

Resistance to covalent Bruton's tyrosine kinase inhibitors in diffuse large B-cell lymphoma: Molecular mechanisms and therapeutic implications (Review)

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Abstract. B-cell receptor (BCR) signaling is essential for the survival and proliferation of normal B cells, and contributes substantially to the pathogenesis of diffuse large B-cell lymphoma (DLBCL). Bruton's tyrosine kinase (BTK), a key downstream component of the BCR pathway, has emerged as an important therapeutic target in B-cell malignancies. However, the clinical efficacy of BTK inhibitors (BTKi) in DLBCL remains heterogeneous and is strongly influenced by molecular subtype and signaling dependency. Accumulating evidence indicates that BTKi resistance in DLBCL is driven by diverse molecular mechanisms, including BTK mutations, gain-of-function alterations in phospholipase C γ 2, activation of the caspase recruitment domain family member 11-B-cell lymphoma/leukemia 10-mucosa-associated lymphoid tissue lymphoma translocation protein 1 complex and dysregulation of myeloid differentiation primary response 88, cluster of differentiation 79B, tumor necrosis factor- α -induced protein 3 and Kelch-like protein 14. In addition to these genetic determinants, non-genetic adaptive mechanisms, such as compensatory phosphoinositide 3-kinase/AKT/mechanistic target of rapamycin and mitogen-activated protein kinase/extracellular signal-regulated kinase signaling, epigenetic reprogramming,

tumor microenvironment remodeling, metabolic rewiring and endoplasmic reticulum stress, may further contribute to therapeutic escape. Emerging approaches, including next-generation covalent and non-covalent BTKi, dual-mode inhibitors, BTK-targeting degraders, allosteric inhibitors and multi-target strategies, are currently being investigated as potential strategies to address BTKi resistance. The present review summarized the molecular mechanisms underlying BTKi resistance in DLBCL and discussed their potential implications for biomarker development, rational combination therapy and future precision treatment.

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1. Introduction

Diffuse large B-cell lymphoma (DLBCL) remains the most prevalent form of aggressive lymphoma in adults, accounting for 30-40% of all newly diagnosed cases of non-Hodgkin lymphomas (NHL) (1,2). DLBCL exhibits high heterogeneity in cellular morphology, genetic subtypes, clinical manifestations, immunophenotype, treatment response and clinical prognosis. Initiated by gene expression profiling analysis, DLBCL was categorized into two distinct cell-of-origin (COO) subtypes: Germinal center B-cell-like (GCB) and activated B-cell-like (ABC), with a subset of cases remaining unclassified (3,4). Subsequent advances in genomic technologies have further refined the classification of DLBCL subtypes (5,6). The LymphGen probabilistic classification tool has identified seven distinct genetic subtypes of DLBCL, including the MCD [myeloid differentiation primary response 88 (MYD88)

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L265P and cluster of differentiation 79B (CD79B) mutations], BN2 [B-cell lymphoma (BCL)6 fusion and notch receptor (NOTCH)2 mutations], N1 (NOTCH1 mutations) subtype, EZB (enhancer of zeste homolog 2 mutations and BCL2 translocations)-MYC⁺, EZB-MYC⁺, A53 (aneuploid with TP53 inactivation) and ST2 (SGK1 and TET2 mutations) subtypes. However, ~40% of cases remain unclassifiable, posing an important challenge to precision treatment in DLBCL (7).

The rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP) regimen forms the backbone of first-line immunochemotherapy for DLBCL (8). While 60-70% of patients achieve cure with this combination treatment, 30-40% of patients experience early relapse or are refractory to front-line treatment, and this relapsed or refractory (R/R) subset generally faces a worse prognosis (9,10). From the perspective of COO classification, R-CHOP has substantially improved outcomes for patients with the GCB subtype. By contrast, only ~40% of those with the ABC subtype are cured with currently available R-CHOP-based therapy, and their survival outcomes are significantly inferior to those of patients with the GCB subtype (11). Collectively, the conventional non-targeted R-CHOP regimen achieves only partial efficacy, and the remarkable heterogeneity of DLBCL poses a major barrier to further improvements in therapeutic outcomes. Thus, there is an urgent need to develop novel targeted strategies in an optimal manner to address these treatment challenges, particularly for the ABC subtype, which carries a worse prognosis.

The B-cell receptor (BCR) signaling pathway plays an indispensable role in the proliferation, differentiation, maturation and survival of normal B cells, as well as in adaptive immunity (12). Dysregulation of this pathway contributes to the pathogenesis of various B-cell malignancies, including DLBCL (13). Bruton's tyrosine kinase (BTK) is a key downstream component of the BCR signaling cascade; it amplifies BCR signals and activates a complex network of downstream pathways, including nuclear factor of activated T cells (NFAT), mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK), and nuclear factor- κ B (NF- κ B) pathways (14). The fact that BTK serves as a central signaling node within the BCR pathway underscores the rationale for targeting it in the treatment of multiple B-cell malignancies.

The advent of BTK inhibitors (BTKi) has revolutionized the treatment landscape for B-cell malignancies. However, it must be noted that, in real-world practice, patient responses to BTKi vary across different types of B-cell tumors (15). As monotherapy, BTKi show only limited clinical benefit in DLBCL and fail to induce durable responses (16). The intrinsic genetic heterogeneity of DLBCL has confounded BTKi application in this disease (17). Furthermore, with the widespread use of BTKi in clinical practice, both primary nonresponse and progression after an initial response have been observed in DLBCL (11). BTKi resistance and off-target toxicity are often the main reasons that force patients to discontinue treatment (18). To date, the mechanisms underlying BTKi resistance in DLBCL remain to be fully elucidated. Accordingly, the present review provided an overview of the current understanding of BCR signaling in DLBCL, focused on delineating the established mechanisms that drive BTKi resistance in DLBCL and

explored potential therapeutic strategies to circumvent drug resistance and improve clinical outcomes.

For the present narrative review, PubMed (National Library of Medicine; <https://pubmed.ncbi.nlm.nih.gov/>) was searched for clinical and preclinical studies on BTKi resistance in DLBCL. The final search was conducted in August 2026. The main search terms included 'diffuse large B-cell lymphoma', 'DLBCL', 'Bruton tyrosine kinase inhibitor', 'BTK inhibitor', 'BTKi', 'ibrutinib', 'acalabrutinib', 'zanubrutinib' and 'orelabrutinib', together with resistance-related terms, including 'drug resistance', 'resistance', 'resistant', 'sensitivity', 'tolerance' and 'adaptation'. Titles and abstracts were screened first. Full texts were reviewed when a study was considered relevant. Studies were included if they examined resistance mechanisms or potential strategies to address resistance in DLBCL cell lines, animal models, tumor samples or patients. Relevant abstracts from the American Society of Hematology (ASH) and European Hematology Association (EHA) meetings and preprints were also included. In addition, reference lists of reviews on BTKi resistance in other B-cell malignancies were checked manually to identify additional studies. The evidence was classified as clinical DLBCL data, DLBCL preclinical data, clinical or preclinical evidence from other B-cell malignancies, or biological hypotheses.

2. BCR signaling pathway and BTKi

Structure and function of BTK. The BCR complex is composed of a membrane-bound immunoglobulin (Ig) non-covalently associated with CD79A (Ig α)-CD79B (Ig β) co-receptor heterodimers (19,20). The immunoglobulin on the cell surface itself lacks kinase activity; rather, the BCR complex collectively triggers downstream signal transduction upon antigen engagement. The BCR signaling plays an indispensable role in regulating multiple stages of B cell development and adaptive immune responses (13). BTK functions as a critical signaling node within the BCR pathway. It is expressed predominantly in B cells and certain other hematopoietic cell types, with the exception of T, natural killer and plasma cells (21,22).

BTK is a 77-kDa cytoplasmic non-receptor tyrosine kinase composed of 659 amino acids, belonging to the tyrosine kinase expressed in hepatocellular carcinoma (TEC) family kinase (23). This family also includes interleukin (IL)-2-inducible T cell kinase, TEC, resting lymphocyte kinase and bone marrow expressed kinase (24). Structurally, BTK contains five functional domains arranged from the N-terminus to the C-terminus: A pleckstrin homology (PH) domain, a proline-rich TEC homology domain, SRC homology (SH) 3 and SH2 domains, and a carboxyl kinase domain (Fig. 1) (25-27). The PH domain facilitates BTK recruitment to the plasma membrane, while the protein kinase domain carries out catalytic activity. Two key tyrosine phosphorylation sites are essential for BTK activation: Y551 within the tyrosine kinase domain and Y223 in the SH3 domain. Their phosphorylation is required for full BTK enzymatic activation and conformational stability (14,23). Given the high structural similarity among kinases within the TEC family, BTKi often exhibit non-specific binding to other family members, which may lead to off-target side effects in therapeutic contexts (12).

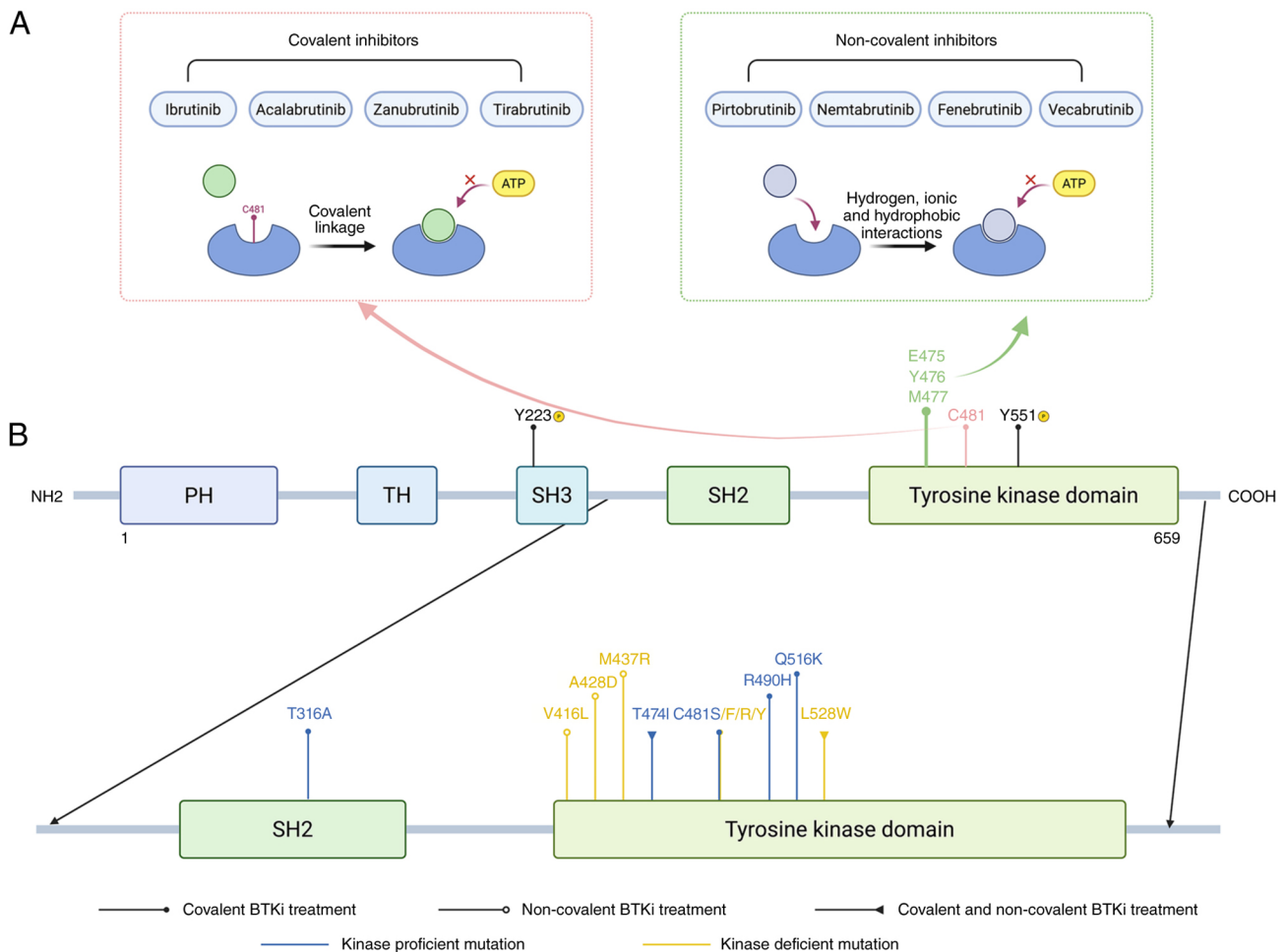


Figure 1. Schematic of BTK domain architecture and resistance mutations. (A) BTKi are mainly grouped into covalent and non-covalent classes according to their mechanism of action. Covalent BTKi, with representative drugs including ibrutinib, acalabrutinib, zanubrutinib and tirabrutinib, irreversibly bind to C481 within the ATP-binding pocket and block ATP docking. Non-covalent BTKi, such as pirtobrutinib, nemtabrutinib, fenebrutinib and vecabrutinib, reversibly bind to E475, Y476 and M477 in the ATP-binding pocket through hydrogen, ionic, and hydrophobic interactions, thereby preventing ATP engagement. (B) BTK is a 659-amino acid protein. From the amino to the carboxyl terminus, it contains a PH domain, a TH domain, an SH3 domain, an SH2 domain, and a tyrosine kinase domain. Resistance mutations are classified as kinase-proficient (blue) or kinase-deficient (yellow) based on the remaining catalytic activity of BTK. Created with BioRender.com. BTK, Bruton's tyrosine kinase; BTKi, BTK inhibitor; PH, pleckstrin homology; SH2, Src homology 2; SH3, Src homology 3; TH, Tec homology.

