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REVIEW

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Strategies for overcoming resistance to Bruton's tyrosine kinase inhibitor zanubrutinib

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Abstract

Bruton's tyrosine kinase (BTK) inhibitors have revolutionized the treatment of B-cell malignancies. They target BTK, a key effector in the B-cell receptor (BCR) signaling pathway, crucial for B-cell survival and proliferation. The first-in-class irreversible BTK inhibitor, ibrutinib, was approved for various B-cell malignancies but has limitations due to off-target effects. Second-generation inhibitors, such as acalabrutinib and zanubrutinib, offer improved selectivity and reduced side effects. However, resistance to BTK inhibitors, driven by BTK mutations, remains a challenge. Combinatorial therapies with PI3K inhibitors, immune checkpoint inhibitors, BH3 mimetics, and anti-CD20 antibodies show promise in overcoming resistance. Noncovalent BTK inhibitors and proteolysis-targeting chimeras (PROTACs) are emerging strategies with potential to combat resistance. Overall, advancements in BTK-targeted therapies provide hope for improved outcomes in patients with B-cell malignancies and a promising avenue to address drug resistance. Further research is needed to optimize combination therapies and identify optimal treatment regimens.

KEYWORDS
BTK, inhibitor, resistance, zanubrutinib

1 | INTRODUCTION

Resistance to Bruton's tyrosine kinase (BTK) inhibitors is a growing concern in the field of cancer treatment. Despite the success of these drugs in treating hematologic malignancies, many patients eventually develop resistance, leading to treatment failure and disease progression.[1–3] Combinatorial therapy has emerged as a promising strategy for overcoming BTK inhibitor resistance and improving patient outcomes. We review the mechanisms of BTK inhibitor resistance and discuss the potential of combinatorial therapy including next-generation inhibitor zanubrutinib as a strategy for avoiding resistance and improving cancer treatment.

BTK inhibitors are effective in selected B-cell malignancies. Their target, the non-receptor tyrosine kinase BTK, is a key effector in the B-cell receptor (BCR) signaling pathway.[4] Once activated, BTK mediates the phosphorylation of phospholipase C-γ2 and affects downstream signaling pathways driven by the release of calcium ions. Transcription factors activated by the BCR pathway allow the cell to proliferate and differentiate. Because of their critical role in B-lymphocyte survival, kinases involved in BCR signaling network are attractive targets in B-cell malignancies.

2 | CLINICALLY APPROVED COVALENT BTK INHIBITORS

Ibrutinib, a first-in-class irreversible BTK inhibitor, was approved by the FDA for mantle cell lymphoma (MCL) and chronic lymphocytic leukaemia (CLL) in 2013 and 2014, respectively, and its indication was further expanded to include small lymphocytic lymphoma (SLL),

Waldenström macroglobulinemia (WM), marginal zone lymphoma (MZL) and chronic graft versus host disease. The introduction of ibrutinib in the treatment of B-cell malignancies has offered a chemotherapy-free regimen, improved the safety profile and reduced treatment-related toxicities in patients.[5]

Ibrutinib covalently (irreversibly) interacts with cysteine 481 in the ATP-binding pocket of BTK. By intervening ATP binding, the activity of BTK is suppressed, thereby interfering with the BCR signaling pathway and inhibiting proliferation of tumor cells.[6] BTK also modulates chemokine receptor (CXCR4) signaling, resulting in impaired cell homing. Consequently, inhibition of homing and re-homing leads to redistribution of tumor cells from the tissue to the peripheral blood and promotes lymphocytosis.[7,8] Although ibrutinib has made a significant contribution to the chemotherapy-free treatment of hematological malignancies, its associated side effects remain a concern. Some patients have to discontinue treatment due to evolving side effects stemming from the off-target activity. The binding site in BTK is not strictly conserved and the same druggable amino acid residue can be found in other kinases. Among these, kinases of the EGFR, SRC and TEC families are potentially responsible for adverse effects (AE) such as atrial fibrillation, rash, diarrhea or bleeding.[9–11] Novel inhibitors with more efficient BTK kinase specificity are therefore being sought and explored.

