Stevens DA, Janssens A, Offner F, Mayer J, O'Dwyer M, Hellmann A, Schuh A, Siddiqi T, Polliack A, Tam CS, Suri D, Cheng M, Clow F, Styles L, James DF, Kipps TJ. Ibrutinib as initial therapy for patients with chronic lymphocytic leukemia. *N Engl J Med*. 2015;373(25):2425–37.
52. Witzig TE, Geyer SM, Ghobrial I, Inwards DJ, Fonseca R, Kurtin P, Ansell SM, Luyun R, Flynn PJ, Morton RF, Dakhil SR, Gross H, Kaufmann SH. Phase II trial of single-agent temsirolimus (CCI-779) for relapsed mantle cell lymphoma. *J Clin Oncol*. 2005;23(23):5347–56.
53. Ansell SM, Inwards DJ, Rowland KM Jr, Flynn PJ, Morton RF, Moore DF Jr, Kaufmann SH, Ghobrial I, Kurtin PJ, Maurer M, Allmer C, Witzig TE. Low-dose, single-agent temsirolimus for relapsed mantle cell lymphoma: a phase 2 trial in the North Central Cancer treatment group. *Cancer*. 2008;113(3):508–14.
54. Hess G, Herbrecht R, Romaguera J, Verhoef G, Crump M, Gisselbrecht C, Laurell A, Offner F, Strahs A, Berkenblit A, Hanushevsky O, Clancy J, Hewes B, Moore L, Coiffier B. Phase III study to evaluate temsirolimus compared with investigator's choice therapy for the treatment of relapsed or refractory mantle cell lymphoma. *J Clin Oncol*. 2009;27(23):3822–9.
55. Dreyling M, Jurczak W, Jerkeman M, Silva RS, Rusconi C, Trneny M, Offner F, Caballero D, Joao C, Witzens-Harig M, Hess G, Bence-Bruckler I, Cho SG, Bothos J, Goldberg JD, Enny C, Traina S, Balasubramanian S, Bandyopadhyay N, Sun S, Vermeulen J, Rizo A, Rule S. Ibrutinib versus temsirolimus in patients with relapsed or refractory mantle-cell lymphoma: an international, randomised, open-label, phase 3 study. *Lancet*. 2016;387(10020):770–8.
56. Chu Y, Lee S, Shah T, Yin CH, Barth M, Miles RR, Ayello J, Morris E, Harrison L, van de Ven C, Galardy P, Goldman SC, Lim MS, Hermiston M, McAllister-Lucas LM, Giulino-Roth L, Perkins SL, Cairo MS. Ibrutinib significantly inhibited Bruton's tyrosine kinase (BTK) phosphorylation, in-vitro proliferation and enhanced overall survival in a preclinical Burkitt lymphoma (BL) model *Oncoimmunology*, 2018. Published online: 11 Oct 2018.
57. Chen J, Kinoshita T, Sukbuntherng J, Chang BY, Elias L. Ibrutinib inhibits ERBB receptor tyrosine kinases and HER2-amplified breast Cancer Cell growth. *Mol Cancer Ther*. 2016;15(12):2835–44.
58. Barf T, Covey T, Izumi R, van de Kar B, Gulrajani M, van Lith B, van Hoek M, de Zwart E, Mittag D, Demont D, Verkaik S, Krantz F, Pearson PG, Ulrich R, Kaptein A. Acalabrutinib (ACP-196): a covalent Bruton tyrosine kinase inhibitor with a differentiated selectivity and in vivo potency profile. *J Pharmacol Exp Ther*. 2017;363(2):240–52.

59. Patel V, Balakrishnan K, Bibikova E, Ayres M, Keating MJ, Wierda WG, Gandhi V. Comparison of Acalabrutinib, a selective Bruton tyrosine kinase inhibitor, with Ibrutinib in chronic lymphocytic leukemia cells. *Clin Cancer Res.* 2017;23(14):3734–43.
60. Covey T, Barf T, Gulrajani M, Krantz F, Lith BV, Bibikova E, van de Kar B, Zwart ED, Hamdy A, Izumi R, Kaptein A. ACP-196: a novel covalent Bruton's tyrosine kinase (Btk) inhibitor with improved selectivity and in vivo target coverage in chronic lymphocytic leukemia (CLL) patients. *Cancer Res.* 2015;75(15 Supplement):2596.
61. Byrd JC, Harrington B, O'Brien S, Jones JA, Schuh A, Devereux S, Chaves J, Wierda WG, Awan FT, Brown JR, Hillmen P, Stephens DM, Ghia P, Barrientos JC, Pagel JM, Woyach J, Johnson D, Huang J, Wang X, Kaptein A, Lannutti BJ, Covey T, Fardis M, McCreivry J, Hamdy A, Rothbaum W, Izumi R, Diacovo TG, Johnson AJ, Furman RR. Acalabrutinib (ACP-196) in relapsed chronic lymphocytic leukemia. *N Engl J Med.* 2016;374(4):323–32.
62. Wang M, Rule S, Zinzani PL, Goy A, Casasnovas O, Smith SD, Damaj G, Doorduijn J, Lamy T, Morschhauser F, Panizo C, Shah B, Davies A, Eek R, Dupuis J, Jacobsen E, Kater AP, Le Gouill S, Oberic L, Robak T, Covey T, Dua R, Hamdy A, Huang X, Izumi R, Patel P, Rothbaum W, Slatter JG, Jurczak W. Acalabrutinib in relapsed or refractory mantle cell lymphoma (ACE-LY-004): a single-arm, multicentre, phase 2 trial. *Lancet.* 2018;391(10121):659–67.
63. Yasuhiro T, Yoshizawa T, Daub H, Weber C, Narita M, Kawabata K. ONO-WG-307, a novel, potent and selective inhibitor of Bruton's tyrosine kinase (Btk), results in sustained inhibition of the ERK, AKT and PKD signaling pathways. *Cancer Res.* 2012;72(suppl 8). Abstract 2021
64. Kozaki R, Hutchinson C, Sandrine J, Dyer MJS. Kinome reprogramming in DLBCL by the BTKspecific inhibitor ONO4059 highlights synergistic combinations for clinical application. *Haematologica.* 2014;99(S1):137–8.
