The Role of Noncovalent BTK Inhibitors in the Era of Covalent BTK Inhibitors

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Keywords
Bruton kinase inhibitors, covalent BTK inhibitors, ibrutinib, nemtabrutinib, noncovalent BTK inhibitors, pirtobrutinib

Abstract: Despite significantly improving outcomes in patients with B-cell malignancies, covalent Bruton tyrosine kinase (BTK) inhibitors are limited by toxicities and the development of resistance. Some toxicities can be life-threatening, such as cardiotoxicity. These toxicities result from off-target effects of covalent BTK inhibitors and frequently lead to dose reductions and discontinuations of the drug. Noncovalent BTK inhibitors bind BTK in a unique fashion and, to date, have demonstrated an excellent safety profile as well as efficacy against a variety of B-cell malignancies. In addition, noncovalent BTK inhibitors have, for the first time, demonstrated efficacy in patients who progressed on other BTK inhibitors. Long-term data and comparative studies are needed to further investigate their efficacy and role in the landscape covalent BTK Inhibitors.

Introduction

Bruton tyrosine kinase (BTK) is a member of the TEC family of nonreceptor tyrosine kinases. Through its action on the B-cell receptor (BCR), BTK plays an essential role in B-cell survival and proliferation and has become a key target for the management of B-cell malignancies (Figure 1).[1] It also acts on B-cell trafficking and homeostasis through its action downstream on chemokine receptors CXCR4 and CXCR5 as well as class switching and antibody production through the toll-like receptor.[1-3] BTK expression in myeloid cells, particularly macrophages, alters chemokine and vascular endothelial growth factor expression and promotes tumor angiogenesis, invasion, and metastasis.[4,5] In addition, BTK inhibition leads to the downregulation of checkpoint molecules on CD4+ and CD8+ T cells.[6] Preclinical studies in chronic lymphocytic leukemia (CLL) suggest enhanced T-cell function with ibrutinib (Imbruvica, Pharmacyclics/Janssen) by decreasing programmed death 1 expression on CD8+ T cells, decreasing cytotoxic T-lymphocyte–associated protein 4 expression on T cells, increasing Th17 cells, and decreasing the regulatory T cell to CD4+ T cell ratio.[7] Therefore, inhibition of BTK