Acalabrutinib is a second-generation BTK inhibitor designed to improve the selectivity and safety profile of BTK-targeted treatment. It was first approved in 2017 and has since been used in both monotherapy in MCL, CLL, SLL. Acalabrutinib inhibits only three other kinases with submicromolar IC_{50} values (EGFR, BMX, TEC).[12] Due to its higher selectivity, the frequency of side effects in patients is reduced compared to ibrutinib. Adverse events did not recur in more than a third of patients who were previously intolerant to ibrutinib.[13] As a result, patients on acalabrutinib were less likely to discontinue treatment than those on ibrutinib in a head-to-head clinical trial.[14]

Zanubrutinib is another second-generation BTK inhibitor approved by the FDA in 2019. This novel BTK-targeting agent is used as monotherapy or in combination with other drugs in CLL/SLL, WM, follicular lymphoma (FL), MZL and MCL. Like other covalent BTK inhibitors, zanubrutinib irreversibly binds to cysteine 481 in BTK. In a comparative biochemical studies on BTK inhibitors, zanubrutinib displays similar potency as ibrutinib. Compared to acalabrutinib, zanubrutinib inhibits several other kinases (EGFR family) at clinically significant levels, which may contribute to AEs in some patients. Nonetheless, the overall selectivity profile of zanubrutinib remains significantly improved and more favorable than that of ibrutinib.[15]

In head-to-head clinical trials with ibrutinib, zanubrutinib was associated with significantly lower cardiotoxicity, consistent with the in vitro results.[16,17] Overall, zanubrutinib demonstrates superiority to ibrutinib therapy, exhibiting longer progression-free survival in CLL patients[18] and better response and safety in patients intolerant to both ibrutinib and acalabrutinib.[19] However, no study to evaluate and compare efficacy between zanubrutinib an acalabrutinib has been published yet.

3 | MECHANISMS OF COVALENT BTK INHIBITOR RESISTANCE

Even though the ibrutinib treatment demonstrated a good safety profile and solid efficacy in high-risk CLL/SLL patients in long-term follow-up study,[20] resistance (namely secondary resistance) remains an Achilles' heel of the treatment. Next-generation covalent BTK inhibitors such as zanubrutinib may improve tolerability and reduce toxicity, but they still fail to prevent resistance from developing, leading to disease progression. As patients are continuously exposed to the drugs, spontaneous mutations, conferring resistance to treatment. Through the selection of favorable mutations, the tumor population becomes enriched with these cells, ultimately leading to the development of drug resistance.[21]

Resistance in patients treated with novel BTK inhibitors is less well described than for ibrutinib. While all approved covalent inhibitors irreversibly bind to cysteine 481, the mechanisms of resistance share common features. In general, unresponsiveness to BTK inhibitors can result from both primary and acquired resistance. Intrinsic resistance may result from pre-existing genetic or epigenetic alterations in key proteins and pathways, which bypass BTK inhibition and contribute to cancer cell survival. However, the mechanism is not yet well understood.[1]

Acquired (secondary) resistance typically arises through mutations in the *BTK* gene or other genes in the BCR signaling pathway. It manifests in patients who initially respond to treatment but relapse or progress with prolonged therapy. In CLL patients, primary resistance to ibrutinib occurs in 10%–16% of cases, and secondary resistance develops in 10%–18% of patients who progress.[2] Both primary and secondary resistance are more common in patients with MCL. Primary resistance is observed in up to 35% of cases, and many others relapse or progress with acquired resistance (17%–54%).[22] There are much fewer studies and less information available for novel BTK inhibitors, but according to the latest reports, from 12% to 15% of CLL patients develop acquired resistance to acalabrutinib and zanubrutinib, based on mutations previously associated with ibrutinib resistance.[3,23]

BTK point mutations have been identified as a common mechanism of acquired resistance to ibrutinib, zanubrutinib and acalabrutinib. Several studies have demonstrated the prevalence of C481S mutations in patients who have received ibrutinib therapy.[24,25] This most common mutation occurs in the BTK binding site, reducing the binding affinity of BTK inhibitors to the protein. Other point mutations in the *BTK* gene have also been identified both in vitro and in patients, potentially acting as resistance mechanisms.[26]