65. Kozaki R, Yoshizawa T, Tohda S, Yasuhiro T, Hotta S, Ariza Y, Ueda Y, Narita M, Kawabata K. Development of a bruton's tyrosine kinase (btk) inhibitor, ONOWG307: efficacy in ABCDLBCL xenograft model potential treatment for Bcell malignancies. *Blood.* 2011;118(21):3731.
66. Walter HS, Rule SA, Dyer MJ, Karlin L, Jones C, Cazin B, Quittet P, Shah N, Hutchinson CV, Honda H, Duffy K, Birkett J, Jamieson V, Courtenay-Luck N, Yoshizawa T, Sharpe J, Ohno T, Abe S, Nishimura A, Cartron G, Morschhauser F, Fegan C, Salles G. A phase 1 clinical trial of the selective BTK inhibitor ONO/GS-4059 in relapsed and refractory mature B-cell malignancies. *Blood.* 2016;127(4):411–9.
67. Tam C, Grigg AP, Opat S, Ku M, Gilbertson M, Anderson MA, Seymour JF, Ritchie DS, Dicorleto C, Dimovski B, Hedrick E, Yang J, Wang L, Luo L, Xue L, Roberts AW. The BTK inhibitor, Bgb-3111, is safe, tolerable, and highly active in patients with relapsed/ refractory B-cell malignancies: initial report of a phase 1 first-in-human trial. *Blood.* 2015;126(23):832.
68. Li N, Sun Z, Liu Y, Guo M, Zhang Y, Zhou D, Zhang B, Su D, Zhang S, Han J, Gao Y, Guo Y, Wang Z, Wei M, Luo L, Wang L. BGB-3111 is a novel and highly selective Bruton's tyrosine kinase (BTK) inhibitor. *Cancer Res.* 2015;75(15):2597.
69. Zhang SQ, Smith SM, Zhang SY, Lynn Wang Y. Mechanisms of ibrutinib resistance in chronic lymphocytic leukaemia and non-Hodgkin lymphoma. *Br J Haematol.* 2015;170(4):445–56.
70. Maddocks KJ, Ruppert AS, Lozanski G, Heerema NA, Zhao W, Abruzzo L, Lozanski A, Davis M, Gordon A, Smith LL, Mantel R, Jones JA, Flynn JM, Jaglowski SM, Andritsos LA, Awan F, Blum KA, Grever MR, Johnson AJ, Byrd JC, Woyach JA. Etiology of Ibrutinib therapy discontinuation and outcomes in patients with chronic lymphocytic leukemia. *JAMA Oncol.* 2015;1(1):80–7.
71. Komarova NL, Burger JA, Wodarz D. Evolution of ibrutinib resistance in chronic lymphocytic leukemia (CLL). *Proc Natl Acad Sci USA.* 2014;111(38):13906–11.
72. Woyach JA, Furman RR, Liu TM, Ozer HG, Zapatka M, Ruppert AS, Xue L, Li DH, Steggerda SM, Versele M, Dave SS, Zhang J, Yilmaz AS, Jaglowski SM, Blum KA, Lozanski A, Lozanski G, James DF, Barrientos JC, Lichter P, Stilgenbauer S, Buggy JJ, Chang BY, Johnson AJ, Byrd JC. Resistance mechanisms for the Bruton's tyrosine kinase inhibitor ibrutinib. *N Engl J Med.* 2014;370(24):2286–94.

73. Albitar A, Ma W, De Dios I, Estrella J, Farooqui M, Wiestner A, Albitar M. High sensitivity testing shows multiclonal mutations in patients with CLL treated with BTK inhibitor and lack of mutations in Ibrutinib-naive patients. *Blood*. 2015;126:716.
74. 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.
75. Furman RR, Cheng S, Lu P, Setty M, Perez AR, Guo A, Racchumi J, Xu G, Wu H, Ma J, Steggerda SM, Coleman M, Leslie C, Wang YL. Ibrutinib resistance in chronic lymphocytic leukemia. *N Engl J Med*. 2014;370(24):2352–4.
76. Sharma S, Galanina N, Guo A, Lee J, Kadri S, Van Slambrouck C, Long B, Wang W, Ming M, Furtado LV, Segal JP, Stock W, Venkataraman G, Tang WJ, Lu P, Wang YL. Identification of a structurally novel BTK mutation that drives ibrutinib resistance in CLL. *Oncotarget*. 2016;7(42):68833–41.
77. Fama R, Bomben R, Rasi S, Dal Bo M, Ciardullo C, Monti S, Rossi F, D'Agaro T, Zucchetto A, Gattei V, Gaidano G, Rossi D. Ibrutinib-naive chronic lymphocytic leukemia lacks Bruton tyrosine kinase mutations associated with treatment resistance. *Blood*. 2014;124(25):3831–3.
78. Lenz G, Balasubramanian S, Goldberg J, Rizo A, Schaffer M, Phelps C, Rule S, Dreyling MH. Sequence variants in patients with primary and acquired resistance to ibrutinib in the phase 3 MCL3001 (RAY) trial. *J Clin Oncol*. 2016;34(15_suppl):7570.
79. Alvarado Y, Giles FJ, Swords RT. The PIM kinases in hematological cancers. *Expert Rev Hematol*. 2012;5(1):81–96.
80. Kuo HP, Ezell SA, Hsieh S, Schweighofer KJ, Cheung LW, Wu S, Apatira M, Sirisawad M, Eckert K, Liang Y, Hsu J, Chen CT, Beaupre D, Chang BY. The role of PIM1 in the ibrutinib-resistant ABC subtype of diffuse large B-cell lymphoma. *Am J Cancer Res*. 2016;6(11):2489–501.