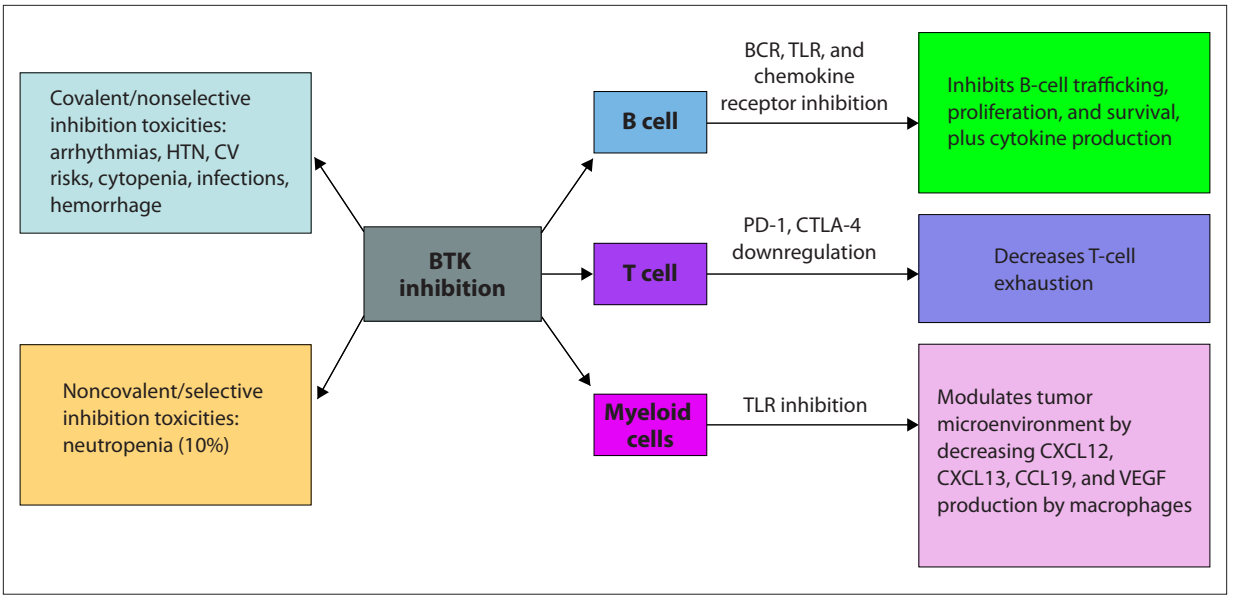

Figure 1. Role of BTK in B cells, T cells, and myeloid cells.
BCR, B-cell receptor; BTK, Bruton tyrosine kinase; CTLA-4, cytotoxic T-lymphocyte–associated antigen 4; CV, cardiovascular; HTN, hypertension; PD-1, programmed death 1; TLR, toll-like receptor; VEGF, vascular endothelial growth factor.

plays a vital role in the treatment of many B-cell malignancies. Several BTK inhibitors have been developed that show promising results in multiple clinical studies against a variety of mature B-cell malignancies.[8-15]

Covalent BTK Inhibitors

The initial class of BTK inhibitors to be developed for targeting the BTK enzyme in B-cell malignancies was covalent BTK inhibitors. These oral agents include ibrutinib, acalabrutinib (Calquence, AstraZeneca), and zanubrutinib (Brukinsa, BeiGene). Each of these agents is currently approved for 1 or more indications, such as CLL, Waldenström macroglobulinemia (WM), marginal zone lymphoma (MZL), and mantle cell lymphoma (MCL).

Ibrutinib, the first-in-class covalent BTK inhibitor, is an irreversible small-molecule BTK inhibitor that works by covalently binding the cysteine 481 site of the BTK enzyme.[16] It was initially developed in preclinical studies for rheumatoid arthritis in 2006.[16] Its efficacy against B-cell malignancies in preclinical studies was first demonstrated in 2010 by Honigberg and colleagues.[17] It was first used in a phase 1/2 clinical trial in 2013.[18] In this study of 85 patients, both the 240 mg and 420 mg doses of ibrutinib demonstrated durable responses, with an overall response rate (ORR) of 71% in patients with relapsed CLL, including those with *TP53* mutations. Although effective, ibrutinib also inhibits EGFR, ITK, and TEC family kinases. This results in off-target toxicities, limiting its use. As a result, the second-generation covalent BTK inhibitors, including zanubrutinib and acalabrutinib, were developed and approved for clinical use.[19,20] These second-generation BTK inhibitors are more selective for BTK and have less off-target inhibition, resulting in similar efficacy and an improved safety profile compared with ibrutinib.[15,21-24] They have been US Food and Drug Administration (FDA)-approved for the management of CLL, MCL, and WM in either the first-line or relapsed setting. Despite the multitude of options, even second-generation covalent BTK inhibitors produce off-target toxicities and the development of resistance.

Off-Target Toxicities

The off-target toxicities of covalent BTK inhibitors occur due to the effect of BTK inhibitors on receptor tyrosine kinases and nonreceptor tyrosine kinases, including EGFR, ITK, TEC, ERBB4, BMK, JAK3, and HER2.[25] These toxicities cause drug intolerance, leading to frequent discontinuations and dose reductions. In a long-term safety analysis of the 3 pivotal trials for ibrutinib, 13% of patients required dose reduction and 11% required permanent discontinuation of ibrutinib. The most common reported adverse events (AEs) were diarrhea (52%) and fatigue (36%), whereas the most common grade 3/4 AEs were neutropenia (18%) and pneumonia (12%). Cardiotoxicity is a potentially fatal toxicity that has been reported with ibrutinib. Although the initial studies reported an elevated risk of atrial fibrillation with ibrutinib,[26] later