The C481F/Y/R/G, T474I/S, L528W, T316A, V537I, G164D, R490H, Q516K, R28S, A428D, M437R and V416L mutations have been observed in patients with progressive disease (Figure 1).[2,27–30] Only a few of these mutations have been functionally studied, and their clinical significance in the context of resistance to both reversible and irreversible BTK inhibitors may vary. For example, in the study by Wang et al, of the non-C481 BTK mutations, only A428D and L528W conferred impaired binding of covalent inhibitors,

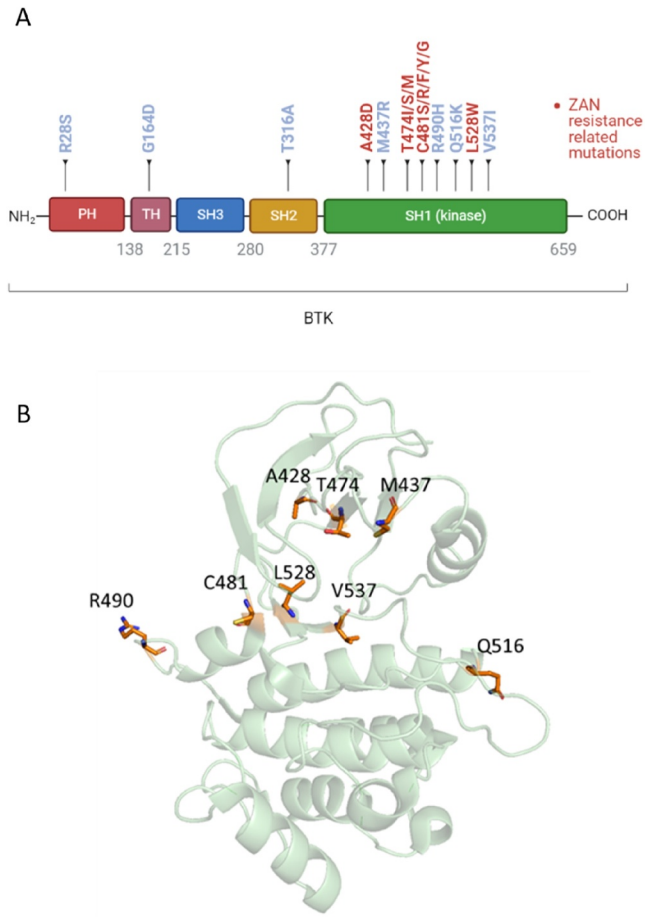

FIGURE 1 Positions of known point mutations in the whole Bruton's tyrosine kinase (A) and its SH1 (kinase) domain (B) contributing to zanubrutinib resistance.

whereas others (V416L, M437R and T474I) only affected non-covalent drugs.[30]

Estupinan et al. identified three double mutations that confer super-resistance to all three irreversible inhibitors used in clinical practice - T474I/C481S, T474M/C481S and T474M/C481T.[31] Interestingly, T474I/C481S and T474S/C481S have also been observed in patients treated with ibrutinib.[32,33]

While C481S does not influence enzymatic activity and partial PLCγ2 signaling,[34] C481F/R, L528W, V416L, M437R and A428D mutants nearly completely loss their activity.[35] However, except C481F/R, all of the other mentioned kinase-dead mutations show active downstream signaling, independent of PLCγ2. This may occur through observed interaction of non-C481 mutated BTK with LYN and HCK kinases.[34] This way, downstream BCR signaling is still activated with PLCγ2-dependent pathway being bypassed. These findings should be taken in consideration as possible mechanism and contribution to developed resistance toward both covalent and noncovalent inhibitors.

In patients treated with zanubrutinib, C481S/R or L528W mutations in BTK have been identified and reported as potentially responsible for resistance.[36,37] Some patients may harbor both mutations at once, which has been reported previously in patients treated with ibrutinib.[33] L528W is the predominant mutation in zanubrutinib-treated patients who progressed the disease (54%), whereas it is a quite rare in patients treated with ibrutinib (4%).[38] The mechanism by which L528W contributes to resistance to BTK inhibitors has been described for ibrutinib. The mutation in L528 forms a steric barrier for the interaction of ibrutinib with C481, thereby increasing the free energy for the covalent inhibitor to bind to the active site.[39] The impaired binding affinity of zanubrutinib has also been observed in *in vitro* models of A428D and T474I mutants,[30] suggesting that these changes may contribute to zanubrutinib resistance in some cases.