81. Treon SP, Xu L, Yang G, Zhou Y, Liu X, Cao Y, Sheehy P, Manning RJ, Patterson CJ, Tripsas C, Arcaini L, Pinkus GS, Rodig SJ, Sohani AR, Harris NL, Laramie JM, Skifter DA, Lincoln SE, Hunter ZR. MYD88 L265P somatic mutation in Waldenstrom's macroglobulinemia. *N Engl J Med*. 2012;367(9):826–33.
82. Hunter ZR, Xu L, Yang G, Zhou Y, Liu X, Cao Y, Manning RJ, Tripsas C, Patterson CJ, Sheehy P, Treon SP. The genomic landscape of Waldenstrom macroglobulinemia is characterized by highly recurring MYD88 and WHIM-like CXCR4 mutations, and small somatic deletions associated with B-cell lymphomagenesis. *Blood*. 2014;123(11):1637–46.
83. Xu L, Tsakmaklis N, Yang G, Chen JG, Liu X, Demos M, Kofides A, Patterson CJ, Meid K, Gustine J, Dubeau T, Palomba ML, Advani R, Castillo JJ, Furman RR, Hunter ZR, Treon SP. Acquired mutations associated with ibrutinib resistance in Waldenstrom macroglobulinemia. *Blood*. 2017;129(18):2519–25.
84. Treon SP, Xu L, Hunter Z. MYD88 mutations and response to Ibrutinib in Waldenstrom's Macroglobulinemia. *N Engl J Med*. 2015;373(6):584–6.
85. Cao Y, Hunter ZR, Liu X, Xu L, Yang G, Chen J, Patterson CJ, Tsakmaklis N, Kanan S, Rodig S, Castillo JJ, Treon SP. The WHIM-like CXCR4(S338X) somatic mutation activates AKT and ERK, and promotes resistance to ibrutinib and other agents used in the treatment of Waldenstrom's Macroglobulinemia. *Leukemia*. 2015;29(1):169–76.
86. Dimopoulos MA, Trotman J, Tedeschi A, Matous JV, Macdonald D, Tam C, Tournilhac O, Ma S, Oriol A, Heffner LT, Shustik C, Garcia-Sanz R, Cornell RF, de Larrea CF, Castillo JJ, Granell M, Kyrtonis MC, Leblond V, Symeonidis A, Kastiris E, Singh P, Li J, Graef T, Bilotti E, Treon S, Buske C. Ibrutinib for patients with rituximab-refractory Waldenstrom's macroglobulinaemia (iNNOVATE): an open-label substudy of an international, multicentre, phase 3 trial. *Lancet Oncol*. 2017;18(2):241–50.
87. Wu C, de Miranda NF, Chen L, Wasik AM, Mansouri L, Jurczak W, Galazka K, Dlugosz-Danecka M, Machaczka M, Zhang H, Peng R, Morin RD, Rosenquist R, Sander B, Pan-Hammarstrom Q. Genetic heterogeneity in primary and relapsed mantle cell lymphomas: impact of recurrent CARD11 mutations. *Oncotarget*. 2016;7(25):38180–90.

88. Zhang L, Nomie K, Zhang S, Liu Y, Guo H, Huang S, Wang J, Lopez E, Zhang H, Lorence EA, Merolle M, Balaji S, Ahmed M, Jiang C, Wang L, Wang M. Molecular pathways associated with Ibrutinib resistance in mantle Cell Lymphoma. *Blood*. 2017;130:2738.
89. Burger JA, Landau DA, Taylor-Weiner A, Bozic I, Zhang H, Sarosiek K, Wang L, Stewart C, Fan J, Hoellenriegel J, Sivina M, Dubuc AM, Fraser C, Han Y, Li S, Livak KJ, Zou L, Wan Y, Konoplev S, Sougnez C, Brown JR, Abruzzo LV, Carter SL, Keating MJ, Davids MS, Wierda WG, Cibulskis K, Zenz T, Werner L, Dal Cin P, Kharchenko P, Neuberger D, Kantarjian H, Lander E, Gabriel S, O'Brien S, Letai A, Weitz DA, Nowak MA, Getz G, Wu CJ. Clonal evolution in patients with chronic lymphocytic leukaemia developing resistance to BTK inhibition. *Nat Commun*. 2016;7:11589.
90. Kadri S, Lee J, Fitzpatrick C, Galanina N, Sukhanova M, Venkataraman G, Sharma S, Long B, Petras K, Theissen M, Ming M, Kobzev Y, Kang W, Guo A, Wang W, Niu N, Weiner H, Thirman M, Stock W, Smith SM, Nabhan C, Segal JP, Lu P, Wang YL. Clonal evolution underlying leukemia progression and Richter transformation in patients with ibrutinib-relapsed CLL. *Blood Adv*. 2017;1(12):715–27.
91. Thompson PA, O'Brien SM, Wierda WG, Ferrajoli A, Stingo F, Smith SC, Burger JA, Estrov Z, Jain N, Kantarjian HM, Keating MJ. Complex karyotype is a stronger predictor than del(17p) for an inferior outcome in relapsed or refractory chronic lymphocytic leukemia patients treated with ibrutinib-based regimens. *Cancer*. 2015;121(20):3612–21.
92. Moyo TK, Wilson CS, Moore DJ, Eischen CM. Myc enhances B-cell receptor signaling in pre-cancerous B cells and confers resistance to Btk inhibition. *Oncogene*. 2017;36(32):4653–61.
93. Rubio-Moscardo F, Blesa D, Mestre C, Siebert R, Balasas T, Benito A, Rosenwald A, Climent J, Martinez JJ, Schilhabel M, Karran EL, Gesk S, Esteller M. 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):3214–22.
94. Rinaldi A, Mian M, Kwee I, Rossi D, Deambrogi C, Mensah AA, Forconi F, Spina V, Cencini E, Drandi D, Ladetto M, Santachiara R, Marasca R, Gattei V, Cavalli F, Zucca E, Gaidano G, Bertoni F. Genome-wide DNA profiling better defines the prognosis of chronic lymphocytic leukaemia. *Br J Haematol*. 2011;154(5):590–9.