studies have also reported risks of supraventricular arrhythmias and life-threatening ventricular arrhythmias.[27,28] In a recent study of hospitalized patients, ibrutinib was independently associated with a greater than 2-fold increase in the rate of atrial fibrillation–related complications and bleeding while hospitalized as well as higher rates of new heart failure.[29] Cardiotoxicity is not limited to ibrutinib, as similar concerns were appreciated with acalabrutinib. After studying 1063 person-years of acalabrutinib exposure, an 8-fold increased risk of ventricular arrhythmias and sudden cardiac death was appreciated.[30] The risk of cardiotoxicity was especially high in older patients, who are enriched for baseline cardiac comorbid conditions.

In addition to cardiac toxicities, another class effect of covalent inhibitors is the risk of bleeding. Because BTK plays a role in platelet signaling through GP1b and GPVI, BTK inhibition through ibrutinib causes bleeding owing to platelet dysfunction.[31] In a systematic review of 11 randomized clinical trials including 4288 patients, ibrutinib was associated with a 2-fold increased risk of bleeding.[32] The risk of bleeding especially becomes significant in the case of atrial fibrillation and the use of vitamin K antagonists, which are generally not recommended for patients taking covalent BTK inhibitors.[33] Although described more commonly with ibrutinib, bleeding events are reported with all classes of covalent BTK inhibitors, including a 66% rate (3% grade 3/4) with acalabrutinib[14] and a 26% rate with zanubrutinib.[34]

Several real-world studies have assessed the rates of AEs as well as treatment discontinuation with ibrutinib. In a large real-world study of 11,807 Medicare patients, the rate of ibrutinib discontinuation was 65%, with 69% of patients discontinuing it in the first 12 months of therapy.[35] In another real-world study, the most common reason for treatment discontinuation was infections, and the rate of cardiovascular AEs was similar to that previously reported in clinical trials.[36]

Resistance Against Covalent BTK Inhibitors

Despite their profound efficacy, most patients will eventually experience resistance to covalent BTK inhibitors. Primary resistance to covalent BTK inhibitors is reported in 13% of 30% of CLL cases and is mostly observed in CLL patients with underlying Richter transformation (RT) and in patients with MCL.[33,37] Secondary resistance occurs during the course of treatment with covalent BTK inhibitors and may be due to the clonal shift of the CLL cells under persistent inhibition of BTK.[38] Two well-established mechanisms of resistance involve a cysteine-to-serine mutation in BTK at the binding site of ibrutinib and downstream mutations in PLCγ2. The point mutation leading to serine residues in the C481 position prevents the irreversible binding of all covalent BTK inhibitors, resulting in disease progression.[39,40] Mutations in PLCγ2 are gain-in-function mutations that cause BCR signaling activation despite BTK inhibition, leading to resistance against covalent inhibitors.[41] Some additional mechanisms of resistance to ibrutinib have been described, including deletion of the short arm of chromosome 8,[38] gain of the short arm of chromosome 2,[42] and mutations in *SF3B1*, *MGA*, *BIRC3*, *NFKBIE, CARD11,* and *XPO1* genes (Figure 2).[43,44]

Role of Noncovalent BTK inhibitors

Noncovalent BTK inhibitors bind to BTK using hydrogen bonds, ionic bonds, and hydrophobic interactions in a mechanism that is independent of the C481-containing binding site. Because of this, they can overcome common causes of resistance associated with covalent BTK inhibitors. In addition, owing to their noncovalent reversible binding and more selective activity against BTK, they may offer a more favorable safety profile than the covalent BTK inhibitors currently available.

A total of 4 noncovalent BTK inhibitors are available or under development. These include pirtobrutinib (Jaypirca, Lilly), vecabrutinib, fenebrutinib, and nemtabrutinib.