BCR signaling cascade pathway. In the absence of antigen stimulation, BCR transmits low-level tonic signals, which depend primarily on phosphoinositide 3-kinase (PI3K) activity to sustain the survival of mature B cells. Upon antigen engagement, however, BCR complexes undergo aggregation and rapid phosphorylation, thereby initiating downstream signaling cascades. A schematic representation of the BCR signaling cascade and its key downstream effectors is provided in Fig. 2.

Specifically, antigen binding to the membrane-bound Ig triggers BCR clustering and conformational changes, leading to the activation of SRC family kinases such as Lck/Yes-related novel protein tyrosine kinase (LYN). Activated LYN subsequently phosphorylates tyrosine residues within the immunoreceptor tyrosine-based activation motifs (ITAMs) of the associated CD79A/B heterodimers. The resulting dually phosphorylated ITAMs then function as docking sites for spleen tyrosine kinase (SYK), which engages via its N-terminal tandem SH2 domains, ultimately activating SYK. Activated SYK phosphorylates and thereby activates the B-cell co-receptor CD19 and the B-cell PI3K adaptor protein. This phosphorylation

provides binding sites for the P85 subunit of PI3K, leading to PI3K recruitment and activation (28,29). Activated PI3K then phosphorylates phosphatidylinositol-4,5-bisphosphate (PIP₂) to generate phosphatidylinositol-3,4,5-trisphosphate.

Activation of the BCR promotes the transition of BTK from a cytoplasmic autoinhibited state to a catalytically active conformation and its subsequent translocation to the plasma membrane (30). BTK then undergoes sequential phosphorylation at residue Y551 within its kinase domain by SYK, followed by autophosphorylation at residue Y223 in its SH3 domain. This autophosphorylation stabilizes BTK in its active conformation and enables full catalytic activity (31). One of the major downstream effectors of BTK is phospholipase C γ 2 (PLC γ 2). Upon activation, PLC γ 2 hydrolyzes PIP₂ to generate two second messengers: Inositol-1,4,5-trisphosphate (IP₃) and diacylglycerol (DAG). IP₃ induces intracellular calcium mobilization, which activates the transcription factor NFAT via calmodulin and calcineurin. NFAT subsequently translocates into the nucleus to regulate B cell proliferation and survival. In the presence of calcium, DAG activates protein kinase C β

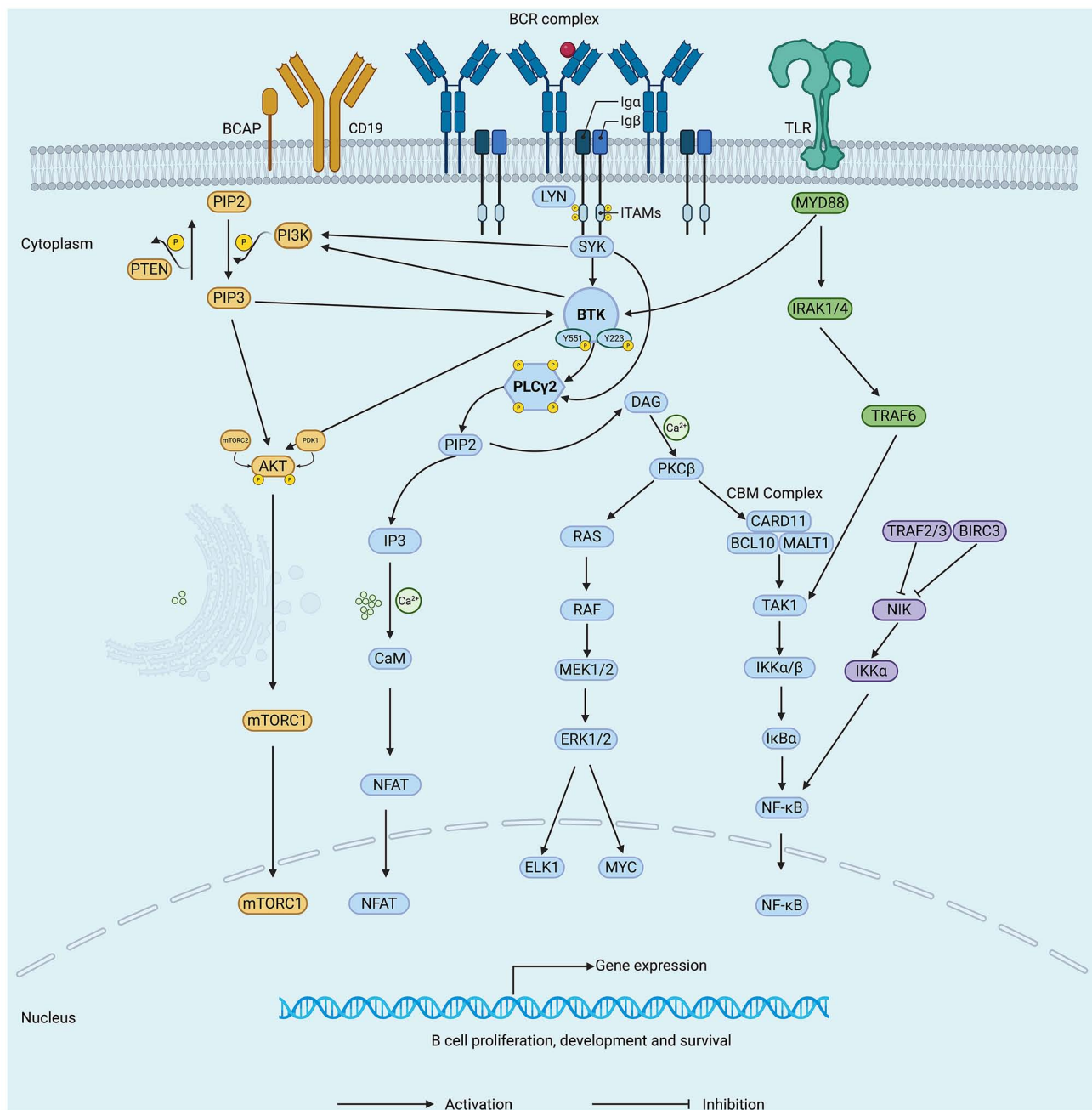


Figure 2. Schematic overview of BCR signaling pathway. Upon antigen engagement, the BCR complex triggers activation of LYN and SYK, which in turn activate PI3K and generate PIP3. BTK is recruited to the membrane and fully activated, followed by PLCγ2 activation. PLCγ2 hydrolyzes PIP2 into IP3 and DAG. IP3 mobilizes intracellular calcium and activates NFAT through calmodulin and calcineurin. DAG activates PKCβ and promotes assembly of the CBM complex (CARD11-BCL10-MALT1), which drives canonical NF-κB signaling via TAK1 and IKK. DAG also initiates the RAS-RAF-MEK-ERK cascade. In parallel, TLR signaling through MYD88 recruits IRAK1/4 and TRAF6, which also activate TAK1 and NF-κB, and this pathway can crosstalk with BCR signaling to amplify survival signals. These pathways converge on transcription factors including NFAT, NF-κB, ELK1, and MYC, which regulate B cell proliferation, survival, and development. Created with BioRender.com. BCR, B-cell receptor; BTK, Bruton's tyrosine kinase; CBM, CARD11-BCL10-MALT1 complex; NFAT, nuclear factor of activated T cells; PI3K, phosphoinositide 3-kinase; PLCγ2, phospholipase Cγ2.

(PKCβ). Activated PKCβ initiates the RAF/MEK/ERK1/2 signaling cascade (23,32). Another DAG substrate is the caspase recruitment domain family member 11 (CARD11). Phosphorylated CARD11 assembles with mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1) and B-cell lymphoma/leukemia 10 (BCL10) to form the CBM adapter complex. This complex then activates downstream kinases such as transforming growth factor β activated kinase 1 and inhibitor of κB (IκB) kinase (IKK) α/β. IKK

phosphorylates IκB, triggering its ubiquitin-dependent degradation, and ultimately facilitating the nuclear translocation of NF-κB to regulate B cell proliferation and adaptive immune responses (23,33).

BTKi classification. The fact that BTK is at the center of the BCR signaling cascade, coupled with its restricted expression in other cell types, highlights BTK as an attractive therapeutic target for several B-cell-derived malignancies (34). The

clinical introduction of BTKi has remarkably transformed the treatment paradigms for these B-cell malignancies. Currently, BTKi are distinguished into covalent and non-covalent classes based on the binding mode to the BTK active site (35). Covalent inhibitors irreversibly bind to the C481 residue within the ATP-binding pocket of the BTK kinase domain, disrupt auto-phosphorylation at Y223 and block ATP docking, substrate phosphorylation and downstream signaling. Non-covalent inhibitors, by contrast, reversibly bind to the ATP-binding pocket or deeper into the H3 pocket of the catalytic domain through hydrogen, ionic and hydrophobic interactions, thereby interfering with phosphorylation at both Y223 and Y551 and suppressing downstream target activation and signal propagation (36,37).

In an initial phase I/II trial, the first-generation BTKi ibrutinib monotherapy delivered selective clinical activity in patients with R/R ABC-subtype DLBCL, achieving an overall response rate (ORR) of 37%. However, the median progression-free survival (PFS) was short, with most patients experiencing rapid disease progression, indicating that ibrutinib alone fails to produce durable responses (11). In addition, ibrutinib modestly inhibits other protein kinases, and these off-target effects contribute to side effects such as atrial fibrillation, bleeding and impairment of macrophage phagocytosis (18,38,39). Such off-target toxicity remains a significant clinical challenge that has limited the broader use of ibrutinib, while also fueling the development of more selective covalent BTKi.

Second-generation BTKi, including acalabrutinib, zanubrutinib, tirabrutinib and orelabrutinib, offer improved BTK target selectivity and reduced off-target activities compared with ibrutinib (36). However, both first- and second-generation covalent BTKi rely on forming a covalent bond with C481 within the BTK active site and mutations at this residue can trigger resistance and limit long-term efficacy (40). Consequently, treatment discontinuation due to acquired resistance or therapy-related toxicity has accelerated the development of non-covalent BTKi, which aim to circumvent resistance to covalent agents and minimize adverse events to improve outcomes for patients with B-cell malignancies. Third-generation non-covalent BTKi, represented by pirtobrutinib, also include agents such as fenebrutinib, nemtabrutinib and vecabrutinib, which bind non-covalently to the BTK active site and suppress BTK kinase activity regardless of C481 mutation status (14).

However, with the widespread clinical use of BTKi, non-C481 BTK mutations have emerged that confer resistance to irreversible inhibitors. Furthermore, complex resistance mechanisms may limit the clinical efficacy of non-covalent BTKi, prompting the development of next-generation BTK-targeted strategies. These topics are discussed in detail in the following sections.

3. Mechanisms of resistance to BTKi in DLBCL

Despite the clinical success of BTKi in multiple B-cell malignancies, their activity in DLBCL remains heterogeneous. In patients with R/R ABC-DLBCL treated with ibrutinib monotherapy, objective responses were observed, but the median PFS was only 6.41 months (11). This short median PFS may

partly reflect the emergence of acquired resistance, but this endpoint alone cannot distinguish primary non-response from progression after an initial response. For the purposes of the present review, primary resistance refers to failure to achieve a complete or partial response to BTKi-based treatment, whereas acquired resistance refers to disease progression after a documented initial response. In preclinical studies, acquired resistance is used only when initially sensitive cells or tumors lose sensitivity after prolonged BTKi exposure. Lack of BTK dependency is considered a biological basis of intrinsic insensitivity rather than acquired resistance. Treatment intolerance refers to dose reduction, interruption or discontinuation because of adverse events and is not considered resistance. Disease progression without a documented prior response is therefore not classified as acquired resistance.

Resistance to second-generation covalent and non-covalent BTKi has been characterized mainly in chronic lymphocytic leukemia (CLL) and other B-cell malignancies (41). In DLBCL, reduced sensitivity to BTKi may result from an intrinsic lack of BTK dependency or from genetic and non-genetic changes that emerge during treatment. The following sections discussed these mechanisms, including molecular subtype, mutations affecting BCR signaling, activation of alternative pro-survival pathways, anti-apoptotic signaling, epigenetic alterations, metabolic reprogramming and remodeling of the tumor microenvironment (TME) (42). Clinical evidence for BTK-binding-site mutations is derived mainly from other B-cell malignancies, whereas their relevance in DLBCL remains uncertain (15). An integrated overview is presented in Fig. 3, and the disease source and evidence category for each mechanism are summarized in Table I.