95. Chapiro E, Leporrier N, Radford-Weiss I, Bastard C, Mossafa H, Leroux D, Tigaud I, De Braekeleer M, Terre C, Brizard F, Callet-Bauchu E, Struski S, Veronese L, Fert-Ferrer S, Taviaux S, Lesty C, Davi F, Merle-Beral H, Bernard OA, Sutton L, Raynaud SD, Nguyen-Khac F. Gain of the short arm of chromosome 2 (2p) is a frequent recurring chromosome aberration in untreated chronic lymphocytic leukemia (CLL) at advanced stages. *Leuk Res*. 2010;34(1):63–8.
96. Turner JG, Dawson J, Sullivan DM. Nuclear export of proteins and drug resistance in cancer. *Biochem Pharmacol*. 2012;83(8):1021–32.
97. Blum KA. B-cell receptor pathway modulators in NHL. *Hematology Am Soc Hematol Educ Program*. 2015;2015:82–91.
98. Hayden MS, Ghosh S. Shared principles in NF-kappaB signaling. *Cell*. 2008;132(3):344–62.
99. Cildir G, Low KC, Tergaonkar V. Noncanonical NF-kappaB signaling in health and disease. *Trends Mol Med*. 2016;22(5):414–29.
100. Rahal R, Frick M, Romero R, Korn JM, Kridel R, Chan FC, Meissner B, Bhang HE, Ruddy D, Kauffmann A, Farsidjani A, Derti A, Rakiec D, Naylor T, Pfister E, Kovats S, Kim S, Dietze K, Dorken B, Steidl C, Tzankov A, Hummel M, Monahan J, Morrissey MP, Fritsch C, Sellers WR, Cooke VG, Gascoyne RD, Lenz G, Stegmeier F. Pharmacological and genomic profiling identifies NF-kappaB-targeted treatment strategies for mantle cell lymphoma. *Nat Med*. 2014;20(1):87–92.
101. Werner M, Hobeika E, Jumaa H. Role of PI3K in the generation and survival of B cells. *Immunol Rev*. 2010;237(1):55–71.
102. Chiron D, Di Liberto M, Martin P, Huang X, Sharman J, Blecua P, Mathew S, Vijay P, Eng K, Ali S, Johnson A, Chang B, Ely S, Elemento O, Mason CE, Leonard JP, Chen-Kiang S. 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):1022–35.
103. Ma J, Lu P, Guo A, Cheng S, Zong H, Martin P, Coleman M, Wang YL. Characterization of ibrutinib-sensitive and -resistant mantle lymphoma cells. *Br J Haematol.* 2014;166(6):849–61.
 104. Zhang L, Nomie K, Zhang S, Liu Y, Guo H, Huang S, Jeffrey Wang J, Lopez E, Zhang H, Lorence EA, Merolle M, Balaji S, Ahmed M, Jiang C, Wang L, Wang M. Molecular pathways associated with Ibrutinib resistance in mantle Cell Lymphoma. *Blood.* 2017;130:2738.
 105. Jain N, Sehgal L, Shuttleworth SJ, Samaniego F. Targeting PI3 pathway in ibrutinib resistant diffuse large B cell lymphoma. *Cancer Res.* 2017;77(13 Supplement)
 106. Paulus A, Akhtar S, Yousaf H, Manna A, Paulus SM, Bashir Y, Caulfield TR, Kuranz-Blake M, Chitta K, Wang X, Asmann Y, Hudec R, Springer W, Ailawadhi S, Chanan-Khan A. Waldenstrom macroglobulinemia cells devoid of BTK(C481S) or CXCR4(WHIM-like) mutations acquire resistance to ibrutinib through upregulation of Bcl-2 and AKT resulting in vulnerability towards venetoclax or MK2206 treatment. *Blood Cancer J.* 2017;7(5):e565.
 107. Tsujimoto Y, Finger LR, Yunis J, Nowell PC, Croce CM. Cloning of the chromosome breakpoint of neoplastic B cells with the t(14;18) chromosome translocation. *Science.* 1984;226(4678):1097–9.
 108. Ashkenazi A, Fairbrother WJ, Levenson JD, Souers AJ. From basic apoptosis discoveries to advanced selective BCL-2 family inhibitors. *Nat Rev Drug Discov.* 2017;16(4):273–84.
 109. Deng J, Isik E, Fernandes SM, Brown JR, Letai A, Davids MS. Bruton's tyrosine kinase inhibition increases BCL-2 dependence and enhances sensitivity to venetoclax in chronic lymphocytic leukemia. *Leukemia.* 2017;31(10):2075–84.
 110. Brocco F, Burg HT, Fernandes S, Tam CS, Forconi F, Guerra RM, Bird G, Walensky LD, Brown JR, Kater AP, Eldering E. Dissecting the role of individual Bcl-2 members in response and resistance to Ibrutinib or Venetoclax in CLL. *Blood.* 2017;130:262.
 111. Kuo H-P, Crowley R, Xue L, Schweighofer KJ, Cheung LW, Hsieh S, Eckert K, Versele M, Chang BY. Combination of Ibrutinib and BCL-2 or SYK inhibitors in Ibrutinib resistant ABC-subtype of diffuse large B-Cell lymphoma. *Blood.* 2014;124:505.
 112. Stacey DW. Cyclin D1 serves as a cell cycle regulatory switch in actively proliferating cells. *Curr Opin Cell Biol.* 2003;15(2):158–63.
 113. Bea S, Valdes-Mas R, Navarro A, Salaverria I, Martin-Garcia D, Jares P, Gine E, Pinyol M, Royo C, Nadeu F, Conde L, Juan M, Clot G, Vizan P, Di Croce L, Puente DA, Lopez-Guerra M, Moros A, Roue G, Aymerich M, Villamor N, Colomo L, Martinez A, Valera A, Martin-Subero JI, Amador V, Hernandez L, Rozman M, Enjuanes A, Forcada P, Muntanola A, Hartmann EM, Calasanz MJ, Rosenwald A, Ott G, Hernandez-Rivas JM, Klapper W, Siebert R, Wiestner A, Wilson WH, Colomer D, Lopez-Guillermo A, Lopez-Otin C, Puente XS, Campo E. Landscape of somatic mutations and clonal evolution in mantle cell lymphoma. *Proc Natl Acad Sci USA.* 2013;110(45):18250–5.