Pirtobrutinib. Pirtobrutinib is a third-generation, highly selective, noncovalent BTK inhibitor and the only noncovalent BTK with an FDA approval to date in relapsed or refractory (R/R) MCL. Owing to noncovalent reversible binding, 300-fold selectivity for BTK, and potency against wild-type and C481-mutated BTK, pirtobrutinib is a highly effective drug against B-cell malignancies, even those resistant to earlier BTK inhibitors.[45,46] Its efficacy on the C481-mutated BTK occurs because it does not directly interact with the C481 domain. Instead, pirtobrutinib binds through hydrogen bonds with the backbone of E475 and M477 in the hinge region, through water-mediated hydrogen bonds with K430 and D539, and through an edge-to-face pi-stacking interaction with F540.[47] In contrast to covalent BTK inhibitors, which only inhibit autophosphorylation of Y223, pirtobrutinib also prevents phosphorylation of Y551 (upstream kinase phosphorylation). This results in the stabilization of BTK in a closed, inactive conformation at a significantly higher temperature than covalent BTK inhibitor–bound BTK, which in turn may lead to fewer interactions of BTK to the upstream kinases and therefore inhibit kinase-independent BTK cellular signaling.[47]

Pirtobrutinib has shown a dose-dependent growth inhibition of BTK-dependent tumors from human lymphoma lines implanted into immunodeficient mice. In a preclinical study by Aslan and colleagues, it inhibited BTK activation and downstream signaling in CLL cells

overexpressing BTK wild-type, BTK with cysteine to serine alteration, or BTK with cysteine to arginine alteration in both in vitro and in vivo murine models.[45]

BRUIN, the first-in-human phase 1/2 trial of pirtobrutinib, included 323 patients.[48] The median age was 68 years (62-74) and approximately 90% of patients with CLL and MCL had received a previous BTK inhibitor. In the phase 1 dose-escalation part of the trial, no dose-limiting toxicities were observed across any dose level (25-300 mg). Based on pharmacokinetics, the 200-mg dose was chosen for phase 2 of the trial. The ORR was 63% in CLL and 52% in MCL; notably, most patients had prior exposure to covalent BTK inhibitors. Response rates of 25% to 75% were observed in other B-cell phenotypes, such as follicular lymphoma (FL) and aggressive B-cell lymphomas. An update was presented at the 2022 American Society of Hematology (ASH) Annual Meeting that included 773 patients with R/R B-cell malignancies, including CLL or SLL (296), MCL (150), WM (78), and RT (57).[49-52]

In the updated analysis of BRUIN, pirtobrutinib demonstrated durable response and efficacy in patients with prior BTK inhibitor use.[50,53] The ORR in the 247 CLL patients pretreated with other BTK inhibitors was 82.2%, including 4 patients with a complete response (CR), 177 with a partial response (PR), and 22 with a PR with lymphocytosis.[53] In addition, there was no difference in PFS in patients with C481S mutation compared with those with wild-type C481. Among the 90 MCL patients pretreated with other BTK inhibitors, the ORR was 58%, including 18 with a CR and 34 with a PR.[54] Pirtobrutinib also demonstrated efficacy in other B-cell malignancies. The ORR was 54% (including 5 patients with a CR) among 50 patients with RT and 68% (including 17 patients with a very good PR and 32 with a PR) among the 72 patients with WM.[49,52] In the other subgroups, responses were observed in 4 of 8 patients with FL, 6 of 25 patients with DLBCL, and 2 of 9 patients with MZL.