Genetic subtypes as determinants of intrinsic resistance. As aforementioned, DLBCL represents a highly heterogeneous B-cell malignancy. While the survival and proliferation of DLBCL cells rely on BCR signaling activation, the specific modes of BCR dependence vary across molecular subtypes. This distinction is evident in the differential treatment responses to ibrutinib among patients with distinct genetic subtypes. For example, the ABC subtype shows an ORR of 37% compared with only 5% in the GCB subtype. Further studies have revealed that ABC DLBCL predominantly exhibits a 'chronic active BCR signaling' profile (43) characterized by constitutive activation of the pro-survival NF- κ B pathway, which is critically required for the survival and proliferation of ABC-type cells (44). By contrast, GCB DLBCL displays a 'tonic BCR signaling' pattern, which differs from both the tonic signaling in normal B cells and the chronic active signaling observed in ABC tumors (5,45). The GCB subtype shows increased dependence on the PI3K and BCL2 signaling pathways (43,46). The differential reliance on distinct BCR signaling cascades may provide the rationale for the primary resistance to BTKi frequently observed in patients with the GCB subtype.

Advances in genomic research have refined the molecular classification of DLBCL, while further revealing heterogeneity in the response to BTKi therapy even within the ABC subtype. A genetically engineered DLBCL mouse model carrying concurrent oncogenic MYD88 and CD79B mutations showed marked and selective sensitivity to ibrutinib

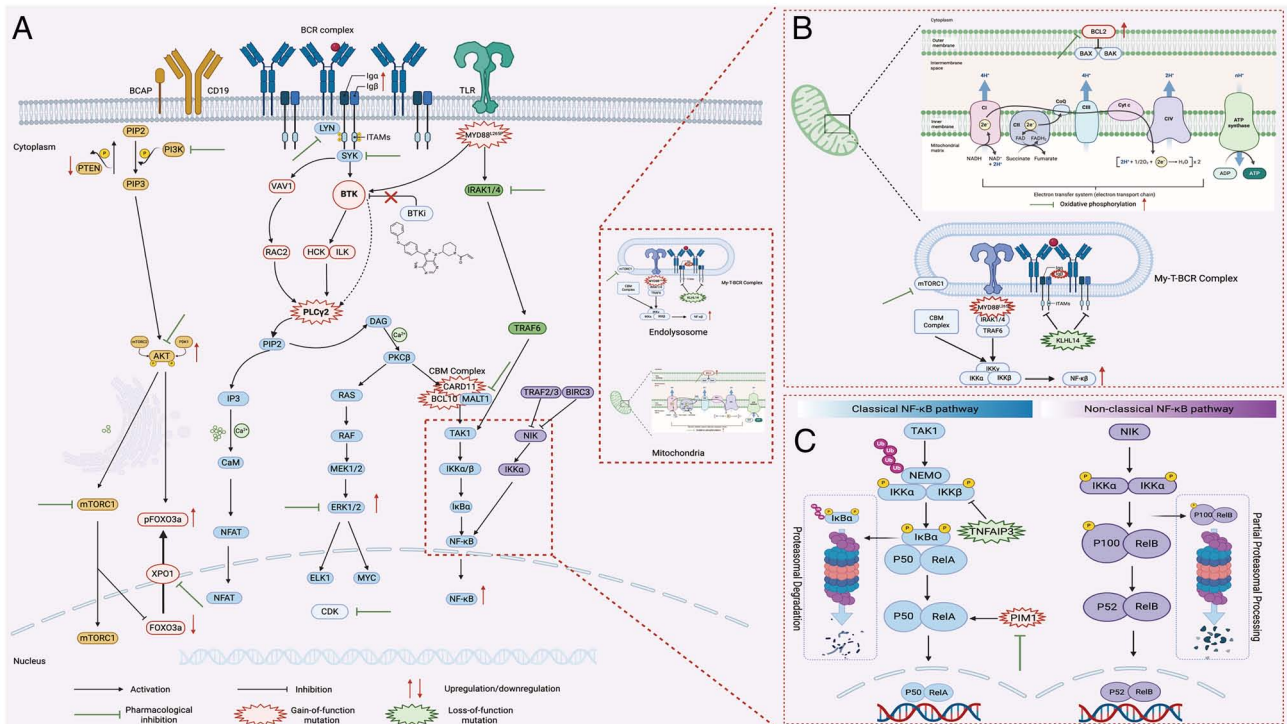


Figure 3. Overview of BTKi resistance mechanisms in DLBCL. (A) Resistance to BTKi in DLBCL arises from multiple interconnected alterations affecting BCR signaling hub, survival pathways, and metabolic reprogramming. These include BTK mutations and their acquired oncogenic scaffolding activity, gain-of-function mutations in PLCγ2, and activating mutations in CARD11 and BCL10 within the CBM complex. Compensatory pro-survival pathways are upregulated, such as PI3K/AKT/mTOR, ERK1/2, canonical NF-κB, and the TLR/MYD88 axis driven by MYD88L265P mutations. Metabolic reprogramming further contributes to resistance. (B) Mitochondrial events and metabolic shifts: upregulation of anti-apoptotic BCL-2 protein sustains cell survival. Resistant cells switch toward OXPHOS, as seen in IL4I1 downregulation and EGR1-mediated PDP1 activation. Loss-of-function mutations in KLHL14 disrupt its tumor suppressor role, promoting assembly of the My-T-BCR supercomplex and constitutive NF-κB activation. (C) Mutations driving canonical NF-κB pathway activation: inactivating mutations in TNFAIP3 relieve negative regulation of IKK, while MYD88L265P and CD79B mutations hyperactivate NF-κB via TLR-MYD88 and chronic BCR signaling. PIM1 overexpression stabilizes RelA, further amplifying NF-κB activity. Created with BioRender.com. BCR, B-cell receptor; BTK, Bruton's tyrosine kinase; BTKi, BTK inhibitor; CBM, CARD11-BCL10-MALT1 complex; DLBCL, diffuse large B-cell lymphoma; My-T-BCR, MYD88-TLR9-BCR supercomplex; OXPHOS, oxidative phosphorylation; PLCγ2, phospholipase Cγ2.

treatment (47). Consistently, patients with the MCD subtype, characterized by concurrent MYD88L265P and CD79B mutations, have been reported to achieve an ORR as high as 80% to ibrutinib, which is substantially greater than that achieved by patients with only single mutations. This suggests that the dual-mutated genotype is hyper-dependent on BCR-driven NF-κB signaling and highly sensitive to ibrutinib (11,48). A subsequent post hoc analysis of the phase III PHOENIX trial also suggested that, among younger patients (≤60 years) with ABC DLBCL, those with MCD or N1 subtypes who received ibrutinib plus R-CHOP reached 3-year event-free survival and overall survival (OS) rates of 100%, compared with ≤50% in the R-CHOP-alone arm (49). Mechanistically, the increased responsiveness of patients with MCD-subtype to BTKi may be attributed to the formation of a multiprotein super-complex termed My-T-BCR, which assembles MYD88L265P, TLR9 and BCR (5,47). My-T-BCR activates pro-survival NF-κB and mTOR signaling, while ibrutinib interferes with its assembly and function, thereby suppressing tumor survival (5). Recent research has uncovered a non-canonical form of chronic selective autophagy in MCD DLBCL. This process enables BTKi to promote the degradation of MYD88L265P through a TBK1-dependent autophagic pathway, which likely explains the pronounced efficacy of ibrutinib observed in this molecular subgroup (17).

While these mechanisms may explain the high BTKi sensitivity of the MCD phenotype, the basis for ibrutinib responsiveness in the N1 subtype remains elusive. It has been hypothesized that recurrent mutations involving genes such as BCL10 enhance BCR signaling and confer BTK dependency (49). Additionally, CD5+ DLBCL exhibits transcriptional signatures of BCR activation and is enriched for BCR-activating mutations associated with BTKi sensitivity. A CD5-based gene-expression signature may therefore serve as a biomarker to identify DLBCL cases likely to respond to BTKi-based therapies. Consequently, the susceptibility of this subtype to BTKi warrants further investigation (50). Patients with high co-expression of BCL2 and MYC have also been identified as a subset of non-GCB DLBCL that may show preferential responsiveness to ibrutinib (51). Thus, further extensive preclinical and clinical research is needed to delineate genetic subgroups that could benefit from BTKi monotherapy or BTKi-containing regimens. Such efforts would help improve therapeutic response and survival outcomes, ultimately advancing precision medicine in DLBCL.

Genetic drivers of BTKi resistance in DLBCL

BTK mutations and functional heterogeneity. The mechanisms underlying acquired resistance to BTKi are complex, arising from recurrent mutations in several key genes. The

Table I. Mechanisms of resistance to BTK inhibitors in diffuse large B-cell lymphoma and potential therapeutic approaches.

Key alterations	Resistance mechanisms	Evidence category	Potential therapeutic approaches
Subtype-driven intrinsic resistance GCB subtype (tonic BCR signaling)	PI3K and BCL2 pathway dependence	Clinical DLBCL; preclinical DLBCL	/
Genetic drivers of BTKi resistance BTK mutations	Disrupt covalent/non-covalent binding; Kinase-impaired mutants acquire neo-morphic scaffolding function	Preclinical DLBCL; evidence from other B-cell malignancies	Non-covalent BTKi, BTK PROTACs, BCL2 inhibitor
PLCγ2 gain-of-function mutations	Bypass BTK and directly activate calcium flux, ERK, and NF-κB	Evidence from other B-cell malignancies; biological hypothesis in DLBCL	RAC2, LYN, SYK inhibitors
MYD88 ^{L265P} mutations	TLR signaling and NF-κB dependence	Clinical DLBCL; preclinical DLBCL	IRAK1/4 inhibitors
CARD11 mutations	Constitutive CBM complex assembly leading to NF-κB activation	Single case of primary cutaneous DLBCL; preclinical DLBCL	MALT1 inhibitor
BCL10 gain-of-function mutations	Reduce CARD11 dependency and drive NF-κB activation	Preclinical DLBCL	MALT1 inhibitor
TNFAIP3 loss-of-function mutations	Relief of IKK inhibition and hyperactivation of canonical NF-κB	Clinical DLBCL; preclinical DLBCL	/
PIM1 mutations	Stabilize RelA and enhance NF-κB activity	Clinical DLBCL; preclinical DLBCL	PIM inhibitor
KLHL14 loss-of-function mutations	Promote My-T-BCR super-complex assembly and sustain NF-κB signaling	Preclinical DLBCL	mTOR inhibitor
Parallel survival pathway activation BTK-independent proximal BCR signaling	Maintain proximal BCR signaling and promote MYC upregulation	Preclinical DLBCL	Pan-SRC kinase inhibitor
PI3K/AKT/mTOR pathway activation	Bypass BTK blockade to sustain cell survival	Preclinical DLBCL	PI3K, mTOR, XPO1 inhibitors
MAPK/ERK pathway activation	Bypass BTK inhibition and promote cell survival	Preclinical DLBCL	MEK, BET inhibitors
BCL2 upregulation	Overexpression of anti-apoptotic protein blocks drug-induced apoptosis	Preclinical DLBCL	BCL2 inhibitor
Epigenetic alterations TCF4-RAC2 axis	BTK-independent RAC2-PLCγ2 activation	Preclinical DLBCL	RAC1/2 inhibitor
YTHDF2 upregulation	m6A-dependent ACER2 mRNA stabilization	Preclinical DLBCL	YTHDF2 suppression
FTO upregulation	m6A demethylation and MYC mRNA stabilization	Preclinical DLBCL	FTO suppression
14q32 miRNA cluster upregulation	PTEN suppression and PI3K/AKT/mTOR activation	Preclinical DLBCL; evidence from other B-cell malignancies	miR-494/miR-495 inhibition
Tumor microenvironment remodeling M2 TAM polarization	Immunosuppressive signaling and tumor-cell protection	Preclinical DLBCL	Tumor microenvironment modulator

Table I. Continued.