Downstream gain-of-function mutations in PLCG2 (R665W, S707Y, L845F), known from prolonged exposure to ibrutinib, also play a role in the development of resistance and have been described in clinical patients as well. Acquired mutations in the *PLCG2* gene have recently been described in patients treated with zanubrutinib and acalabrutinib.[23,40] Based on experience with ibrutinib therapy, these mutations may contribute to potential resistance to zanubrutinib. A recent report confirmed also TP53 and CARD11/BCL10 mutations in patients treated with zanubrutinib; they are associated with poor prognosis and worse response to BTK inhibitors.[36]

4 | COMBINATORIAL THERAPY AS A STRATEGY FOR AVOIDING RESISTANCE

Combinatorial therapy may help to overcome BTK inhibitor resistance by targeting alternative signaling or cancer cell vulnerabilities that are not affected by BTK inhibitors alone. Several preclinical and clinical studies have demonstrated the potential of combinatorial therapy to overcome BTK inhibitor resistance, including combinations with other targeted therapies.

4.1 | Combinations with PI3K inhibitors

Combining BTK and PI3K inhibitors has been proposed as a potential strategy to overcome resistance and improve treatment outcomes in patients with B-cell malignancies. The BTK and PI3K signaling pathways are both important for BCR signaling, which plays a critical role in the survival and proliferation of B-cell malignancies. BTK inhibitors such as zanubrutinib block BCR signaling by inhibiting the downstream kinase BTK. However, BTK inhibitors may not completely abrogate BCR signaling, and resistance can develop through activation of alternative signaling pathways. PI3K inhibitors, on the other hand, target a different downstream protein of the BCR pathway and may complement the activity of BTK inhibitors.[25]

PI3K contributes to signal transfer in BCR signaling pathway where it is tightly bound to BTK activity. When the BCR receptor is stimulated, PI3K is activated. The enzyme phosphorylates PI(3,4)P2 to generate PI(3,4,5)P3, which serves as a ligand for the PH domain of BTK and enables attachment of the kinase to the membrane. Once bound via PIP3, BTK can be fully activated via transphosphorylation,[4] and further mediates signal transfer downstream the BCR pathway.

In contrast to BTK downstream signaling, PI3K-dependent signaling involves different pathways (AKT/mTOR, Akt/Raf) leading to the activation of transcription factors in the nucleus (Figure 2). This action complements the activity of BTK in B-cells, which provides cancer cells with critical pro-survival signals.[41]

Hence, PI3K emerges as another promising targed, particularly in CLL. While PI3K inhibitors are less frequently employed in treatment due to their higher toxicity, ongoing research focuses on developing more selective and safer inhibitors. For instance, BGB-10188, a novel highly selective PI3K inhibitor,[42] is currently under investigation in a phase 2 clinical trial in patients with relapsed/refractory (R/R) mature B-cell malignancies. In addition to monotherapy, the trial includes combination with zanubrutinib (Table 1). As both pathways work in synergy, there is a potential benefit in inhibiting two components that mediate cell proliferation and survival. However, further studies are needed to fully assess the safety and efficacy of these combinations.

The efficacy of such a combination may be further improved by adding an anti-CD20 agent to the regimen. The combination of the PI3K inhibitor idelalisib and the monoclonal antibody rituximab showed improved response rate and overall survival in CLL patients.[43] However, idelalisib was withdrawn from use in FL and SLL. Among the FDA-approved BTK inhibitors, acalabrutinib is being studied in combination with the PI3K inhibitor umbralisib and the anti-CD20 antibody ublituximab.[44] Combining more pathways at once could potentially extend the treatment options with zanubrutinib to help overcome resistance.