In addition, pirtobrutinib demonstrated lower rates of toxicity than observed with covalent BTK inhibitors, although no head-to-head data are available. The most common grade 3 or higher AE was neutropenia (20.4%), followed by anemia (8.8%).[50] Hypertension of any grade occurred in 9.2% of patients, atrial fibrillation/flutter of any grade occurred in 2.8% of patients, and grade 3 or higher hemorrhage was present in 1.8% of patients. Pirtobrutinib was well tolerated in patients who had documented intolerance to prior BTK inhibitors.[55] Among the 123 patients who discontinued BTK inhibitors, including 118 patients on ibrutinib, 29 patients on acalabrutinib, and 6 patients on zanubrutinib, only 45 discontinued pirtobrutinib. Twenty-eight of these discontinuations were due to disease progression and 9 were for AEs, including 4 patients who had treatment-related AEs: myalgia, neutropenia, maculopapular rash, and staphylococcal sepsis. Therefore, pirtobrutinib demonstrated a favorable safety profile among patients who developed intolerance to other BTK inhibitors.

Currently, several clinical trials are ongoing to assess the efficacy of pirtobrutinib compared with or in combination with other drugs, including the following:

(1) a phase 3 study of pirtobrutinib vs investigator's choice of covalent BTK inhibitor in patients with previously treated BTK inhibitor-naive MCL (BRUIN-MCL-321; NCT04662255);

(2) a phase 3 study of pirtobrutinib vs investigator's choice of idelalisib plus rituximab or bendamustine plus rituximab in patients with previously treated CLL/SLL (BRUIN CLL-321; NCT04666038);

(3) a phase 3 study of fixed-duration pirtobrutinib plus venetoclax and rituximab vs venetoclax and rituximab in previously treated CLL/SLL (BRUIN CLL-322; NCT04965493);

(4) a phase 3 study of pirtobrutinib vs ibrutinib in patients with CLL/SLL (BRUIN-CLL-314; NCT05254743); and

(5) a phase 3 study of pirtobrutinib vs bendamustine and rituximab in untreated CLL/SLL (BRUIN-CLL-313; NCT05023980).

Vecabrutinib. Vecabrutinib, also known as SNS-062, reversibly binds to BTK at the ATP binding pocket, independent of the C481 residue.[56] In preclinical studies, vecabrutinib has shown efficacy in both wild-type BTK and C481S-mutated BTK, and has led to a decrease in tumor burden and improved survival in murine models.[56] Vecabrutinib was examined in a phase 1b study of 39 patients with B-cell malignancies, 77% of whom had CLL.[57] All patients in the trial had prior BTK inhibitor treatment, 45% had a BTK C481S mutation, and 18% had a PLCγ2 mutation. The most common AE was anemia (31%), followed by nausea, fatigue, headache, and dyspnea (21% each). Despite the favorable safety profile, vecabrutinib demonstrated modest evidence of clinical benefit, with only 1 patient having a PR and 13 patients having stable disease. Given its modest clinical efficacy, further clinical development of this drug has been terminated.

Fenebrutinib. Fenebrutinib reversibly binds to the K430, M477, and D539 residues of BTK through hydrogen bonds with more than 100-fold selectivity to BTK compared with other kinases.[58] Fenebrutinib targets BTK with a minimal number of off-target effects and a low disassociation rate from BTK, making the extent of treatment comparable to that of irreversible covalent BTK inhibitors.[59] Clinically, fenebrutinib was tested in a phase 1 dose-escalation trial with 24 patients, including 25% of