Key alterations	Resistance mechanisms	Evidence category	Potential therapeutic approaches
IL-16/CD9 axis upregulation	PI3K δ -AKT/ERK survival signaling	Preclinical DLBCL; evidence from other B-cell malignancies	IL-16/CD9 axis blockade
Direct cell-ECM adhesion	Reversible AKT/mTOR-associated survival signaling	Preclinical DLBCL	/
Metabolic reprogramming			
IL4I1 downregulation and EGR1 upregulation	Metabolic shift toward sustained OXPHOS	Preclinical DLBCL	OXPHOS inhibitor IACS-010759
mTOR and mitochondrial program upregulation	Metabolic adaptation and resistant-clone expansion	Clinical DLBCL; preclinical DLBCL	Targeted miR-28 nanoparticles
UPR downregulation	ER stress	Preclinical DLBCL	UPR activator 2-DG

2-DG, 2-deoxy-D-glucose; ACER2, alkaline ceramidase 2; BCL2, B-cell lymphoma 2; BCL10, B-cell lymphoma/leukemia 10; BCR, B-cell receptor; BET, bromodomain and extra-terminal domain; BTK, Bruton's tyrosine kinase; BTKi, BTK inhibitor; CARD11, caspase recruitment domain family member 11; CBM, CARD11-BCL10-MALT1 complex; CD9, cluster of differentiation 9; DLBCL, diffuse large B-cell lymphoma; ECM, extracellular matrix; EGR1, early growth response 1; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; FTO, fat mass and obesity-associated protein; GCB, germinal center B-cell-like; IKK, I κ B kinase; IL-16, interleukin-16; IL4I1, interleukin-4-induced 1; KLHL14, kelch-like protein 14; LYN, Lck/Yes-related novel protein tyrosine kinase; m6A, N6-methyladenosine; MALT1, mucosa-associated lymphoid tissue lymphoma translocation protein 1; MAPK, mitogen-activated protein kinase; miRNA, microRNA; MYD88, myeloid differentiation primary response 88; My-T-BCR, MYD88-TLR9-BCR supercomplex; OXPHOS, oxidative phosphorylation; PI3K, phosphoinositide 3-kinase; PIM1, proviral insertion in murine 1; PLC γ 2, phospholipase C γ 2; PROTAC, proteolysis-targeting chimera; PTEN, phosphatase and tensin homolog; SYK, spleen tyrosine kinase; TAM, tumor-associated macrophage; TNFAIP3, tumor necrosis factor α -induced protein 3; UPR, unfolded protein response; XPO1, exportin 1; YTHDF2, YTH m6A RNA-binding protein 2; /, no specific therapeutic approach identified. The listed therapeutic approaches are investigational and should not be interpreted as recommendations for routine clinical practice.

most frequently implicated targets are components of the BCR signaling pathway, including BTK itself and its downstream substrate PLC γ 2 (52). However, clinical evidence for BTK and PLC γ 2 resistance mutations is derived mainly from CLL, and their relevance in DLBCL remains uncertain.

The most common BTK resistance mutation occurs at the binding residue C481, with the predominant substitution being C481S, in which serine replaces cysteine and prevents covalent BTKi binding, ultimately driving acquired resistance (52). In a MYD88-mutant ABC-DLBCL cell model, BTKC481S reactivated the BTK/PLC γ 2/ERK1/2 axis during ibrutinib exposure. This ERK1/2 reactivation also led to the release of pro-survival and pro-inflammatory cytokines, including IL-6 and IL-10, which exert paracrine protective effects on BTK wild-type (WT) cells (53). Using protein engineering, all possible amino acid substitutions at the C481 residue resulting from single nucleotide changes were generated, along with threonine, which requires two nucleotide changes, yielding a series of C481X mutants. Among these, the C481T substitution conferred catalytic activity comparable to that of C481S and similarly mediated genetic resistance to ibrutinib. By contrast, substitutions with arginine, phenylalanine, tryptophan, tyrosine or glycine severely impaired or abolished BTK kinase activity, though these variants have also been speculated to contribute to ibrutinib resistance. Notably, non-C481S substitutions are expected to occur at low frequency clinically and have yet to be validated in large cohorts of patients with ibrutinib-resistant DLBCL (54). While the aforementioned

study deepened the mechanistic understanding of lymphoma resistance and provided a scientific foundation for resistance monitoring and the development of next-generation agents, BTK C481 substitutions have not been established as clinical resistance drivers in longitudinal DLBCL cohorts.

Beyond mutations at the covalent binding residue, genetic resistance to ibrutinib can also arise from alterations at the gatekeeper residue T474 within the BTK kinase domain. These mutations do not always occur in isolation; compound BTK mutations affecting both C481 and T474 have been documented, including T474I/C481S, T474M/C481S and T474M/C481T. Estupinan *et al* (55) demonstrated that these double variants remained insensitive to irreversible BTKi at concentrations ≥ 16 -fold above the pharmacological serum concentration and were therefore classified as super-resistant. These variants were characterized in engineered systems and have not been reported as acquired resistance mechanisms in patients with DLBCL.

The emergence of BTKC481S as a mechanism disrupting covalent inhibitor binding and conferring resistance to irreversible agents accelerated the development of reversible BTKi, which exert inhibitory activity against both C481S-mutant and WT BTK through non-covalent binding. However, longitudinal studies in patients with CLL treated with non-covalent BTKi identified multiple non-C481 BTK mutations, including V416L, A428D, M437R, T474I and L528W, all located within the BTK catalytic domain (41,56). These non-C481 mutations confer resistance to non-covalent BTKi. Notably, the

gatekeeper residue mutation T474I and the kinase-impaired residue mutation L528W also induce cross-resistance to covalent BTK inhibitors. The BTKL528W mutation confers resistance to ibrutinib and zanubrutinib, while the BTKT474I mutation confers resistance to acalabrutinib (57). Comparable longitudinal evidence is not available in DLBCL.

Functional studies have classified BTK resistance variants as kinase-proficient or kinase-impaired according to their catalytic activity relative to WT BTK (Fig. 1). Kinase-proficient mutants, such as C481S/T, T474I and T316A, retain enzymatic function and sustain BCR signaling. Kinase-deficient mutants, including C481F/R/Y, V416L, A428D, M437R and L528W, exhibit partial or complete loss of catalytic activity. Instead, these kinase-dead BTK mutant proteins adopt non-catalytic scaffolding functions and physically interact with alternative kinases such as hematopoietic cell kinase (HCK) and integrin-linked kinase (ILK), thereby bypassing the requirement for BTK autophosphorylation, and maintaining downstream PLC γ 2 activation and BCR signal propagation (22,58,59). These mechanisms were defined in engineered systems, including TMD8 DLBCL cells, rather than in longitudinal samples from BTKi-treated patients with DLBCL.

PLC γ 2 gain-of-function mutations. A less frequent genetic resistance mechanism involves gain-of-function mutations in PLC γ 2, the downstream substrate of BTK, which confer BTK-independent resistance in DLBCL cells. Mutated PLC γ 2 exhibits enhanced functional activity and drives BCR signaling downstream of BTK, promoting calcium flux, ERK phosphorylation and NF- κ B activation, which is likely to cause resistance to BTK degraders (60). Isolated PLC γ 2 mutations represent a rare mechanism of ibrutinib resistance and are often found in the context of concurrent BTK mutations (15,61). Gain-of-function PLC γ 2 mutations are established downstream escape mechanisms in CLL. However, this possibility has not been well established in DLBCL.

Overall, clinical evidence for BTK and PLC γ 2 resistance mutations comes mainly from CLL. Evidence in DLBCL is limited to mechanistic cell models and paired pretreatment and progression samples are scarce. The prevalence and clinical significance of these mutations in DLBCL therefore remain unknown. Long-term follow-up of patients with DLBCL treated with BTKi-containing regimens is currently underway, and is expected to enable precise interpretation of the BTK and PLC γ 2 resistance mutation spectrum in this population.

Genetic determinants of BTKi resistance beyond BTK and PLC γ 2. Beyond BTK and PLC γ 2 mutations, various other genomic alterations outside of these genes have also been identified in DLBCL as contributors to genetic resistance. As aforementioned, while the MCD subtype of DLBCL responds favorably to ibrutinib, patients with solitary MYD88L265P mutations or isolated CD79B mutations exhibit reduced response rates (11), suggesting that single mutations in MYD88 or CD79B may contribute to primary resistance to ibrutinib (48). MYD88L265P is the most recurrent point mutation in the ABC subtype, occurring in ~29% of cases. Evidence indicates a functional crosstalk between MYD88L265P and chronic active BCR signaling. This gain-of-function mutation in the adaptor protein MYD88 constitutively activates NF- κ B

via the TLR-MYD88 pathway. Knockdown of MYD88L265P in ABC cell lines markedly reduces NF- κ B activity, while ectopic expression of the mutant rescues cell survival and reverses the decline in NF- κ B signaling (62,63). Consequently, ABC-DLBCL harboring the L265P mutation becomes dependent on MYD88-driven NF- κ B activation and remains insensitive to ibrutinib (11,62,64). In ABC-DLBCL cells, CD79B upregulation mediates primary resistance to ibrutinib through the activation of compensatory pro-survival signaling pathways. Conversely, CD79B depletion restores sensitivity to ibrutinib in primarily refractory ABC-DLBCL cells, supporting its potential as a biomarker for predicting responses to BTKi in DLBCL (65), as detailed in subsequent sections.

Oncogenic gain-of-function mutations targeting the coiled-coil domain of the signaling adapter CARD11 were first reported in DLBCL in 2008 (66). These mutations drive spontaneous assembly of the CBM scaffold complex downstream of the BCR signaling pathway, ultimately leading to constitutive NF- κ B activation and promoting resistance to BTKi (43,66,67). Nagel *et al* (68) showed that MALT1 protease activity depended on BTK in CD79-mutant ABC-DLBCL cells but remained active independently of BTK in CARD11-mutant cells. Ectopic expression of oncogenic CARD11 in CD79-mutant cells conferred ibrutinib resistance. These findings provide direct preclinical evidence that oncogenic CARD11 can sustain CBM-mediated NF- κ B signaling despite BTK inhibition. Clinical evidence remains limited. Caeser *et al* (69) identified an acquired CARD11K215T mutation in a patient with primary cutaneous DLBCL who relapsed after 13 months of combined BTK and SYK inhibition. The mutation was absent in pretreatment biopsies and detected at relapse. Expression of CARD11K215T in U2932 cells reduced the ibrutinib sensitivity by three orders of magnitude, supporting its role in acquired resistance to BCR-pathway inhibition. Notably, however, the signaling potency of various CARD11 mutant isoforms differs substantially. In certain cases, cells harboring weak CARD11 alleles may remain highly dependent on upstream survival signals and, conversely, prove sensitive to ibrutinib intervention. Prospective functional and clinical evaluation of recurrent CARD11 variants is required before they can inform BTKi selection in DLBCL (67). The scaffold protein MALT1 participates in the formation of the CBM adapter complex to activate canonical NF- κ B signaling. In addition, MALT1 physically interacts with TRAF3, a negative regulator of NIK, thereby modulating non-canonical NF- κ B signaling (70). A previous study identified the deubiquitinating enzyme cylindromatosis (CYLD) as a downstream effector of MALT1 and its downregulation correlated with poor prognosis in patients with DLBCL. Under conditions of chronic BCR signaling, MALT1 mediates the cleavage and inactivation of CYLD, which promotes NF- κ B activation as well as the proliferation of BCR-dependent DLBCL cells, ultimately rendering these cells less sensitive to BTKi (71). Previous studies revealed that gain-of-function mutations in the signaling protein BCL10, another component of the CBM multiprotein complex, drive resistance to BTKi (72,73). These findings underscore the central role of the CBM complex in mediating BTKi resistance in DLBCL and highlight the clinical potential of targeting CBM complex constituents as an attractive therapeutic strategy to overcome such resistance.

Activation of canonical NF- κ B signaling promotes resistance to BTKi in DLBCL. Downstream of this pathway, tumor necrosis factor- α -induced protein 3 (TNFAIP3) negatively regulates IKK activity. Loss-of-function mutations in TNFAIP3 (also known as A20) provoke hyperactivation of NF- κ B signaling independently of upstream BTK activity, thereby conferring ibrutinib resistance in ABC-DLBCL cells (11,43,63,74). Proviral insertion in murine 1 (PIM1), a serine/threonine kinase, activates NF- κ B signaling by stabilizing RelA, and regulates cellular proliferation, differentiation, apoptosis and survival (23,75). Kuo *et al* (76) identified PIM1 mutations in six patients with DLBCL, five of whom had ABC-DLBCL. In total, four of these five patients had poor responses to ibrutinib. In ibrutinib-resistant ABC-DLBCL cells, PIM1 was upregulated. Expression of the L2V, P81S and S97N variants increased PIM1 protein stability and reduced ibrutinib sensitivity, while combined PIM and BTK inhibition suppressed cell proliferation *in vitro* and tumor growth in xenograft models *in vivo*. These findings provide exploratory clinical and preclinical DLBCL evidence, but do not establish PIM1 as a validated resistance biomarker.

Platelet-derived growth factor D (PDGFD) has also been linked to reduced ibrutinib response in DLBCL. Jin *et al* (77) analyzed gene-expression data and tumor tissues from ibrutinib-treated patients with DLBCL and found higher PDGFD expression in samples with reduced drug response. In acquired-resistant TMD8 and HBL1 cells, PDGFD was upregulated, whereas PDGFD silencing reduced EGFR expression, lowered the half maximal inhibitory concentration (IC₅₀) of ibrutinib and increased apoptosis. These findings provide exploratory clinical and preclinical DLBCL evidence, but require validation in an independent clinical cohort.

Kelch-like protein 14 (KLHL14) is a tumor suppressor that localizes in proximity to BCR in cell line models of MCD DLBCL, promotes the turnover of immature glycoforms of BCR subunits and downregulates total cellular BCR levels, thereby functioning as a negative regulator of BCR signaling. Loss-of-function mutations in KLHL14 are frequently observed in DLBCL of the MCD molecular subtype. Inactivation of KLHL14 facilitates the assembly of the My-T-BCR signaling complex and the initiation and maintenance of NF- κ B signaling, thus contributing to relative resistance to ibrutinib in MCD cells (78). These preclinical findings identify KLHL14 loss as a candidate determinant of reduced ibrutinib sensitivity in MCD-subtype DLBCL. However, its predictive value has not been evaluated prospectively in BTKi-treated patients.