4.2 | Combinations with immune checkpoint inhibitors

Simultaneous targeting of BTK and immune checkpoint proteins has been proposed as a potential strategy to enhance the anti-tumor immune response and improve treatment outcomes and responses in patients with B-cell malignancies. The immune checkpoint protein PD-1 and its ligands PD-L1 and PD-L2 are expressed on malignant B cells and contribute to tumorigenesis and cell survival in several cancers. Anti-PD-1 and PD-L1 antibodies are key players in cancer immunotherapy, with eight agents approved by the FDA to date. In the search for other inhibitors of the PD-1 pathway to improve treatment outcomes, the kinase ITK has been identified as a key component of the pathway. Inhibition of ITK results in impaired PD-1 function, thereby enhancing the effect of immune checkpoint antibody monotherapy.[45] ITK is a member of the TEC kinase family, plays a role in the immune response and, most importantly, is also sensitive to BTK inhibitors.[46] Thus, in addition to inhibiting BTK in malignant B cells, treatment with BTK inhibitors can also suppress the function of ITK.

Previous preclinical studies have shown that the combination of BTK inhibitors with immune checkpoint inhibitors has the potential to make a positive contribution to the therapy of hematological malignancies.[47] The study by Long et al. reported ibrutinib as a promising T-cell immunomodulatory agent in CLL patients, helping to overcome immune suppression in these patients. The observed effects are proposed to be the result of off-target inhibition of ITK. In comparison, acalabrutinib showed no efficacy in inducing an immune response due to its impaired ability to inhibit ITK-driven signaling.[48] Zanubrutinib is 20-fold less potent in ITK inhibition than the less selective ibrutinib, but more potent than acalabrutinib.[49] Thus, the synergistic effect of combining PD-1/PD-L-1 antibodies and BTK inhibitors that target both BTK and ITK may be beneficial in indicated patients.

A combination of immune checkpoint inhibitors and BTK inhibitors has also been proposed for PCNSL patients.[50] Targeted therapy for PCNSL patients includes both PD-1/PD-L1 antibodies

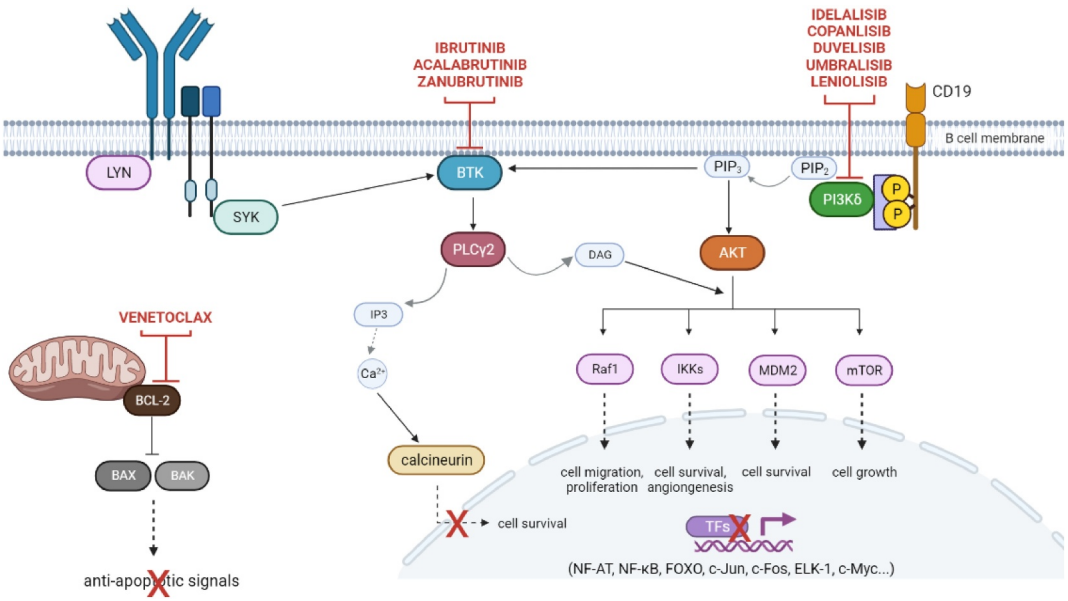

FIGURE 2 BCR signaling and potential therapeutical targets. BTK inhibitors can be used in combination with PI3K inhibitors, targeting two diverse branches of the signaling pathway simultaneously and deepening the anti-cancer effect of the treatment. Aside from components of BCR signaling, BH3 mimetics act in synergy with BTK inhibition, sensitizing the cells toward the treatment.

TABLE 1 Clinical trials of zanubrutinib in combination with other targeted drugs.