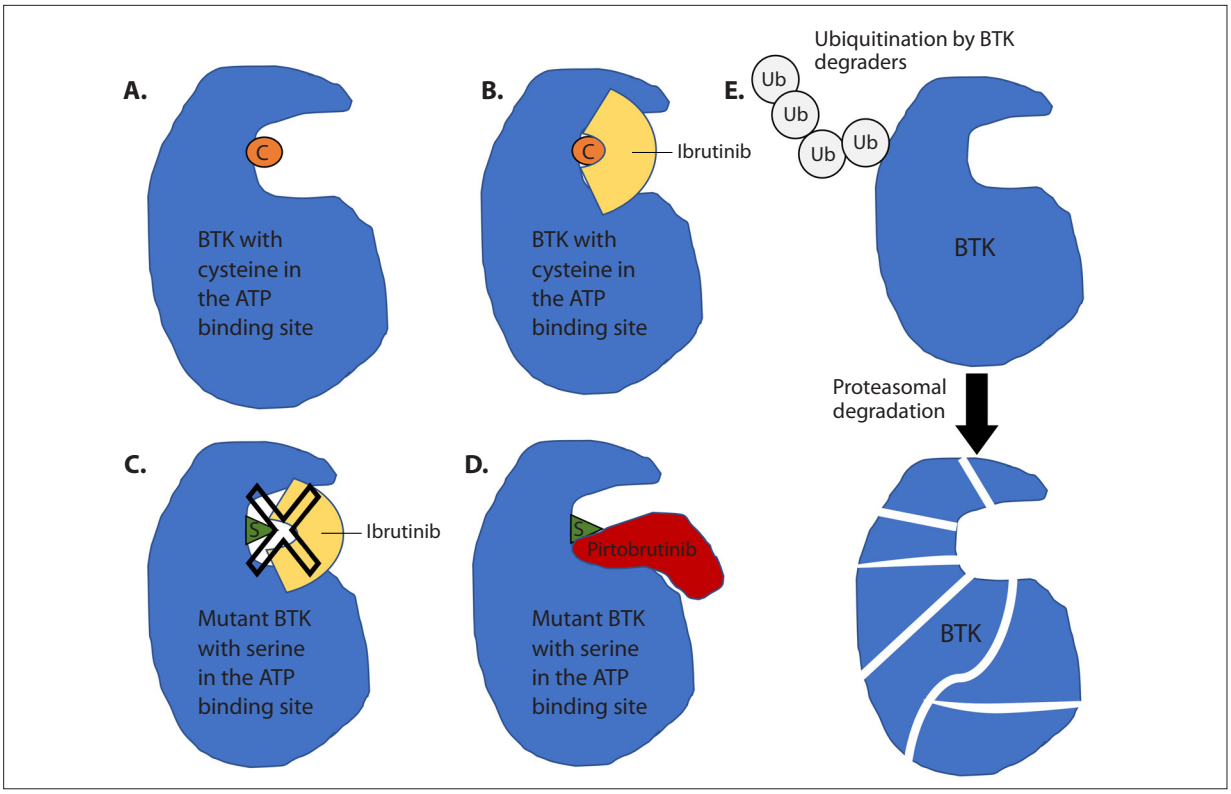

Figure 2. Mechanisms of resistance against covalent BTK inhibitors with mutated C481. **A,** Wild-type BTK with cysteine in the ATP binding site. **B,** Binding of ibrutinib to the wild-type BTK. **C,** Resistance to ibrutinib binding with BTK with mutated C481S. **D,** Pirtobrutinib with binding to mutated BTK with mutated C481S. **E,** BTK with BTK degradation leading to proteasomal degradation of the BTK enzyme and inhibition of the downstream pathway.
BTK, Bruton kinase inhibitor; C, cysteine; S, serine; Ub, ubiquitination.

patients with a BTK C481S mutation. Fenebrutinib was well-tolerated overall, with the most common AE being fatigue (37%), followed by nausea (33%) and diarrhea (29%). Two fatal AEs occurred during the study, which were H1N1 influenza and influenza infections. In terms of its efficacy, the ORR was 33% in all patients, whereas the response rate in patients with CLL was 50%. Although this is not being further studied in B-cell malignancies, multiple studies have shown its efficacy in autoimmune diseases, including chronic spontaneous urticaria, rheumatoid arthritis, and systemic lupus erythematosus.[60-62] In addition, studies are ongoing to assess fenebrutinib's safety and efficacy in patients with relapsing and primary progressive multiple sclerosis (NCT04544449, NCT04586023, NCT04544449).