 114. Mohanty A, Sandoval N, Das M, Pillai R, Chen L, Chen RW, Amin HM, Wang M, Marcucci G, Weisenburger DD, Rosen ST, Pham LV, Ngo VN. CCND1 mutations increase protein stability and promote ibrutinib resistance in mantle cell lymphoma. *Oncotarget.* 2016;7(45):73558–72.
 115. Son B, Lee S, Youn H, Kim E, Kim W, Youn B. The role of tumor microenvironment in therapeutic resistance. *Oncotarget.* 2017;8(3):3933–45.
 116. Saba NS, Liu D, Herman SE, Underbayev C, Tian X, Behrend D, Weniger MA, Skarzynski M, Gyamfi J, Fontan L, Melnick A, Grant C, Roschewski M, Navarro A, Bea S, Pittaluga S, Dunleavy K, Wilson WH, Wiestner A. Pathogenic role of B-cell receptor signaling and canonical NF-kappaB activation in mantle cell lymphoma. *Blood.* 2016;128(1):82–92.
 117. Zhao X, Lwin T, Silva A, Shah B, Tao J, Fang B, Zhang L, Fu K, Bi C, Li J, Jiang H, Meads MB, Jacobson T, Silva M, Distler A, Darville L, Han Y, Rebatchouk D, Di Liberto M, Moscinski LC, Koomen JM, Dalton WS, Shain KH, Wang M, Sotomayor E. Unification of de novo and acquired ibrutinib resistance in mantle cell lymphoma. *Nat Commun.* 2017;8:14920.
 118. Byrd JC, Smith S, Wagner-Johnston N, Sharman J, Chen AI, Advani R, Augustson B, Marlton P, Renee Commerford S, Okrah K, Liu L, Murray E, Penuel E, Ward AF, Flinn IW. First-in-

- human phase 1 study of the BTK inhibitor GDC-0853 in relapsed or refractory B-cell NHL and CLL. *Oncotarget*. 2018;9(16):13023–35.
119. Fabian CA, Reiff SD, Guinn D, Neuman L, Fox JA, Wilson W, Byrd JC, Woyach JA, Johnson AJ. SNS-062 demonstrates efficacy in chronic lymphocytic leukemia in vitro and inhibits C481S mutated Bruton tyrosine kinase. *Cancer Res*. 2017;77(13 supplement):1207.
 120. Johnson AR, Kohli PB, Katewa A, Gogol E, Belmont LD, Choy R, Penuel E, Burton L, Eigenbrot C, Yu C, Ortwine DF, Bowman K, Franke Y, Tam C, Estevez A, Mortara K, Wu J, Li H, Lin M, Bergeron P, Crawford JJ, Young WB. Battling Btk mutants with noncovalent inhibitors that overcome Cys481 and Thr474 mutations. *ACS Chem Biol*. 2016;11(10):2897–907.
 121. Reiff SD, Guinn D, Mantel R, Smith L, Cheney C, Johnson AJ. Evaluation of the novel Bruton's tyrosine kinase (BTK) inhibitor GDC-0853 in chronic lymphocytic leukemia (CLL) with wild type or C481S mutated BTK. *J Clin Oncol*. 2016;34(15 supplement):7530.
 122. Paul J, Soujon M, Wengner AM, Zitzmann-Kolbe S, Sturz A, Haike K, Keng Magdalene KH, Tan SH, Lange M, Tan SY, Mumberg D, Lim ST, Ziegelbauer K, Liu N. Simultaneous inhibition of PI3Kdelta and PI3Kalpha induces ABC-DLBCL regression by blocking BCR-dependent and -independent activation of NF-kappaB and AKT. *Cancer Cell*. 2017;31(1):64–78.
 123. Scuoppo C, Jiguang W, Persaud M, Mittan S, Pasqualucci L, Rabadan R, Grandori C, Dalla-Favera R. Repurposing Dasatinib for Ibrutinib-resistant diffuse large B-cell lymphoma. *Blood*. 2017;130:3843.
 124. Jones R, Axelrod MJ, Tumas D, Quéva C, Julie Di Paolo J. Combination effects of B cell receptor pathway inhibitors (Entospletinib, ONO/GS-4059, and Idelalisib) and a BCL-2 inhibitor in primary CLL cells. *Blood*. 2015;126(23):1749.
 125. Morschhauser F, Danilov AV, Hodson DJ, Salles GA, Starodub A, Mitra S, Yang Y, Walter H, Fegan C. Preliminary results of A phase 1B study of Tirabrutinib (GS-4059/ONO-4059) in combination with entospletinib in patients with B-cell malignancies. *Hematol Oncol*. 2017;35(S2)
 126. Niemann CU, Mora-Jensen HI, Dadashian EL, Krantz F, Covey T, Chen SS, Chiorazzi N, Izumi R, Ulrich R, Lannutti BJ, Wiestner A, Herman SEM. Combined BTK and PI3Kdelta inhibition with Acalabrutinib and ACP-319 improves survival and tumor control in CLL mouse model. *Clin Cancer Res*. 2017;23(19):5814–23.
 127. Pike KG, Malagu K, Hummersone MG, Menear KA, Duggan HM, Gomez S, Martin NM, Ruston L, Pass SL, Pass M. Optimization of potent and selective dual mTORC1 and mTORC2 inhibitors: the discovery of AZD8055 and AZD2014. *Bioorg Med Chem Lett*. 2013;23(5):1212–6.