Src homology 2 domain-containing phosphatase 1 (SHP1) is a cytosolic protein tyrosine phosphatase responsible for negatively regulating the BCR signaling pathway (79). Promoter hypermethylation of SHP1 accounts for reduced SHP1 protein expression in DLBCL tumor cells. SHP1 depletion in BCR-dependent ABC- and GCB-DLBCL cell lines enhances proximal BCR signaling activity and sensitivity to ibrutinib. Conversely, re-expression of SHP1 in knockout cells restores basal BCR signaling intensity and confers ibrutinib resistance. Pharmacological inhibition of SHP1 synergizes with ibrutinib to suppress the proliferation of DLBCL cell lines and the growth of patient-derived primary tumor cells. Collectively, these preclinical findings indicate that SHP1 loss may increase ibrutinib sensitivity in DLBCL. However,

its value as a predictive biomarker has not been assessed in BTKi-treated cohorts (80).

Despite the identification of multiple complicated genetic mechanisms driving resistance to BTKi, emerging evidence suggests that the clinical emergence of resistance largely results from the competitive selection of pre-existing resistant subclones present at treatment initiation. Current limitations in detecting low-abundance resistant subclones and pre-existing resistance mutations pose marked challenges to the comprehensive understanding of BTKi resistance mechanisms. Addressing these technological and analytical gaps represents a critical focus for future investigations (60).

Non-genetic drivers of resistance to BTKi in DLBCL. In B-cell malignancies, >20% of patients with ibrutinib resistance have been reported to lack identifiable genetic resistance alterations (81). This observation suggests that tumor cells may exploit non-genetic mechanisms to adapt to the inhibition of the BCR-associated kinases, thereby driving resistance to BTKi. These non-genetic drivers of BTKi resistance encompass compensatory activation of alternative pro-survival pathways, epigenetic alterations, remodeling of the TME and metabolic reprogramming, as subsequently discussed.

Lack of BTK dependency and persistent proximal BCR signaling. Lack of BTK dependency represents an important mechanism of intrinsic insensitivity to selective BTK inhibition in DLBCL. Battistello *et al* (82) showed that ibrutinib suppressed BTK phosphorylation in primary DLBCL samples and in both ABC- and GCB-DLBCL cells, whereas the phosphorylation of CD19 and GSK3 β was not consistently inhibited. In ibrutinib-insensitive cells, BTK inhibition was accompanied by MYC upregulation. A similar increase in MYC expression was observed in a BCL6-driven lymphoma mouse model that was insensitive to ibrutinib. Mechanistically, the proximal SRC-family kinases LYN, FYN and BLK exhibited functional redundancy. Neither individual kinase loss nor combined loss of LYN and FYN impaired lymphoma cell proliferation, whereas simultaneous disruption of all three kinases reduced cell viability. The pan-SRC kinase inhibitor masitinib suppressed multiple BCR signaling outputs, including the phosphorylation of BTK, CD19 and GSK3 β , reduced MYC expression and inhibited tumor growth in both cell-line-derived and patient-derived DLBCL xenograft models. Collectively, these preclinical findings indicate that selective BTK inhibition may be insufficient in BTK-independent tumors, as BTK blockade could shift tumor-cell dependency toward parallel BCR pathways that sustain MYC expression and lymphoma-cell survival.

BTK-independent activation of bypass survival pathways. The PI3K/AKT/mTOR signaling pathway is known to be dysregulated in various cancer types, where it regulates tumor cell proliferation, survival and angiogenesis (83). This signaling cascade exhibits crosstalk with the BCR signaling pathway. Activation of PI3K signaling has been shown to rescue mature B cells from apoptosis in the absence of BCR signals (84,85). Consequently, aberrant activation of this pathway has been found to bypass BTK and overcome BTKi-mediated inhibition in B-cell malignancies. Zhang *et al* (86) observed

that PI3K signaling pathway members were upregulated in patient-derived xenograft models of DLBCL with acquired resistance to ibrutinib. Additionally, Jain *et al* (87) reported that enhanced PI3K/AKT/mTOR signaling was a hallmark of ibrutinib-resistant DLBCL cells. Compensatory upregulation of the PI3K/AKT axis drives acquired ibrutinib resistance in DLBCL and targeting the upregulated activity of PI3K in ABC-DLBCL cells has been shown to reduce tumorigenicity and survival while reversing resistance. Mechanistically, Paul *et al* (88) identified reciprocal feedback between the BTK and PI3K/AKT pathways in ABC-DLBCL cells and patient-derived xenograft models. PI3K inhibition increased BTK phosphorylation, whereas BTK inhibition induced rebound AKT phosphorylation. Dual PI3K α/δ inhibition suppressed AKT and NF- κ B signaling, and induced tumor regression in a CD79B WT/MYD88 mutant ABC-DLBCL xenograft model that was unresponsive to ibrutinib. Similarly, Kapoor *et al* (89) reported that ABC-DLBCL cells refractory to ibrutinib were characterized by reduced nuclear levels of forkhead box O 3a (FOXO3a) and phosphatase and tensin homolog (PTEN), along with hyperactivation of PI3K/AKT/mTOR signaling. AKT serves as a key upstream regulator of tumor suppressor FOXO3a, directly phosphorylating it, and thereby promoting its cytoplasmic retention and subsequent degradation. Consequently, less unphosphorylated FOXO3a translocates into the nucleus to activate transcription of apoptosis-related genes (90). Inhibition of hyperactivated PI3K and AKT attenuates pathway activity, restores nuclear FOXO3a abundance and sensitizes resistant cells to ibrutinib-induced apoptosis. The aforementioned study further highlights that dysregulated nuclear/cytoplasmic subcellular localization of FOXO3a contributes to BTKi resistance in DLBCL. Enhancing nuclear accumulation of FOXO3a to reactivate its transcriptional functions holds promise for improving BTKi efficacy and represents a potential strategy to restore ibrutinib sensitivity. Notably, exportin 1 inhibitors have demonstrated synergistic antitumor effects with ibrutinib in preclinical studies and are being actively investigated in clinical trials as rational combination regimens for treating BTKi-resistant B-cell malignancies (89).

The MAPK pathway, particularly the ERK branch, functions downstream of the BCR/BTK/PLC γ 2 axis to regulate lymphoma cell proliferation and survival. Compensatory upregulation of the ERK MAPK signaling cascade has been shown to bypass BTK inhibition and promote resistance to BTKi in DLBCL. Specifically, as aforementioned, CD79B overexpression activates both AKT and MAPK signaling in ABC-DLBCL, thus contributing to ibrutinib resistance. Knockdown of CD79B in ABC-DLBCL cells reduces phosphorylated MAPK levels and sensitizes primary refractory cells to ibrutinib. Furthermore, pharmacological inhibition of MAPK in combination with ibrutinib may represent a strategy to address ibrutinib resistance in DLBCL cells (65). Further studies have uncovered a mechanism by which the MAPK/ERK signaling pathway protects tumor cells lacking genetic alterations from adapting to BTK inhibition through a paracrine effect. Specifically, cells harboring BTK mutations activate ERK1/2 signaling and release pro-survival chemokines, thereby shielding their non-mutated counterparts (53). Most recently, ubiquitin-specific peptidase 13 (USP13)-mediated ERK activation has been

identified as a post-translational regulatory mechanism driving ibrutinib resistance in DLBCL. USP13 expression is elevated in ibrutinib-resistant cells and further upregulated upon drug exposure. Mechanistically, USP13 stabilizes four and a half LIM domains 1 through deubiquitination, leading to sustained ERK1/2 phosphorylation, downregulation of the pro-apoptotic protein Bax and upregulation of the anti-apoptotic protein Bcl-xL, thereby promoting cell survival and reducing ibrutinib sensitivity (91). Upon phosphorylation, ERK translocates to the nucleus and regulates the transcriptional activation of targets such as MYC and ELK1. DLBCL-specific evidence linking MYC upregulation to reduced ibrutinib sensitivity is discussed in the present review.

Furthermore, mutations in multiple core genes that initiate the activation of the canonical NF- κ B signaling pathway have been implicated in resistance to BTKi in DLBCL, as aforementioned. In addition, resistance to apoptosis induced by BTKi represents a fundamental mechanism underlying acquired resistance to BTK inhibition in DLBCL. This process is characterized by upregulation of anti-apoptotic BCL2 family members, including BCL2 itself. Increased BCL2 expression was detected in patients with ABC-DLBCL exhibiting poor response to ibrutinib. Notably, ibrutinib-resistant TMD8 cells harboring elevated BCL2 gene expression exhibited enhanced sensitivity to BCL2 inhibition. Combination of a BCL2 inhibitor with ibrutinib demonstrated synergistic effects both *in vitro* and *in vivo*, suppressing proliferation and inducing apoptosis in resistant cells, regardless of whether resistance arose from genetic mutations or drug-induced adaptation. These findings position BCL2 inhibition as a plausible therapeutic combination strategy to circumvent BTKi resistance (92).

Epigenetic alterations contributing to BTKi resistance. Epigenetic dysregulation has recently emerged as a key contributor to treatment resistance in cancer, prompting renewed interest in therapeutic strategies targeting epigenetic modifiers (93). Shaffer *et al* (16) elucidated a mechanism by which epigenetic rewiring of BCR signaling underlies a novel BTKi resistance mode in ABC DLBCL. The aforementioned study first demonstrated that treatment with a histone deacetylase inhibitor, alone or in combination with a DNA methylation inhibitor, resensitized ibrutinib-resistant pools to ibrutinib, supporting the notion that resistance in these pools was epigenetically driven. Subsequently, the study showed that the transcriptional regulatory network in ibrutinib-resistant pools becomes preferentially dependent on the transcription factor TCF4 and its downstream target genes. This TCF4-driven program upregulates genes, including *RAC2*, *TLR9* and *BCL2*, that enable cells to evade apoptosis, rewire signaling dependencies and adapt to microenvironmental stress. Mechanistically, increased chromatin accessibility at the enhancer domain enables the TCF4-driven upregulation of *RAC2*, a Rho family GTPase. Elevated *RAC2* interacts more robustly with PLC γ 2, bypassing BTK to directly activate downstream PLC γ 2 and calcium flux, thereby re-routing BCR signals to NF- κ B and conferring resistance to ibrutinib. These findings position small-molecule *RAC1/2* inhibitors as promising therapeutic agents to address ibrutinib resistance. In addition, ibrutinib-resistant pools (polyclonal resistant populations) exhibit a shift in the burden of NF- κ B activation from

the CBM signalosome toward the TLR9/MYD88 module, along with increased reliance on BCL2 and nuclear regulators such as MYC and bromodomain-containing protein 4 (BRD4), ultimately orchestrating epigenetic resistance to ibrutinib. The study by Shaffer *et al* (16) introduced a paradigm of epigenetic reprogramming as a basis for BTKi resistance in DLBCL. Currently, multiple agents targeting epigenetic resistance mechanisms are under investigation for lymphoid malignancies, offering promise for more effective, personalized treatment strategies (16,94).

RNA modification represents an important mechanism of epigenetic regulation. These modifications influence RNA stability, translation efficiency and protein interactions, thereby shaping cell fate (93). YTH N6-methyladenosine (m6A) RNA binding protein 2 (YTHDF2) serves as a key m6A-binding protein that facilitates the degradation or translation of m6A-modified mRNAs. YTHDF2 is overexpressed in DLBCL, where it acts in a pro-tumorigenic manner and correlates with unfavorable prognosis. Inhibiting YTHDF2 enhances the sensitivity of DLBCL cells to ibrutinib *in vitro* (95).

Beyond m6A recognition by YTHDF2, Fat mass and obesity-associated protein (FTO)-mediated m6A demethylation has been associated with reduced ibrutinib sensitivity in DLBCL. FTO silencing increased m6A enrichment on MYC mRNA, reduced its stability and enhanced ibrutinib sensitivity in DLBCL cells. Conversely, FTO overexpression increased MYC expression, raised the IC₅₀ of ibrutinib and attenuated ibrutinib-induced apoptosis in DLBCL cells. MYC overexpression also reduced ibrutinib sensitivity, whereas FTO knockdown partially reversed this effect *in vitro* and in xenograft models. These findings support a role for FTO-mediated m6A demethylation and stabilization of MYC mRNA in reducing ibrutinib sensitivity in DLBCL. However, whether this mechanism contributes to acquired resistance remains unknown and requires validation in acquired-resistance models and tumor samples (96).

In addition, non-coding RNA alterations have gained increasing recognition as epigenetic contributors to BTKi resistance. Among these, microRNAs (miRNAs/miRs) have been found to regulate BCR signaling and its modulators, including PTEN, SHIP-1, ZAP-70, GAB1 and BTK, positioning them as potential predictive and prognostic markers for clinical outcomes and treatment response (97). In acquired ibrutinib-resistant TMD8 and RIVA cells, Kapoor *et al* (98) found coordinated upregulation of the 14q32 miRNA cluster, including miR-494, miR-495 and miR-543, accompanied by PTEN suppression and PI3K/AKT/mTOR activation. Inhibition of miR-494 and miR-495 restored PTEN expression and ibrutinib sensitivity *in vitro*. Analyses of BTKi-resistant patient samples in the same study were performed in CLL rather than DLBCL. Clinical validation of this mechanism in DLBCL is therefore still lacking. In ABC-DLBCL cell models, another study implicated the miR-30 family and its target BCL6 in ibrutinib resistance. Inhibition of BCL6 restored ibrutinib sensitivity in resistant DLBCL cells, further supporting the role of miRNA-mediated epigenetic regulation in BTKi resistance (99).