Nemtabrutinib. Formerly known as MK1026 and ARQ-531, nemtabrutinib binds to E475 and Y476 through the formation of hydrogen bonds.[63] In addition to BTK, nemtabrutinib also binds to SRC, AKT, and ERK, as well as BTK with C481S BTK and autoactivating PLCγ2 mutations. In vivo, nemtabrutinib demonstrated an increased survival over ibrutinib in murine models of CLL and RT. Similarly, when combined with venetoclax, nemtabrutinib prolonged survival in mice compared with ibrutinib.[64]

In a phase 1 dose-escalation study evaluating nemtabrutinib in 40 patients with R/R B-cell lymphoid malignancies, the most common AEs were nausea (10%) and diarrhea (10%) and the most common grade 3/4 AEs were neutropenia (in 3 patients), febrile neutropenia, cellulitis, thrombocytopenia, increase in lipase, and rash (in 1 patient each).[65] Responses were seen in all B-cell malignancies, including a PR in 7 patients with CLL, 1 patient with RT, 1 patient with DLBCL, and 1 patient with FL.

In the updated analysis of this phase 1/2 study that included 112 patients with R/R B-cell malignancies, nemtabrutinib demonstrated favorable toxicity and durable antitumor activity in R/R CLL.[66] Among the 57 patients with CLL/SLL, the ORR was 56%, including 2 patients with a CR, 15 patients with a PR, and 15 patients with a PR with lymphocytosis. The median duration of response was 24.4 months and the median PFS among responders

was 26.3 months. In addition, nemtabrutinib produced responses in 95% of patients with CLL who had prior covalent BTK inhibitor exposure. The most common AEs of any grade were dysgeusia (21%), neutropenia (20%), and fatigue (13%). The most common grade 3 or higher AE was neutropenia, which occurred in 17% of patients.

Other ongoing studies for nemtabrutinib include:

(1) a phase 3 randomized, open-label, multicenter study to evaluate the efficacy and safety of nemtabrutinib compared with investigator's choice of chemoimmunotherapy in patients with previously untreated CLL/SLL without *TP53* aberrations (BELLWAVE-008; NCT05624554);

(2) a phase 3 study of nemtabrutinib and venetoclax vs venetoclax and rituximab as second-line or later treatment for R/R CLL/SLL (BELLWAVE-010; NCT05947851); and

(3) a phase 2 study of zilovertamab vedotin as monotherapy and in combination with nemtabrutinib in patients with aggressive and indolent B-cell malignancies (MK-2140-006; NCT05458297).

Other Noncovalent BTK Inhibitors. Several other noncovalent BTK inhibitors are currently under development in preclinical studies. XMU-MP-3 has shown an elevated selectivity toward BTK, including BTK with mutated C481, both in vitro and in murine models.[67] CB1763, also known as AS-1763, is another highly selective, orally available reversible noncovalent inhibitor of wild-type BTK and mutated C481S. In a preclinical study, CB1763 demonstrated excellent antitumor activity in the BTK-driven ABC-type DLBCL cell line xenograft mouse model.[68] Two other noncovalent BTK inhibitors, GNE-431 and CGI-1746, have shown activity against BTK with mutated C481S in vitro. There have not been studies in vivo so far.[69-71]

Resistance Against Noncovalent BTK Inhibitors. Recent studies have shown that, similarly to covalent BTK inhibitors, resistance against the noncovalent BTK inhibitors can occur owing to the development of new mutations that render the agent ineffective. This has been best described with pirtobrutinib, given the large clinical experience surrounding this agent. The mutations that create resistance to noncovalent BTK inhibitors are different from the mutation seen in the C481 position with covalent inhibitors. In a single-center study, using patient samples from the BRUIN study, Wang and colleagues performed next-generation sequencing in the peripheral blood of CLL patients who progressed after pirtobrutinib.[72] Nine of these 55 patients were found to have mutations, including at the V146L, A428D, M437R, T474I, and L528W sites in the kinase domain of BTK. Although there was low BTK activity, there was a sustained activation of AKT, ERK, and hyperactive calcium ion flux in the downstream pathway in the presence of these mutations. In addition, mutations were also found in the downstream signaling kinase PLCγ2. These mutations conferred resistance against both covalent and noncovalent BTK inhibitors, bringing to light a concern that although noncovalent inhibitors have been effective after covalent, the opposite may not be true.