 128. Ezell SA, Mayo M, Bihani T, Tepsuporn S, Wang S, Passino M, Grosskurth SE, Collins M, Parmentier J, Reimer C, Byth KF. Synergistic induction of apoptosis by combination of BTK and dual mTORC1/2 inhibitors in diffuse large B cell lymphoma. *Oncotarget*. 2014;5(13):4990–5001.
 129. Yahiaoui A, Meadows SA, Sorensen RA, Cui ZH, Keegan KS, Brockett R, Chen G, Queva C, Li L, Tannheimer SL. PI3Kdelta inhibitor idelalisib in combination with BTK inhibitor ONO/GS-4059 in diffuse large B cell lymphoma with acquired resistance to PI3Kdelta and BTK inhibitors. *PLoS One*. 2017;12(2):e0171221.
 130. Platanias LC. Map kinase signaling pathways and hematologic malignancies. *Blood*. 2003;101(12):4667–79.
 131. Zhao Y, Adjei AA. The clinical development of MEK inhibitors. *Nat Rev Clin Oncol*. 2014;11(7):385–400.
 132. Chen JG, Liu X, Munshi M, Xu L, Tsakmaklis N, Demos MG, Kofides A, Guerrero ML, Chan GG, Patterson CJ, Meid K, Gustine J, Dubeau T, Severns P, Castillo JJ, Hunter ZR, Wang J, Buhrlage SJ, Gray NS, Treon SP, Yang G. BTK(Cys481Ser) drives ibrutinib resistance via ERK1/2 and protects BTK(wild-type) MYD88-mutated cells by a paracrine mechanism. *Blood*. 2018;131(18):2047–59.
 133. Ding N, Li X, Shi Y, Ping L, Wu L, Fu K, Feng L, Zheng X, Song Y, Pan Z, Zhu J. Irreversible dual inhibitory mode: the novel Btk inhibitor PLS-123 demonstrates promising anti-tumor activity in human B-cell lymphoma. *Oncotarget*. 2015;6(17):15122–36.

134. Wu H, Hu C, Wang A, Weisberg EL, Chen Y, Yun CH, Wang W, Liu Y, Liu X, Tian B, Wang J, Zhao Z, Liang Y, Li B, Wang L, Wang B, Chen C, Buhrlage SJ, Qi Z, Zou F, Nonami A, Li Y, Fernandes SM, Adamia S, Stone RM, Galinsky IA, Wang X, Yang G, Griffin JD, Brown JR, Eck MJ, Liu J, Gray NS, Liu Q. Discovery of a BTK/MNK dual inhibitor for lymphoma and leukemia. *Leukemia*. 2016;30(1):173–81.
135. Alfaro J, Perez de Arce F, Belmar S, Fuentealba G, Avila P, Ureta G, Flores C, Acuna C, Delgado L, Gaete D, Pujala B, Barde A, Nayak AK, Upendra TVR, Patel D, Chauhan S, Sharma VK, Kanno S, Almirez RG, Hung DT, Chakravarty S, Rai R, Bernales S, Quinn KP, Pham SM, McCullagh E. Dual inhibition of Bruton's Tyrosine Kinase and Phosphoinositide-3-Kinase p110delta as a therapeutic approach to treat non-Hodgkin's B cell malignancies. *J Pharmacol Exp Ther*. 2017;361(2):312–21.
136. Ge Y, Wang C, Song S, Huang J, Liu Z, Li Y, Meng Q, Zhang J, Yao J, Liu K, Ma X, Sun X. Identification of highly potent BTK and JAK3 dual inhibitors with improved activity for the treatment of B-cell lymphoma. *Eur J Med Chem*. 2018;143:1847–57.
137. Davids MS. Targeting BCL-2 in B-cell lymphomas. *Blood*. 2017;130(9):1081–8.
138. Zhao X, Bodo J, Sun D, Durkin L, Lin J, Smith MR, Hsi ED. Combination of ibrutinib with ABT-199: synergistic effects on proliferation inhibition and apoptosis in mantle cell lymphoma cells through perturbation of BTK, AKT and BCL2 pathways. *Br J Haematol*. 2015;168(5):765–8.
139. Tam CS, Anderson MA, Pott C, Agarwal R, Handunnetti S, Hicks RJ, Burbury K, Turner G, Di Iulio J, Bressel M, Westerman D, Lade S, Dreyling M, Dawson SJ, Dawson MA, Seymour JF, Roberts AW. Ibrutinib plus Venetoclax for the treatment of mantle-cell lymphoma. *N Engl J Med*. 2018;378(13):1211–23.
140. Kuo HP, Ezell SA, Schweighofer KJ, Cheung LWK, Hsieh S, Apatira M, Sirisawad M, Eckert K, Hsu SJ, Chen CT, Beaupre DM, Versele M, Chang BY. Combination of ibrutinib and ABT-199 in diffuse large B-cell lymphoma and follicular lymphoma. *Mol Cancer Ther*. 2017;16(7):1246–56.
141. Burger JA, Keating MJ, Wierda WG, Hartmann E, Hoellenriegel J, Rosin NY, de Weerd I, Jeyakumar G, Ferrajoli A, Cardenas-Turanzas M, Lerner S, Jorgensen JL, Nogueras-Gonzalez GM, Zacharian G, Huang X, Kantarjian H, Garg N, Rosenwald A, O'Brien S. Safety and activity of ibrutinib plus rituximab for patients with high-risk chronic lymphocytic leukaemia: a single-arm, phase 2 study. *Lancet Oncol*. 2014;15(10):1090–9.
142. Wang ML, Lee H, Chuang H, Wagner-Bartak N, Hagemester F, Westin J, Fayad L, Samaniego F, Turturro F, Oki Y, Chen W, Badillo M, Nomie K, DeLa Rosa M, Zhao D, Lam L, Addison A, Zhang H, Young KH, Li S, Santos D, Medeiros LJ, Champlin R, Romaguera J, Zhang L. Ibrutinib in combination with rituximab in relapsed or refractory mantle cell lymphoma: a single-Centre, open-label, phase 2 trial. *Lancet Oncol*. 2016;17(1):48–56.