TME remodeling in BTKi resistance. The TME is defined as a complex and coordinated ecological network consisting

of cellular components, including cancer, non-hematopoietic stromal and immune cells, as well as non-cellular elements represented by the extracellular matrix (ECM). The dynamic interactions among these constituents regulate immune evasion, tumor progression and responsiveness to anticancer therapies (100).

Among the non-cellular components of the TME, physical contact with the ECM may reduce the response of DLBCL cells to ibrutinib. Ceccato *et al* (101) cultured four DLBCL cell lines on three-dimensional scaffolds prepared from decellularized human bone. Ibrutinib-induced apoptosis was attenuated in ECM-adherent OCI-LY18 and RIVA cells. In OCI-LY18 cells, this protection was confined to the attached population, was absent on PDMS scaffolds lacking native ECM components and was lost after the cells were returned to two-dimensional culture. ECM adhesion was also accompanied by increased phosphorylated AKT levels and S6 expression, consistent with an AKT/mTOR-related survival state. ECM exposure further altered cytokine secretion and promoted lymphoma-cell migration and scaffold colonization. Together, these findings indicate a reversible and cell line-dependent form of micro-environment-mediated protection from ibrutinib rather than stable acquired resistance.

Multiple immune cell types have been identified in close proximity to tumor cells, where they exert either positive or negative regulatory effects on therapeutic resistance through intricate biological interactions (102). Tumor-associated macrophages (TAMs) represent a critical component that remodels an immunosuppressive microenvironment, thereby driving tumor progression and drug resistance. Macrophages can polarize into distinct phenotypes in response to various stimuli, broadly categorized as proinflammatory M1 macrophages and anti-inflammatory M2 macrophages (103). In preclinical DLBCL models, polarization of TAMs toward the M2 phenotype contributed to reduced ibrutinib sensitivity. Song *et al* (104) experimentally demonstrated that, during *in vitro* co-culture, DLBCL cells drove the polarization of M0 macrophages toward a tumor-promoting M2 phenotype. Addition of SYHA1813 to the co-culture system reversed DLBCL-induced M2 polarization in a dose-dependent manner, providing an explanation for the dose-dependent antitumor effects observed in ibrutinib-resistant xenograft models, where SYHA1813 effectively overcame ibrutinib resistance.

In an acquired ibrutinib-resistant model, IL-16 expression and secretion were increased. Conditioned medium from resistant cells reduced ibrutinib sensitivity in parental cells, while recombinant IL-16 reproduced this phenotype. Mechanistically, IL-16 engaged CD9-enriched membrane microdomains and activated PI3K δ , thereby sustaining AKT and ERK signaling, stabilizing MYC, activating NF- κ B and increasing BFL1 expression. In TMD8 ABC-DLBCL cells, CD9 silencing blocked the IL-16-induced AKT activation and BFL1 upregulation, and reversed IL-16-driven ibrutinib resistance. Pharmacologically blocking IL-16 or CD9 also enhanced R-CHOP sensitivity in ABC-DLBCL cell lines. These findings provide DLBCL preclinical support for the IL-16/CD9 axis, although the acquired-resistance model was derived from marginal zone lymphoma, and no BTKi-treated DLBCL samples were examined (105).

However, the precise role of immune microenvironment reshaping in mediating BTKi resistance in DLBCL remains to be fully elucidated. Our recent comprehensive review systematically examined the mechanisms by which the TME modulates resistance to BTKi across B-cell lymphoproliferative disorders, with an emphasis on CLL, mantle cell lymphoma and Waldenström macroglobulinemia (106). That review underscored that exploring immune microenvironment alterations and their impact on BTKi efficacy in DLBCL represents an important area for future study.

Rewiring of cellular metabolism in BTKi resistance. Tumor cell metabolism encompasses aerobic glycolysis, amino acid metabolism and fatty acid metabolism. It is well established that lymphoma cells rewire their metabolic pathways to support the increased energy demands imposed by rapid proliferation. This adaptive metabolic reprogramming fuels tumor growth, sustains disease progression and orchestrates resistance to anticancer agents (42).

A hallmark of cancer cells is the Warburg effect, defined as the preferential conversion of glucose to lactate via glycolysis even under normoxic conditions, bypassing the more efficient oxidative phosphorylation (OXPHOS) pathway. This phenomenon is also referred to as aerobic glycolysis (23). DLBCL cells similarly rely on aerobic glycolysis to fuel their energetic needs. However, in ibrutinib-resistant ABC-DLBCL, a metabolic shift occurs toward sustained OXPHOS. Mechanistically, multi-omics analysis revealed downregulation of IL-4-induced 1, a key metabolic regulator, resulting in elevated levels of tricarboxylic acid cycle enzymes and driving the metabolic transition toward OXPHOS in ibrutinib-resistant DLBCL cells. In line with this, resistant cells exhibited heightened sensitivity to the OXPHOS inhibitor IACS-010759 (107). Liu *et al* (108) uncovered a new mechanism driving the metabolic shift toward OXPHOS in DLBCL. The authors found that the transcription factor early growth response gene 1 (EGR1) is upregulated in ibrutinib-tolerant ABC-DLBCL cells. Overexpression of EGR1 induces the transcriptional activation of PDP1, which triggers a metabolic shift toward OXPHOS and ultimately promotes the development of ibrutinib resistance. Targeting OXPHOS suppressed the proliferation of ibrutinib-resistant lymphoma cells both *in vitro* and *in vivo*. These findings uncover a hallmark metabolic switch associated with BTKi resistance in DLBCL and suggest that targeting metabolic enzymes or metabolites may represent a promising and rational therapeutic option to circumvent BTKi resistance.

A recent study modeled acquired ibrutinib resistance in four ABC-DLBCL cell lines through prolonged exposure to progressively increasing drug concentrations. In MD-901 cells, clonal barcoding demonstrated that ibrutinib exposure progressively selected resistant clones at the expense of clonal diversity. Transcriptomic profiling of resistant MD-901 clones revealed activation of mTOR and mitochondrial gene programs, including mitochondrial translation, respiratory chain assembly and oxidative phosphorylation-related pathways. miR-28 counteracted these adaptive changes, reduced mitochondrial mass and, in MD-901 cells, decreased mitochondria-derived ATP production. It also suppressed mTORC1 activity, as reflected by reduced S6 phosphorylation, and impaired the emergence of resistant cells *in vitro*. Consistently,

miR-28 expression or targeted delivery of miR-28 mimics inhibited the growth of ibrutinib-resistant ABC-DLBCL tumors in xenograft models. In an exploratory analysis of the PHOENIX trial, a lower miR-28-regulated ibrutinib-resistance signature score was associated with longer overall survival among older patients with MCD DLBCL treated with ibrutinib plus R-CHOP, whereas such association was not observed in the R-CHOP-alone group. The clinical relevance of this association remains to be confirmed prospectively (109).

Additionally, altered cellular stress responses may also contribute to BTKi resistance. The synthesis and folding of secreted and membrane proteins take place in the endoplasmic reticulum (ER) (110). When the demand for secreted proteins exceeds the folding capacity of the ER, or under conditions such as hypoxia, misfolded proteins accumulate and trigger ER stress. Chronic ER stress promotes chemotherapy adaptation in tumor cells through multiple mechanisms, including inhibition of apoptosis, metabolic reprogramming, antioxidant defense and drug efflux (111). Upon ER stress, the cell activates the highly conserved unfolded protein response (UPR), which enhances the folding capacity of the ER by upregulating molecular chaperones and reducing protein translation, thereby restoring homeostasis (110). Zhang *et al* (112) observed that UPR marker genes were markedly downregulated in ibrutinib-resistant ABC-DLBCL cells. Combining the UPR activator 2-deoxy-D-glucose with ibrutinib resulted in enhanced growth inhibition, increased apoptosis and stronger suppression of calcium flux compared with either agent alone. These findings suggest that targeting the UPR merits further evaluation in DLBCL. A comprehensive summary of the key resistance mechanisms and their corresponding potential therapeutic counter-strategies is presented in Table I.

4. Emerging BTK-targeted strategies to address BTKi resistance

Although non-covalent ATP-competitive BTKi were developed to block BCR signaling driven by BTKC481 mutant proteins, accumulating evidence reveals that multiple newly identified non-C481 BTK mutations confer broad cross-resistance to both covalent and non-covalent inhibitors. As a result, simply swapping a reversible inhibitor for an irreversible one in sequential treatment regimens may not be effective (57). Consequently, next-generation BTK-targeted therapies are currently undergoing preclinical development and clinical evaluation. These approaches move beyond the binary covalent or non-covalent binding of BTK, employing more diverse strategies including dual-mode BTK inhibition, BTK degradation and allosteric BTK inhibition, with the aim of achieving greater efficacy, reducing off-target side effects and addressing resistance to traditional BTKi (34,57).

The strategies discussed below are supported by evidence of varying strength. Preclinical evidence includes activity in BTKi-resistant cell lines and xenograft models. Clinical evidence consists mainly of preliminary responses in R/R DLBCL or in mixed cohorts that included patients previously exposed to a BTKi. The majority of clinical studies were small, single-arm and early phase. Prior BTKi progression, resistance mutations and response duration were not consistently reported. These findings, therefore, support the potential

of these strategies to address BTKi resistance, but do not establish that resistance has been overcome.

Dual-mode (covalent/non-covalent) BTKi. Dual-binding BTKi engage BTK through both covalent and non-covalent binding modes, thereby suppressing both WT BTK and C481-mutant variants (113). A representative dual-binding BTKi currently undergoing clinical development is rocbrutinib, previously known as LP-168. Rocbrutinib is a novel dual covalent and non-covalent BTKi that exhibits high selectivity for BTK and favorable bioavailability. It dampens BCR signaling, as evidenced by reduced phosphorylation of BTK and its direct downstream substrate PLC γ 2 in CLL cells. In DLBCL, previous *in vitro* studies demonstrated that rocbrutinib exerts moderate cytotoxicity in TMD8 cells harboring BTKC481S and BTKT474I mutations (57,113). Given its preclinical activity, this dual-binding inhibitor has advanced into clinical investigation to evaluate its efficacy and safety. An ongoing open-label phase I trial (NCT04993690) is evaluating rocbrutinib monotherapy in patients with R/R B-cell NHL (B-NHL). Among 33 efficacy-evaluable patients with R/R non-GCB DLBCL, the ORR was 60.6% and the complete response (CR) rate was 33.3%. At a median follow-up of 6.7 months, the duration of response (DOR), PFS and OS data were not yet mature. The most common treatment-emergent adverse events (TEAEs) included neutropenia, leukopenia, thrombocytopenia, anemia, and petechiae, most of which were grade 1 (114,115).

Additionally, another recently registered multi-center clinical study is underway to evaluate the efficacy and safety of rocbrutinib as monotherapy compared with the BR regimen (bendamustine plus rituximab) or the R2 regimen (rituximab plus lenalidomide) in patients with non-GCB DLBCL who are refractory or have relapsed after ≥ 2 prior lines of therapy (NCT07189065), with ORR as the primary endpoint. These findings show preliminary activity in R/R non-GCB DLBCL, but do not establish that rocbrutinib overcomes acquired resistance to covalent BTKi. Further studies in patients with documented progression during prior BTKi treatment are required.

BTK proteolysis targeting chimeras (PROTACs). Recently, targeted protein degradation (TPD) technologies, which aim to induce or enhance the interaction between an E3 ubiquitin ligase and a protein of interest (POI) to facilitate ubiquitin transfer and subsequent targeted degradation, have garnered increasing attention. PROTACs represent one of the two classical modalities in the TPD field. They are essentially heterobifunctional compounds composed of a central chemical linker with an E3 ligase-binding moiety at one end and a POI-binding moiety at the other (116). The molecular mechanism by which BTK PROTACs induce targeted degradation of BTK is depicted in Fig. 4.

BTK PROTACs function by bringing the E3 ubiquitin ligase into close proximity with BTK, forming a ternary complex that promotes ubiquitin transfer to BTK and ultimately leads to its precise degradation by the proteasome (116). Compared with conventional covalent and non-covalent BTKi, BTK degraders offer several notable advantages. First, they exploit two independent ligands to simultaneously recruit BTK and

an E3 ligase, thereby inducing polyubiquitination of BTK and initiating its degradation via the ubiquitin-proteasome system. As a result, BTK degraders exhibit higher selectivity for BTK and correspondingly fewer off-target effects than classical BTKi. Second, unlike classical BTKi, which rely on sustained occupancy of the BTK active site for functional effects, BTK degraders operate through a catalytic mechanism of action, driving multiple rounds of BTK degradation at substoichiometric doses. More importantly, preclinical studies suggest that BTK PROTACs may address resistance mechanisms associated with conventional BTKi. Kinase-deficient BTK mutants acquire a neo-morphic scaffolding function that activates alternative kinases such as HCK and ILK, which in turn phosphorylate PLC γ 2 and sustain BCR signaling, thereby driving resistance to BTK inhibition. BTK PROTACs counteract this by degrading the mutant BTK protein, thereby disrupting its newly acquired kinase-independent scaffolding activity and providing a mechanistic rationale for their evaluation in BTKi-resistant disease (58,116,117).