Target	Combination drug	Identifier	Status	Phase	Condition
CD20	Rituximab	NCT05735834	Recruiting	Phase 3	Splenic marginal zone lymphoma
		NCT05635162	Not yet recruiting	Phase 2	Indolent mantle cell lymphoma
		NCT04458610	Recruiting	Phase 2	Chronic lymphocytic leukemia/Small lymphocytic lymphoma
		NCT04002297	Active	Phase 3	Mantle cell lymphoma
		NCT03520920	Completed	Phase 2	R/R diffuse large B-cell lymphoma (Non-GCB subtype) and R/R indolent lymphoma
	Obinutuzumab	NCT02569476	Completed	Phase 1b	B-cell lymphoid malignancies
		NCT03332017	Active	Phase 2	R/R follicular non-hodgkin lymphoma
PD-1	Tislelizumab	NCT04705129	Recruiting	Phase 2	R/R primary mediastinal large B-cell lymphoma and epstein-barr virus-positive diffuse large B-cell lymphoma
		NCT04271956	Active	Phase 2	Richter transformation
		NCT02795182	Completed	Phase 1b	B-cell lymphoid malignancies
BCL-2	Venetoclax	NCT05650723	Recruiting	Phase 2	Chronic lymphocytic leukemia
		NCT05168930	Recruiting	Phase 2	Chronic lymphocytic leukemia (CLL) or small lymphocytic lymphoma (SLL)
	BGB-11417	NCT04277637	Recruiting	Phase 1a/1b	Mature B-cell malignancies
PI3Kδ	BGB-10188	NCT04282018	Recruiting	Phase 1/2	R/R follicular lymphoma, R/R mantle cell lymphoma or R/R diffuse large B-cell lymphoma

and BTK inhibitors (ibrutinib), suggesting that combining these agents may be beneficial to improve treatment outcomes.

Based on previous results with ibrutinib and the effect on ITK kinase described above, clinical trials of zanubrutinib in combination with the PD-1 antibody tislelizumab have been initiated (Table 1). The combination of these anti-BTK and anti-PD-1 agents may overcome treatment difficulties in patients with Richter's transformation of CLL and other relapsed or refractory B-cell lymphoid malignancies.[51] The combination of zanubrutinib and tislelizumab has been already shown to be beneficial for a subset of DLBCL patients harboring specific gene alterations. Patients with *PD-L1* gene amplification, high-level PD-L1 tumor cells, and high mRNA levels of genes involved in the immune system, CD3D, HLA-DRA, and LAG3, may be more likely to respond to this combination therapy.[52]

4.3 | Combinations with BH3 mimetics

BCL-2 is a member of the BCL-2 protein family and belongs to the group of anti-apoptotic proteins. Its dysregulation plays a critical role in the pathogenesis of B-cell lymphomas and leukaemias, making it an attractive target in these malignancies. As a prosurvival protein, BCL-2 inhibits the action of pro-apoptotic proteins. BCL-2 prevents the oligomerisation-dependent activation of BAK/BAX, which leads to permeabilisation of the mitochondrial outer membrane. Inhibition blocks the subsequent release of cytochrome c from into the cytoplasm, a necessary step for caspase-mediated cell death.[53]

BH3 mimetics are a class of small molecule drugs that antagonise BCL-2 proteins. These compounds act in a supportive manner to induce BAK/BAX activation, blocking the inhibitory interaction of the BH3 domain of pro-apoptotic proteins with the hydrophobic pocket of anti-apoptotic proteins, thus shifting the balance of apoptosis homeostasis in favor of tumor cell death.[53] Based on their pro-apoptotic effect in cancer cells, BH3-mimetics have become an important part of therapies in patients with CLL, AML.