143. Fowler N, Nastoupil L, de Vos S, Knapp M, Flinn IW, Chen R, Advani RH, Bhatia S, Martin P, Mena R, Suzuki S, Beaupre DM, Neuenburg JK, Palomba ML. Ibrutinib plus rituximab in treatment-naive Patients with follicular Lymphoma: results from a multicenter, phase 2 study. *Blood*. 2015;126:470.
144. Fowler NH, Coleman M, Stevens DA, Smith SM, Venugopal P, Martin P, Phillips TJ, Agajanian R, Stephens DM, Izumi R, Cheung J, Slatter JG, Yin M, Hiremath M, Hunder NNH, Christian B, Acalabrutinib alone or in combination with rituximab (R) in follicular lymphoma (FL). *J Clin Oncol*. 2018. 36(suppl; abstr 7549).
145. Younes A, Thieblemont C, Morschhauser F, Flinn I, Friedberg JW, Amorim S, Hivert B, Westin J, Vermeulen J, Bandyopadhyay N, de Vries R, Balasubramanian S, Hellemans P, Smit JW, Fourneau N, Oki Y. Combination of ibrutinib with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) for treatment-naive patients with CD20-positive B-cell non-Hodgkin lymphoma: a non-randomised, phase 1b study. *Lancet Oncol*. 2014;15(9):1019–26.
146. Maddocks K, Christian B, Jaglowski S, Flynn J, Jones JA, Porcu P, Wei L, Jenkins C, Lozanski G, Byrd JC, Blum KA. A phase 1/1b study of rituximab, bendamustine, and ibrutinib in patients with untreated and relapsed/refractory non-Hodgkin lymphoma. *Blood*. 2015;125(2):242–8.

147. Chanan-Khan A, Cramer P, Demirkan F, Fraser G, Silva RS, Grosicki S, Pristupa A, Janssens A, Mayer J, Bartlett NL, Dilhuydy MS, Pylypenko H, Loscertales J, Avigdor A, Rule S, Villa D, Samoiloova O, Panagiotidis P, Goy A, Mato A, Pavlovsky MA, Karlsson C, Mahler M, Salman M, Sun S, Phelps C, Balasubramanian S, Howes A, Hallek M. Ibrutinib combined with bendamustine and rituximab compared with placebo, bendamustine, and rituximab for previously treated chronic lymphocytic leukaemia or small lymphocytic lymphoma (HELIOS): a randomised, double-blind, phase 3 study. *Lancet Oncol.* 2016;17(2):200–11.
148. Wilson WH, Young RM, Schmitz R, Yang Y, Pittaluga S, Wright G, Lih CJ, Williams PM, Shaffer AL, Gerecitano J, de Vos S, Goy A, Kenkre VP, Barr PM, Blum KA, Shustov A, Advani R, Fowler NH, Vose JM, Elstrom RL, Habermann TM, Barrientos JC, McCreivy J, Fardis M, Chang BY, Clow F, Munneke B, Moussa D, Beaupre DM, Staudt LM. Targeting B cell receptor signaling with ibrutinib in diffuse large B cell lymphoma. *Nat Med.* 2015;21(8):922–6.
149. Yasuhiro T, Sawada W, Klein C, Kozaki R, Hotta S, Yoshizawa T. Anti-tumor efficacy study of the Bruton's tyrosine kinase (BTK) inhibitor, ONO/GS-4059, in combination with the glycoengineered type II anti-CD20 monoclonal antibody obinutuzumab (GA101) demonstrates superior in vivo efficacy compared to ONO/GS-4059 in combination with rituximab. *Leuk Lymphoma.* 2017;58(3):699–707.
150. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. *N Engl J Med.* 2011;365(8):725–33.
151. Neelapu SS, Locke FL, Bartlett NL, Lekakis LJ, Miklos DB, Jacobson CA, Braunschweig I, Oluwole OO, Siddiqi T, Lin Y, Timmerman JM, Stiff PJ, Friedberg JW, Flinn IW, Goy A, Hill BT, Smith MR, Deol A, Farooq U, McSweeney P, Munoz J, Avivi I, Castro JE, Westin JR, Chavez JC, Ghobadi A, Komanduri KV, Levy R, Jacobsen ED, Witzig TE, Reagan P, Bot A, Rossi J, Navale L, Jiang Y, Aycocock J, Elias M, Chang D, Wiezorek J, Go WY. Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. *N Engl J Med.* 2017;377(26):2531–44.
152. Ruella M, Kenderian SS, Shestova O, Fraietta JA, Qayyum S, Zhang Q, Maus MV, Liu X, Nunez-Cruz S, Klichinsky M, Kawalekar OU, Milone M, Lacey SF, Mato A, Schuster SJ, Kalos M, June CH, Gill S, Wasik MA. The addition of the BTK inhibitor Ibrutinib to anti-CD19 chimeric antigen receptor T cells (CART19) improves responses against mantle cell lymphoma. *Clin Cancer Res.* 2016;22(11):2684–96.
153. Turtle CJ, Hay KA, Hanafi LA, Li D, Cherian S, Chen X, Wood B, Lozanski A, Byrd JC, Heimfeld S, Riddell SR, Maloney DG. 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):3010–20.
154. Dubovsky JA, Beckwith KA, Natarajan G, Woyach JA, Jaglowski S, Zhong Y, Hessler JD, Liu T-M, Chang BY, Larkin KM, Stefanovski MR, Chappell DL, Frizzera FW, Smith LL, Smucker KA, Flynn JM, Jones JA, Andritsos LA, Maddocks K, Lehman AM, Furman R, Sharman J, Mishra A, Caligiuri MA, Satoskar AR, Buggy JJ, Muthusamy N, Johnson AJ, Byrd JC. Ibrutinib is an irreversible molecular inhibitor of ITK driving a Th1-selective pressure in T lymphocytes. *Blood.* 2013;122(15):2539–49.