Collectively, BTK PROTACs represent an emerging therapeutic strategy that can degrade WT BTK and several resistance-associated BTK variants in preclinical models. Early-phase trials have also reported preliminary responses in heterogeneous R/R B-cell malignancies.

NX-2127 is a heterobifunctional molecule composed of a BTK-binding module and a cereblon E3 ubiquitin ligase-binding module. It promotes ubiquitin-dependent degradation of BTK regardless of mutant BTK functional category, while also stimulating T cell activation through degradation of Ikaros family transcription factors (IKZF1/3) (118,119). Elevated expression of IKZF3 has been linked to resistance against conventional BTKi (120). NX-2127 is an orally available, first-in-class BTK degrader with dual functionality. NX-2127 combines BTK degradation with immunomodulatory activity and provides a mechanistic strategy for targeting both catalytic and kinase-independent BTK functions. Preclinical studies have shown that NX-2127 promotes the degradation of BTKC481S mutant in an ABC-DLBCL cell line and exhibits greater potency than ibrutinib in suppressing the proliferation of BTKC481S-mutant TMD8 cells. In a xenograft model carrying BTKC481S-mutant TMD8 cells, NX-2127 significantly inhibited tumor growth (121). The first-in-human phase I trial of NX-2127 in patients with R/R B-NHL (NCT04830137), in which 72% of patients had received prior BTKi treatment, has yielded initial results. Rapid, robust and sustained BTK degradation was observed across all patients, independent of baseline BTK levels, tumor type or NX-2127 dose. Among the responses, two patients achieved CR, including one with DLBCL. The study remains ongoing. Early clinical findings suggest that NX-2127 has a manageable safety profile and can produce responses in R/R B-NHL. However, prior BTKi exposure, resistance status and response duration were not reported specifically for the DLBCL subgroup (58,122).

NX-5948 is a selective BTK degrader that induces specific degradation of both WT and mutant BTK proteins without affecting IKZF1/3 or other cereblon neo-substrates. *In vitro*, NX-5948 suppressed the proliferation of a BTKC481S mutant ABC-DLBCL cell line with a half-maximal effective concentration (EC_{50}) < 10 nM, outperforming ibrutinib with an $EC_{50} > 1$ μ M. In a TMD8 xenograft mouse model

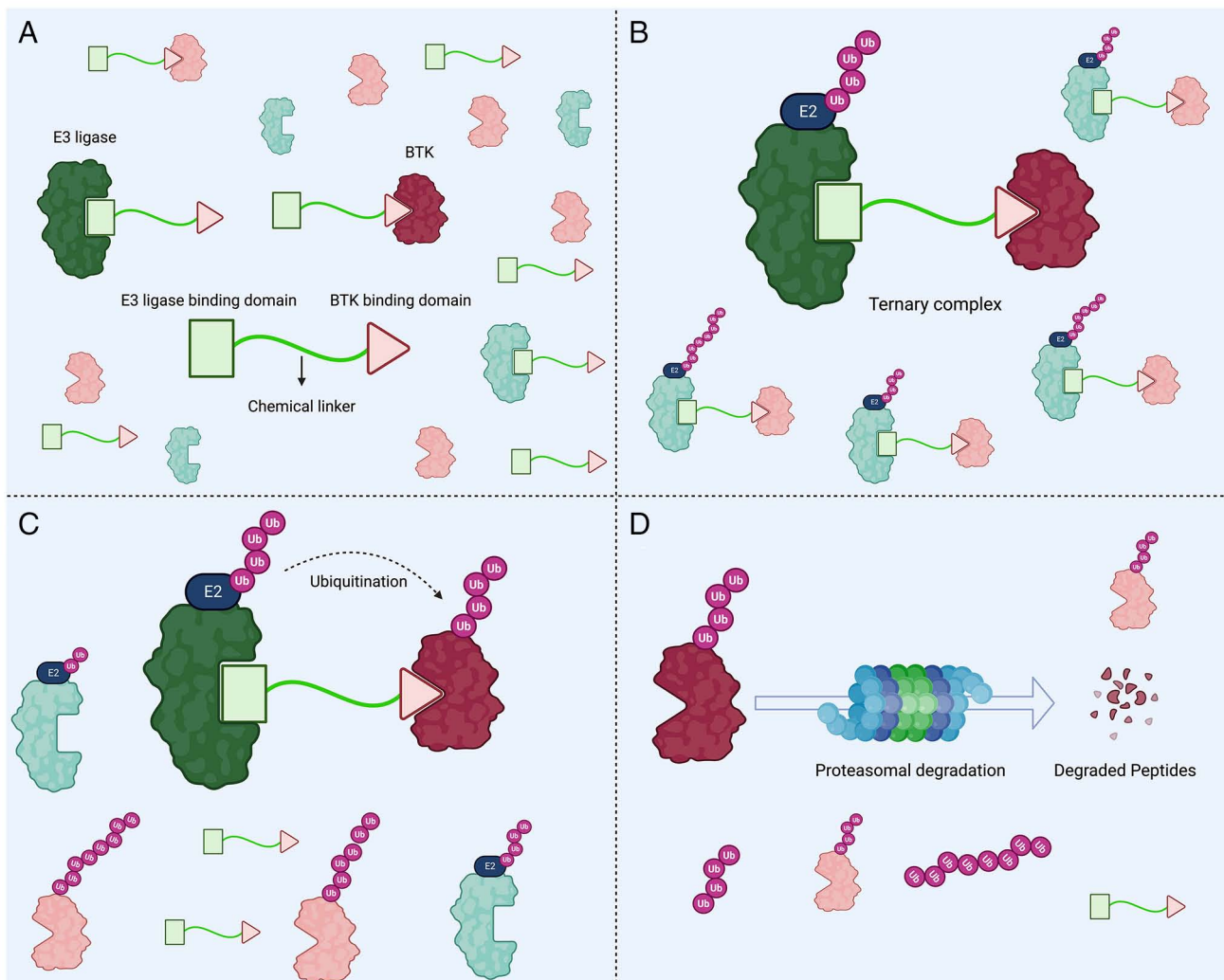


Figure 4. Mechanism of BTK degradation by PROTACs. (A) A BTK PROTAC is a heterobifunctional molecule composed of three parts: An E3 ligase binding domain, a BTK binding domain and a chemical linker. (B) The PROTAC simultaneously recognizes and binds BTK protein regardless of clinically relevant resistance mutations and an E3 ubiquitin ligase, bringing the two proteins into close proximity to form a ternary complex. (C) This ternary complex facilitates the transfer of Ub from the E2 conjugating enzyme to BTK. (D) The polyubiquitinated BTK is then precisely degraded by the proteasome system. Created with BioRender.com. BTK, Bruton's tyrosine kinase; E2, ubiquitin-conjugating enzyme; E3, ubiquitin ligase; PROTAC, proteolysis-targeting chimera; Ub, ubiquitin.

harboring the BTKC481S mutation, NX-5948 achieved superior tumor growth inhibition compared with ibrutinib (123). An ongoing first-in-human phase I trial (trial registration no. NCT05131022) is evaluating the safety and efficacy of NX-5948 in patients with R/R B-cell malignancies. Early enrollment included 10 patients with B-cell NHL, four of whom had DLBCL; half of the patients had received prior BTKi therapy. The initial findings suggest that NX-5948 is generally well tolerated and the most common TEAEs were purpura or contusion (57.1%, all below grade 3), nausea (35.7%, all below grade 3), and thrombocytopenia (35.7%, all below grade 3). Similar to NX-2127, rapid, robust and sustained BTK degradation was observed across all patients (124). Clinical response data for the patients with DLBCL were not available.

BGB-16673 is another orally active BTK-targeting chimeric degradation activation molecule. In preclinical models, it degrades both WT BTK and multiple mutant forms linked to resistance against conventional BTKi. Consequently, BGB-16673 exerts potent anti-proliferative activity in TMD8

cells expressing WT BTK or harboring C481S, T474I or L528W mutants and suppresses xenograft tumor growth driven by these on-target resistance mutations. In these preclinical models, BGB-16673 produced more prolonged tumor control and fewer splenic metastases than ibrutinib or pirtobrutinib. In a first-in-human phase I/II study, BGB-16673 monotherapy has demonstrated a tolerable safety profile and robust anti-tumor responses that deepened over time in heavily pretreated patients with R/R B-cell malignancies (NCT05006716) (125). Building on these results, an ongoing phase I/II trial is evaluating the safety, tolerability and clinical activity of BGB-16673 in combination with other regimens in the R/R setting (NCT06634589). The non-GCB DLBCL substudy of this trial aims to assess BGB-16673 plus glofitamab, a bispecific antibody targeting both CD20 on B cells and CD3 on T cells, in patients with R/R non-GCB DLBCL who have received ≥ 2 prior lines of therapy. The study is currently recruiting participants (126). Detailed clinical trial information for these degraders is summarized in Table II.

Table II. Clinical trials of emerging BTK-targeted and multi-kinase agents in B-cell malignancies.

Category/mechanism of action/ representative agents	Clinical trial identifier	Phase	Patient Population	Regimens
Dual-binding BTKi; engaging both WT and C481-mutant BTK through covalent and non-covalent binding modes				
Rocbrutinib (LP-168)	NCT04993690	I	R/R CLL/SLL, DLBCL, MCL, MZL, FL, PMBCL	Monotherapy
Rocbrutinib (LP-168)	NCT06251180	I	TN DLBCL, MZL, MCL	Rocbrutinib + R-CHOP
Rocbrutinib (LP-168)	NCT07189065	II	R/R DLBCL	Monotherapy
Rocbrutinib (LP-168)	NCT07570017	I	R/R DLBCL	Rocbrutinib + R-GemOx
BTK degrader; targeted degradation of WT and mutant BTK proteins via the ubiquitin-proteasome system				
Zelevbrudomide (NX-2127)	NCT04830137	I	R/R CLL/SLL, DLBCL, FL, MCL, MZL, WM	Monotherapy
Bexobrutideg (NX-5948)	NCT05131022	I	R/R CLL/SLL, DLBCL, MCL, MZL, FL, WM, PCNSL/SCNSL	Monotherapy
Catadegbrutinib (BGB-16673)	NCT05006716	I/II	R/R CLL/SLL, WM, MCL, MZL, DLBCL, FL, RT	Monotherapy
Catadegbrutinib (BGB-16673)	NCT05294731	I/II	R/R CLL/SLL, MZL, MCL, FL, WM, DLBCL, RT	Monotherapy
Catadegbrutinib (BGB-16673)	NCT06634589	I/II	R/R CLL/SLL, FL, MZL, MCL, DLBCL, WM, RT	Catadegbrutinib + sonrotoclast/ zanubrutinib/ mosunetuzumab/ glofitamab/ obinutuzumab
HSK29116	NCT04861779	I	R/R CLL/SLL, MCL, DLBCL, FL, MZL, WM	Monotherapy
AC676	NCT05780034	I	R/R CLL/SLL, DLBCL, FL, MCL, MZL, WM	Monotherapy
ABBV-101	NCT05753501	I	R/R CLL/SLL, DLBCL, MCL, FL, MZL, WM, transformed iNHL	Monotherapy
UBX-303061	NCT06590961	I	R/R CLL/SLL, DLBCL, FL, MCL, WM, MZL	Monotherapy
HZ-Q1070	CTR20240471	I	R/R CLL/SLL, WM, MCL, FL, MZL, DLBCL, PCNSL	Monotherapy
Allosteric BTKi; non-active site binding with kinase inhibition	No clinical studies identified			
Dual and Multi-Target BTKi; simultaneously blocking BTK and other survival-driving kinases				
Birelentinib (DZD8586, BTK/LYN)	NCT05844956	I	R/R CLL/SLL, DLBCL, MCL, FL, MZL, SCNSL	Monotherapy
Birelentinib (DZD8586, BTK/LYN)	NCT05824585	I	R/R CLL/SLL, DLBCL, MCL, FL, MZL, SCNSL	Monotherapy
Birelentinib (DZD8586, BTK/LYN)	NCT06539195	II	R/R DLBCL	Monotherapy
Birelentinib (DZD8586, BTK/LYN)	NCT07059650	II	TN and R/R DLBCL	Birelentinib + R-CHOP/ R-GemOx/BR

Table II. Continued.

Category/mechanism of action/ representative agents	Clinical trial identifier	Phase	Patient Population	Regimens
Luxetpinib (CG-806, BTK/FLT3)	NCT03893682	I	R/R CLL/SLL, B-NHL	Monotherapy
Cerdulatinib (PRT062070, SYK/JAK)	NCT01994382	I/II	R/R CLL/SLL, NHL	Cerdulatinib ± rituximab

B-NHL, B-cell NHL; BR, bendamustine plus rituximab; BTK, Bruton's tyrosine kinase; BTKi, BTK inhibitor; CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; FLT3, FMS-like tyrosine kinase 3; iNHL, indolent NHL; LYN, Lck/Yes-related novel protein tyrosine kinase; MCL, mantle cell lymphoma; MZL, marginal zone lymphoma; NHL, non-Hodgkin lymphoma; PCNSL, primary central nervous system lymphoma; PMBCL, primary mediastinal large B-cell lymphoma; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone; R-GemOx, rituximab, gemcitabine and oxaliplatin; R/R, relapsed or refractory; RT, Richter transformation; SCNSL, secondary central nervous system lymphoma; SYK, spleen tyrosine kinase; TN, treatment-naïve; WM, Waldenström macroglobulinemia; WT, wild-type.