In ibrutinib monotherapy, other mechanisms besides from point mutations mediate patients' unresponsiveness. Among these, upregulation of BCL-2 can contribute to resistance in spite of inhibition of BTK.[54] The use of BH3 mimetics such as venetoclax sensitises CLL and MCL patients to BTK inhibitors, and although they target distinct subpopulations of CLL cells,[55] such drug combination can effectively bypass resistance.[56] A recent report investigated co-treatment of venetoclax with ibrutinib or acalabrutinib in patients with aggressive DLBCL with high expression of BCL-2. Such combination therapy may be potentially beneficial as the study demonstrated a sensitising effect of BTK inhibitors on venetoclax-treated cells. The addition of BTK inhibitors to BH3 mimetics therapy leads to faster tumor regression in the cells with both primary and acquired resistance to venetoclax.[57]

Based on successful outcomes with ibrutinib and acalabrutinib, studies on the combination of the novel inhibitor zanubrutinib and BH3 mimetics have been initiated. Potential combination of zanubrutinib with FDA approved venetoclax is studied in two clinical trials (Table 1). In addition, co-treatment with a novel Bcl-2 inhibitor BGB-11417 is being investigated (Table 1).[58]

4.4 | Combinations with anti-CD20 monoclonal antibodies

CD20 is a glycosylated protein expressed on both healthy and malignant B-cells, interacts with BCR and is involved in downstream signaling of the BCR pathway.[59] The physiological activity and cooperation of CD20 and BCR together play a critical role in healthy humoral immunity. Targeting both components suggests advantages in the therapy of B-cell malignancies.

Trials of combinations of BTK drugs and anti-CD20 antibodies are ongoing, although some previous studies have shown no benefit of adding monoclonal antibodies to ibrutinib therapy in CLL patients.[60] Ibrutinib in higher doses in vitro has been shown to antagonise the cytotoxic effect of FDA-approved rituximab via several mechanisms.[61] The effect of ibrutinib on NK cells and its inhibitory effect on anti-CD20 antibodies is believed to arise from its off-target ITK, which is involved in NK cells fate and survival.[62] Moreover, BTK inhibition affects CXCR4 signaling in CLL cells resulting in suppressed CD20 expression in CLL cells.[63] This observation may explain why co-treatment with ibrutinib and rituximab provided no significantly improved outcomes in CLL patients as was showed in recent trials.[64,65] This obstruction can be solved by combining BTK inhibitor with more potent and cytotoxic anti-CD20 antibody, such is obinutuzumab, which is not affected by lower CD20 levels. Concomitant treatment with ibrutinib has been shown to be an effective and safe chemotherapy-free option for previously untreated CLL and SLL patients.[66] In a study combining acalabrutinib with obinutuzumab, the therapy improved 5-year progression-free survival in CLL patients.[67] A similar benefit may be expected from combination with the next-generation drug zanubrutinib and obinutuzumab. The trial combining these two agents has already been initiated in patients with FL and CLL.[68]

With regard to the therapy of Waldenström's macroglobulinemia, a recent study investigating the combination of ibrutinib and rituximab suggests that such a tandem may hold promise. Treated patients had higher response rates than those treated with anti-CD20 monotherapy. Improved outcomes were observed in patients with both wild-type and mutated MYD88 and/or CXCR4. Based on these results, patients with CXCR4 mutation with poor response to ibrutinib and other ibrutinib-resistant patients may benefit from such a combination.[69] These reports leave room for further investigation of novel inhibitors such as zanubrutinib combined with anti-CD20 antibodies in indicated WM patients.

5 | NON-COVALENT BTK INHIBITORS

While C481 in the ATP-binding pocket is not required for the function of non-covalent inhibitors, they are efficient in both wild-type and C481-BTK mutant cells. The first reversible BTK inhibitor approved by the FDA in 2023 is pirtobrutinib.[70] In preclinical models, pirtobrutinib was reported as a potent and highly selective inhibitor.[71] Pirtobrutinib inhibits cell proliferation in CLL patients with both wild-type and mutated BTK (C481S).[72] However, treatment with pirtobrutinib has been shown to induce other specific resistance-related mutations. Akt signaling was not suppressed in cells harboring the V416L, A428D or L528W mutations. Such cells retain B-cell receptor signaling despite ongoing treatment resulting in resistance toward the inhibitor.[30]

Nemtabrutinib is another reversible inhibitor tested in clinical trials. To date, it has shown to have significant activity against both wild-type and mutant forms of BTK and a promising safety profile in pretreated relapsed or refractory CLL/SLL patients.[73] This compound blocks the ATP binding site of BTK by forming hydrogen bonds with residues other than C481 (e.g., E475 and Y476). Similarly, another clinical candidate fenebrutinib interacts with K430, M477 and D539.