155. Robinson HR, Qi J, Cook EM, Nichols C, Dadashian EL, Underbayev C, Herman SEM, Saba NS, Keyvanfar K, Sun C, Ahn IE, Baskar S, Rader C, Wiestner A. A CD19/CD3 bispecific antibody for effective immunotherapy of chronic lymphocytic leukemia in the ibrutinib era. *Blood.* 2018;132:521–32.
156. Sahakian E, Rock-Klotz J, Shah BD, Powers J, Cultrera JL, Deng S, Woods DM, Nguyen M, Cheng F, Wang H, Perez-Villarroel P, Lienlaf M, Knox T, Chen-Kiang S, Villagra A, Tao J, Pinilla-Ibarz J, Jones SS, Sotomayor EM. Combination of ACY1215, a selective histone Deacetylase 6 (HDAC6) inhibitor with the Bruton tyrosine kinase (BTK) inhibitor, Ibrutinib, represents a novel therapeutic strategy in mantle cell lymphoma (MCL). *Blood.* 2012;120:1660.
157. Mondello P, Brea EJ, De Stanchina E, Toska E, Chang AY, Fennell M, Seshan V, Garippa R, Scheinberg DA, Baselga J, Wendel HG, Younes A. Panobinostat acts synergistically with ibrutinib in diffuse large B cell lymphoma cells with MyD88 L265 mutations. *JCI Insight.* 2017;2(6):e90196.

158. Restelli V, Lupi M, Vagni M, Chila R, Bertoni F, Damia G, Carrassa L. Combining Ibrutinib with Chk1 inhibitors synergistically targets mantle Cell Lymphoma Cell lines. *Target Oncol.* 2018;13(2):235–45.
159. Bedford L, Lowe J, Dick LR, Mayer RJ, Brownell JE. Ubiquitin-like protein conjugation and the ubiquitin-proteasome system as drug targets. *Nat Rev Drug Discov.* 2011;10(1):29–46.
160. Dasmahapatra G, Patel H, Dent P, Fisher RI, Friedberg J, Grant S. The Bruton tyrosine kinase (BTK) inhibitor PCI-32765 synergistically increases proteasome inhibitor activity in diffuse large-B cell lymphoma (DLBCL) and mantle cell lymphoma (MCL) cells sensitive or resistant to bortezomib. *Br J Haematol.* 2013;161(1):43–56.
161. Axelrod M, Ou Z, Brett LK, Zhang L, Lopez ER, Tamayo AT, Gordon V, Ford RJ, Williams ME, Pham LV, Weber MJ, Wang ML. Combinatorial drug screening identifies synergistic co-targeting of Bruton's tyrosine kinase and the proteasome in mantle cell lymphoma. *Leukemia.* 2014;28(2):407–10.
162. Trepel J, Mollapour M, Giaccone G, Neckers L. Targeting the dynamic HSP90 complex in cancer. *Nat Rev Cancer.* 2010;10(8):537–49.
163. Mitra SK, Schlaepfer DD. Integrin-regulated FAK-Src signaling in normal and cancer cells. *Curr Opin Cell Biol.* 2006;18(5):516–23.
164. Rudelius M, Rosenfeldt MT, Leich E, Rauert-Wunderlich H, Solimando AG, Beilhack A, Ott G, Rosenwald A. Inhibition of focal adhesion kinase overcomes resistance of mantle cell lymphoma to ibrutinib in the bone marrow microenvironment. *Haematologica.* 2018;103(1):116–25.
165. Byrd JC, Harrington B, O'Brien S, Jones JA, Schuh A, Devereux S, Chaves J, Wierda WG, Awan FT, Brown JR, Hillmen P, Stephens DM, Ghia P, Barrientos JC, Pagel JM, Woyach J, Johnson D, Huang J, Wang X, Kaptein A, Lannutti BJ, Covey T, Fardis M, McCreivy J, Hamdy A, Rothbaum W, Izumi R, Diacovo TG, Johnson AJ, Furman RR. Acabrutinib (ACP-196) in relapsed chronic lymphocytic leukemia. *N Engl J Med.* 2016;374(4):323–32.
166. Cosson A, Chapiro E, Bougacha N, Lambert J, Herbi L, Cung HA, Algrin C, Keren B, Damm F, Gabillaud C, Brunelle-Navas MN, Davi F, Merle-Beral H, Le Garff-Tavernier M, Roos-Weil D, Choquet S, Uzunov M, Morel V, Leblond V, Maloum K, Lepretre S, Feugier P, Lesty C, Lejeune J, Sutton L, Landesman Y, Susin SA, Nguyen-Khac F. Gain in the short arm of chromosome 2 (2p+) induces gene overexpression and drug resistance in chronic lymphocytic leukemia: analysis of the central role of XPO1. *Leukemia.* 2017;31(7):1625–9.
167. Li J, Wang X, Xie Y, Ying Z, Liu W, Ping L, Zhang C, Pan Z, Ding N, Song Y, Zhu J. The mTOR kinase inhibitor everolimus synergistically enhances the anti-tumor effect of the Bruton's tyrosine kinase (BTK) inhibitor PLS-123 on mantle cell lymphoma. *Int J Cancer.* 2018;142(1):202–13.
168. Gaudio E, Tarantelli C, Kwee I, Barassi C, Bernasconi E, Rinaldi A, Ponzoni M, Cascione L, Targa A, Stathis A, Goodstal S, Zucca E, Bertoni F. Combination of the MEK inhibitor pimasertib with BTK or PI3K-delta inhibitors is active in preclinical models of aggressive lymphomas. *Ann Oncol.* 2016;27(6):1123–8.
169. Patel VK, Lamothe B, Ayres ML, Gay J, Cheung JP, Balakrishnan K, Ivan C, Morse J, Nelson M, Keating MJ, Wierda WG, Marszalek JR, Gandhi V. Pharmacodynamics and proteomic analysis of acalabrutinib therapy: similarity of on-target effects to ibrutinib and rationale for combination therapy. *Leukemia.* 2018;32(4):920–30.