Overall, BTK degraders have retained activity against several resistance-associated BTK variants in preclinical models. Early-phase studies have also reported clinical activity in heterogeneous B-cell malignancy cohorts, but DLBCL-specific clinical evidence remains limited. Furthermore, evidence from other B-cell malignancies indicates that the BTKA428D mutation may confer resistance to BGB-16673 and NX-2127 (127). BTK degraders are therefore not universally active across resistance-associated BTK variants, and their role in BTKi-resistant DLBCL remains to be defined.

However, Hatcher *et al* (128) developed DFCI-002-06, a first-in-class HCK/BTK PROTAC for MYD88-mutated B-cell malignancies. Liu *et al* (129) subsequently showed that, in DLBCL cells expressing the BTKA428D mutation, DFCI-002-06 degrades HCK, LYN and BTK in parallel, promoting apoptosis in cells that are resistant to other investigational PROTACs, including BGB-16673, NX-2127 and NX-5948. These preclinical findings support further evaluation of DFCI-002-06 in BTK-targeted therapy resistance. In experimental models, combining BTK degraders with venetoclax delayed the emergence of BTKA428D-resistant clones, while NX-2127 suppressed both WT and A428D clones (130). The clinical relevance of this combination remains unknown.

BTK degraders therefore remain investigational strategies for addressing resistance to conventional BTKi. The identification of BTKA428D supports prospective evaluation of mutation profiles and treatment sequencing in future clinical studies. These efforts may help expand the potential future applications of these novel drugs.

Allosteric BTKi. Allosteric BTK inhibition is also being explored as a potential approach to resistance associated with acquired BTK mutations (131,132). Allosteric inhibitors are designed to perturb allosteric sites on BTK rather than the ATP binding active site within the catalytic kinase domain that traditional inhibitors target. Through a network of coupled amino acids, these perturbations are transmitted to the active site (orthosteric site), causing a conformational change in BTK. This in turn suppresses BTK kinase activity and blocks the interaction of BTK with downstream substrates required for signal propagation (131,133).

The mechanisms that govern allosteric regulation of BTK are diverse. They include interdomain communication, kinase dimerization, protein-protein interactions, post-translational modifications and ligand binding (131). The interdomain interactions that guide BTK through its activation cycle, from a cytosolic autoinhibited state to membrane-associated and fully activated conformations, have facilitated the identification and understanding of allosteric sites on BTK (134,135). Allosteric inhibitors fall into two major categories: Type III inhibitors, which bind adjacent to the ATP active site without overlapping it and type IV inhibitors, which bind a site remote from the ATP pocket (131). This mode of action provides a mechanistic rationale for testing allosteric inhibitors against mutations affecting the orthosteric site. In BTK, a negatively charged side chain, D656, provides a binding interface for the SH2 domain. Engagement of the SH2 domain with the kinase domain induces the autoinhibited conformation. Conversely, the SH2 domain can also activate the catalytic domain by binding to the N-lobe of the kinase domain (131). Targeting the SH2-kinase domain interface has been established as a primary strategy for allosteric BTK inhibition. Building on this concept, a protein binder named rF10 has been developed in preclinical studies. It displays high specificity for the BTK-SH2 domain over other SH2-containing kinases, and has the ability to inhibit activation of both WT and mutated BTK (136,137).

In parallel, Kueffer *et al* (132) carried out virtual screening against non-active site pockets combined with *in vitro* experiments. The authors identified a series of small molecules that bind BTK outside the ATP active site. These proof-of-principle data demonstrate that BTK can be targeted allosterically with small molecules, offering an alternative to active-site-directed BTKi. Therefore, allosteric sites on BTK deserve exploration for drug development with the goal of achieving more specific BTK targeting and enabling combination with active-site inhibitors. However, the available evidence remains preclinical, and the relevance of allosteric BTK inhibition to BTKi-resistant DLBCL requires further investigation.

Dual and multi-target BTKi. Despite BTKi having profoundly reshaped the treatment paradigm for B-cell malignancies, the clinical use of single-target agents remains constrained by

the emergence and progression of acquired BTKi resistance. Recently, the development of dual or multi-target BTKi that simultaneously block BTK and other kinases has seen tangible progress, offering hope for improved therapeutic outcomes and sustained tumor responses in DLBCL. Multi-kinase inhibitors that target BTK, proximal BCR kinases or compensatory survival pathways are currently being investigated as potential approaches to BTKi resistance (33,138).

Birelentinib (DZD8586) is a novel oral non-covalent dual inhibitor of LYN and BTK. Preclinical activity data show that birelentinib selectively inhibits BTK and LYN with minimal off-target effects against other kinases. In DLBCL cell and xenograft models harboring BTK variants associated with resistance to acalabrutinib and pirtobrutinib, birelentinib retained antiproliferative and antitumor activity (139). Preliminary clinical results in R/R DLBCL showed an ORR of 48.4% and a CRR of 35.5%; however, outcomes according to prior BTKi exposure or resistance status were not reported (140).

Luxepitinib (formerly CG-806) is another oral non-covalent multi-kinase inhibitor that works in a mutation-agnostic manner. It targets pan-BTK, pan-FMS-like tyrosine kinase 3 and also suppresses BCR signaling at multiple nodes, including LYN and SYK. Compared with the classical BTKi ibrutinib, luxepitinib offers distinct advantages. Across a broad range of malignant B cells, luxepitinib suppresses cell proliferation and triggers apoptosis with much greater potency than ibrutinib. It also blocks aberrantly active BCR signaling at multiple nodes, including LYN, SYK, BTK, PLC γ 2, AKT, PKC and ERK, in a cell line-, dose- and time-dependent manner (141,142). Notably, luxepitinib reduces the phosphorylation of LYN and SYK more effectively than ibrutinib does, acting at steps further upstream in the BCR signaling cascade (143).

In addition, luxepitinib drives metabolic reprogramming in B-cell tumor cells. This is reflected by enhanced respiratory reserve capacity, increased glycolytic activity and induction of mitophagy in both parental and ibrutinib-resistant cells (144). The direct and potent killing of a wide range of malignant B cells supports the clinical development of luxepitinib for patients with B-cell malignancies that are insensitive, resistant or refractory to ibrutinib. A phase I clinical trial is currently evaluating the tolerability, antitumor activity and safety of luxepitinib as a single agent in patients with R/R B-cell NHL, nearly 50% of whom had prior exposure to BTKi. Early-phase results indicated a favorable safety profile and antitumor activity in a heavily pretreated population with multiple R/R B-NHL subtypes, including patients previously exposed to ibrutinib (145,146). However, DLBCL-specific enrollment, responses and outcomes after prior BTKi progression remained unreported.

KIN-8194 is another orally available dual-action BTK/HCK inhibitor with favorable bioavailability, pharmacokinetic properties and minimal toxicity (147). Yang *et al* (148) demonstrated that KIN-8194 exerts antitumor activity in MYD88-mutant driven B-cell lymphomas. Furthermore, it kills ABC-DLBCL cells carrying the BTKC481S mutation. In xenograft models of MYD88-mutant ABC DLBCL expressing either WT or C481S mutant BTK, KIN-8194 provided significantly improved survival benefits compared with ibrutinib. Combined with the BCL2 inhibitor venetoclax, it showed synergistic antitumor effects, markedly reducing

tumor growth and extending survival (149). These preclinical findings show that dual BTK/HCK inhibition retains activity in ibrutinib-resistant ABC-DLBCL models. Clinical activity in patients with BTKi-resistant DLBCL has not been evaluated to date. Further clinical development of this dual-target inhibitor is warranted to assess its antitumor activity and tolerability in patients with BTKi-resistant B-cell malignancies. Based on this, a first-in-class dual-targeting PROTAC, namely DFCI-002-06, was designed to concurrently degrade both HCK and BTK. It exerts kinase inhibitory activity irrespective of the functional phenotype of BTK mutations, as aforementioned. In mouse models, DFCI-002-06 achieved superior tumor suppression and prolonged survival compared with KIN-8194 (128).

SRX3333 is a triple-action inhibitor targeting BTK, PI3K and BRD4, which is currently under investigation. Previous *in vitro* studies have demonstrated that it suppresses the proliferation of malignant B cells while showing minimal toxicity to healthy lymphocytes, and it outperforms ibrutinib. Furthermore, SRX3333 triggers apoptosis in malignant B cells, and reduces resistance to cell death by attenuating BCR signaling and lowering MYC expression. SRX3333 reduced the proliferation of ibrutinib-resistant malignant B cells *in vitro*, indicating activity in preclinical resistance models. Its safety and efficacy in patients with BTKi-resistant DLBCL have not been evaluated yet (150).

Apart from the aforementioned dual or multi-target inhibitors, cerdulatinib (PRT062070), a dual SYK/JAK inhibitor, has shown preclinical activity in ibrutinib-resistant DLBCL (33). The preclinical findings and early clinical data from these multi-kinase inhibitors support further exploration as potential therapeutic strategies for addressing BTKi resistance. However, current clinical trials remain in early phases, and the exact efficacy and safety profile in patients with BTKi resistance require continued observation and validation.

Ongoing clinical trials evaluating these emerging BTK-targeted and multi-kinase agents are summarized in Table II. The aforementioned agents have shown activity in preclinical models or early clinical studies, but their role in BTKi-resistant DLBCL remains to be elucidated. Despite these advantages, several key questions in the current BTK-targeted therapy landscape remain unanswered. For example, the optimal sequencing of covalent and non-covalent inhibitors remains unknown. Whether resistance mutation profiles can guide treatment sequencing requires prospective evaluation in DLBCL. Even when a covalent BTKi is selected, head-to-head clinical evidence is lacking to determine whether acalabrutinib or zanubrutinib should be preferred. Available matching-adjusted indirect comparisons have shown mixed results, and are limited by cross-trial differences in baseline characteristics and other confounding factors. New covalent and non-covalent BTKi continue to be designed and developed besides the existing ones, raising the question of whether the best-in-class covalent and non-covalent BTKi are already available or if more are needed. Previous clinical findings have uncovered that the pan-resistant BTKA428D mutation confers co-resistance between a BTK inhibitor and a BTK degrader. This suggests that a simple switch from earlier-generation drugs to next-generation drugs may not be effective. It also prompts a rethinking of whether the focus for overcoming

BTKi resistance should shift to combination strategies. In other B-cell malignancies, fixed-duration combinations of BTKi and BCL2 inhibitors may delay the emergence of resistance mutations. Their ability to prevent or overcome BTKi resistance in DLBCL remains unproven (22,57,133). Prospective studies involving patients with documented progression during BTKi treatment, paired molecular samples and adequate follow-up are required.

5. Conclusions and future perspectives

The BCR signaling pathway plays a central role in the development and progression of B-cell malignancies. As a core node within this pathway, BTK has become a key therapeutic target. BTK-directed therapies have substantially improved tumor responses and clinical outcomes for patients with B-cell lymphomas. However, off-target toxicities that force treatment discontinuation, along with acquired resistance to BTKi, limit their broader clinical application. Therefore, a deeper understanding of the resistance mechanisms to BTKi is critically needed.

The present review summarized the mechanisms associated with reduced sensitivity to BTKi in DLBCL, ranging from subtype-associated intrinsic resistance to genetic and non-genetic mechanisms that may contribute to acquired resistance. At present, no candidate resistance biomarker discussed in the current review has been prospectively validated to guide routine genotyping or the sequencing of BTK-targeted therapies in DLBCL. Associations derived from retrospective clinical analyses remain exploratory. Mechanisms demonstrated in DLBCL cell lines or animal models, as well as those extrapolated from other B-cell malignancies, should be regarded as hypothesis-generating rather than clinically actionable.

Future studies should enroll molecularly characterized DLBCL cohorts, as well as collect paired pretreatment and progression samples for longitudinal analysis. Prospective molecularly selected studies with adequate follow-up are needed to determine whether resistance genotyping or specific treatment sequences improve clinical outcomes. Until such evidence becomes available, these mechanisms and emerging therapeutic strategies should inform study design rather than routine treatment decisions.

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Availability of data and materials

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Authors' contributions

CL conceived the review and drafted the manuscript. CL, TZ, WZ and LW performed the literature search and screening, and contributed to evidence collection, organization and synthesis. TZ, WZ and LW also contributed to drafting and revising the manuscript. LW, CW, QC and LY contributed to funding acquisition. QC and YS made contributions to the analysis and interpretation of the literature relevant to the figures and tables, including the organization and synthesis of mechanistic evidence, and prepared the figures and tables. QC and YS were also involved in critically revising the manuscript for important intellectual content. CW and LY critically revised the manuscript and were responsible for correspondence regarding the manuscript. Data authentication is not applicable. All authors read and approved the final manuscript, and agree to be accountable for all aspects of the work.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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