In another preclinical study, Qi and colleagues utilized the long-term in vitro dose escalation method to generate resistance to ibrutinib and the noncovalent BTK inhibitors, including pirtobrutinib, vecabrutinib, nemtabrutinib, fenebrutinib, and RN-486, using the REC-1 MCL cell line. This resulted in similar resistant mutations described previously by Wang and colleagues, as well as novel mutations, including L528S, G409R, G480R, and D539H. Of note, these mutations in BTK and PLCγ2 were not identified in MCL cells resistant to nemtabrutinib.

Beyond Noncovalent BTK Inhibitors: Next-Generation BTK Degraders. A new class of BTK-targeting drugs is also under development, called BTK degraders. These small-molecule oral agents lead to proteasomal degradation of the entire BTK enzyme, resulting in inhibition and disease response. Although beyond the scope of this paper, several of these small-molecule BTK degraders are being developed and have demonstrated efficacy in preclinical studies.[73-77] These BTK degraders have not only exhibited activity against BTK with mutated C481S, but also BTK with mutated L528W, which causes resistance against pirtobrutinib.

The results of a phase 1 first-in-human study of NX-2127, a novel small molecule that drives targeted BTK and IKZF3 degradation, were reported at the 2022 ASH Annual Meeting.[78] Among the 28 patients enrolled (including 17 patients with CLL), there was 1 dose-limiting toxicity (cognitive impairment). The most common AE was fatigue (62%), and the most common grade 3 or higher AE was neutropenia (39%). Among the 12 CLL patients evaluable for response, the ORR was 33%, including those who were refractory to both BTK inhibitors and venetoclax and those who progressed on a noncovalent BTK inhibitor. Further studies are ongoing.

Conclusion

Although covalent BTK inhibitors have revolutionized the treatment outcomes of B-cell malignancies, the existing class of covalent inhibitors remains challenged by off-target toxicities and the development of resistance. A new class of noncovalent BTK inhibitors has emerged as a treatment option after covalent BTK inhibitor progression, or possibly in the future as an agent that can be used in the upfront setting before a covalent BTK inhibitor.

Noncovalent BTK inhibitors, especially pirtobrutinib, have to date demonstrated an exciting safety and efficacy profile, with durable responses seen even among patients with mutated C481S. Unfortunately, despite the effectiveness of this new class of drugs, resistance to pirtobrutinib has occurred in the setting of on-target BTK mutations (V416L, A428D, M437R, T474I, and L528W) and downstream PLCγ2 mutations.[72] Longer follow-ups from early clinical trials of noncovalent BTK inhibitors and randomized studies comparing them with current standards of care, as well as their safety and tolerability in combination with other agents, are ongoing. The results of these studies will help determine the role of noncovalent BTK inhibitors in the era of covalent BTK inhibitors. In addition, the role of the small-molecule BTK degraders, especially in the setting of resistance to the noncovalent BTK inhibitors, is yet to be determined.

Disclosures

Dr Furqan has no conflicts of interest. Dr Shah reports participation on advisory boards and/or consultancy for Kite Pharma, BMS/Juno, Miltenyi Biotec, Lilly, Ipsen, Incyte, Novartis, Seagen, AbbVie, and Galapagos; has research funding, travel support, and honoraria from Lilly and Miltenyi Biotec; and serves on a scientific advisory board for Tundra Targeted Therapeutics.

Acknowledgments

Dr Shah is a Scholar in Clinical Research of the Leukemia & Lymphoma Society.

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