Non-covalent inhibitors offer a promising solution for patients who have discontinued the treatment with covalent drugs due to the disease progression. However, even with reversible inhibitors, mutations in BTK occur and impede the disease manageability.[30]

6 | PROTACS

Proteolysis-targeting chimera (PROTAC) is a novel and potent approach to cancer therapy in cases of undruggable proteins or resistance to conventionally used low-molecular weight inhibitors. These heterobifunctional molecules mediate targeting protein of interest for its degradation via proteasome. PROTACs consist of two ligands joined with a chemical linker; one selectively interacts with the protein of interest, the second binds E3 ubiquitin ligase. By this action, target protein is poly-ubiquitinated and directed to undergo proteasomal degradation.[74] Due to their catalytic mechanism of action, low doses of PROTACs for shorter period are required, therefore PROTAC treatment may help to prevent developed resistance and avoid AE caused by high drug concentration.[75]

Regarding their crucial characteristics, several BTK targeting PROTACs have been introduced. Amongst them, PROTACs with various BTK linkers have been synthesised: ibrutinib-derived MT-208, P13I, L18I,[76–78] ARQ-531 derived 6e,[79] or compound with novel BTK linker, UBX-382.[80] More interestingly, two of them (Nx-2127 and Nx-5948) have been recently involved in clinical trials (NCT04830137 and NCT05131022, respectively) as first of its kind amongst BTK targeting drugs.[81,82] Moreover, NX-2127 possesses dual degradation mechanism, and besides BTK can target other clinically validated proteins: transcription factors Aiolos (IKZF3) and Ikaros (IKZF1) regulating T-cells function.[82] Both PROTACs were efficient in C481S mutated cells, NX-5948 was proven to induce also degradation of other forms of mutated BTK (T474I, V416L and L528W).[83,84] Expectedly, NX-2127 degraded BTK also in patients who have acquired BTK resistance mutations as a result of prior BTK inhibitor therapies.[35]

7 | CONCLUSION

The introduction of BTK inhibitors into the treatment of B-cell malignancies has enabled chemotherapy-free disease management, reduced the cytotoxicity of standard therapy and improved

progression-free survival in patients. However, their prolonged use may lead to the development of resistance and subsequent disease relapse or progression. Most frequent mechanism of resistance is mutation in the *BTK* gene.[3,27–29] Therefore, the search for novel approaches is necessary and has been actively explored recently. These alternative treatment options can either target mutated variants of BTK, act in non-covalent way, or inhibit multiple signaling pathways simultaneously. Today, ibrutinib can be replaced with the second-generation drugs, developed with more efficient selectivity and safety profiles, ensuring improved outcomes. However, acquired mutations and disease progression remain an issue that needs to be addressed even for zanubrutinib.[85] Its continuous exposure leads to secondary resistance, most commonly arising from mutations in the *BTK* gene.[33,34] Zanubrutinib is being investigated in clinical trials (Table 1) in combination with six other targeted drugs that inhibit the activity of CD20, PI3K, BCL-2 and PD-1, providing the possible solutions to overcome the resistance. While greater selectivity is typically desirable, the sensitivity of ITK to zanubrutinib could significantly improve the efficacy of PD-1/PD-L-1 inhibitors; the extent of this benefit will be assessed in an ongoing clinical trial.

Apart from combination therapies, non-covalent inhibitors provide another strategy that may take over in future management of disease's progression.[72] Also PROTACs possess high potential to overcome mutations-related resistance, with two compounds being already investigated in clinical trials. Recent study with one of them helped to reveal BTK scaffolding function, independent on its kinase activity, and indicate that PROTACs could be even more effective in blocking downstream BCR signaling.[35] Besides BTK, induced degradation of other BCR signaling molecules can provide also interesting option. Only recently PROTACs targeting Akt have been developed.[86,87] One of them even showed synergy with ibrutinib and effectively inhibited MCL cell growth.[87] Thus, combination of Akt degraders and BTK inhibitors such zanubrutinib may offer novel approach in managing resistance and bringing benefit to patients, but possible drug combinations and regimes remain to be explored using suitable experimental models.

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PEER REVIEW

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