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Targeting Bruton's Tyrosine Kinase in CLL

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Targeting the B-cell receptor signaling pathway through BTK inhibition proved to be effective for the treatment of chronic lymphocytic leukemia (CLL) and other B-cell lymphomas. Covalent BTK inhibitors (BTKis) led to an unprecedented improvement in outcome in CLL, in particular for high-risk subgroups with *TP53* aberration and unmutated immunoglobulin heavy-chain variable-region gene (IGHV). Ibrutinib and acalabrutinib are approved by the US Food and Drug Administration for the treatment of CLL and other B-cell lymphomas, and zanubrutinib, for patients with mantle cell lymphoma. Distinct target selectivity of individual BTKis confer differences in target-mediated as well as off-target adverse effects. Disease progression on covalent BTKis, driven by histologic transformation or selective expansion of *BTK* and *PLCG2* mutated CLL clones, remains a major challenge in the field. Fixed duration combination regimens and reversible BTKis with non-covalent binding chemistry hold promise for the prevention and treatment of BTKi-resistant disease.

Keywords: Bruton's tyrosine kinase, chronic lymphocytic leukemia, B-cell receptor signaling pathway, ibrutinib, acalabrutinib, zanubrutinib

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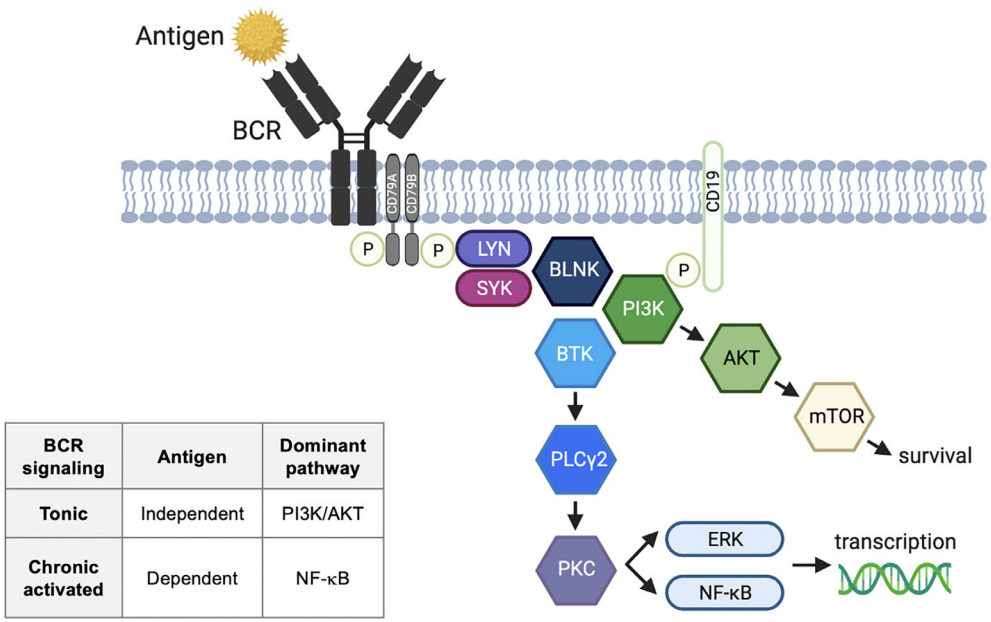
INTRODUCTION

B cell receptor (BCR) signaling is an essential component of normal B cell development and malignant B cell survival (**Figure 1**). There are two types of BCR signaling: chronically activated BCR and tonic BCR. Activated BCR signaling is an antigen-dependent process utilizing the canonical nuclear factor-κB (NF-κB) pathway (1). Antigen binding by surface immunoglobulin initiates BCR signaling, resulting in coupling and autophosphorylation of the CD79A/CD79B heterodimer by Src family kinases (2). The phosphorylation of the immunoreceptor tyrosine-based activation motif recruits a cascade of signaling molecules. These include spleen tyrosine kinase (SYK), Bruton's tyrosine kinase (BTK), phospholipase Cγ2 (PLCγ2), and protein kinase C, which lead to activation of NF-κB, phosphatidylinositol 3-kinase (PI3K) and ERK. Tonic BCR is an antigen-independent process that maintains B cell survival through PI3K-AKT-mTOR signaling rather than NF-κB (3, 4). PI3Kδ is a proximal component of the BCR signaling pathway involved in both tonic and chronic activated signaling.

The distinction between tonic and chronically activated BCR signaling is clinically relevant as it mirrors disease sensitivity to BTK inhibition. BTK inhibition alone is ineffective for the treatment of germinal center B diffuse large B-cell lymphoma (DLBCL) and Burkitt lymphoma with tonic BCR signaling, which require additional therapeutic targets such as SYK and CXCR4 (5–7). In contrast, lymphoma subtypes with activated BCR signaling are sensitive to BTK inhibitors (BTKis), and



BCR signaling	Antigen	Dominant pathway
Tonic	Independent	PI3K/AKT
Chronic activated	Dependent	NF-κB

FIGURE 1 | B cell receptor signaling pathway. Activated B cell receptor (BCR) signaling is an antigen-dependent process utilizing the canonical nuclear factor-κB (NF-κB) pathway. Antigen binding by surface immunoglobulin initiates BCR signaling, resulting in coupling and autophosphorylation of the CD79A/CD79B heterodimer by Src family kinases. The phosphorylation of the immunoreceptor tyrosine-based activation motifs recruits a cascade of signaling molecules. These include LYN tyrosine kinase (LYN), spleen tyrosine kinase (SYK), Bruton's tyrosine kinase (BTK), phospholipase Cγ2 (PLCγ2), and protein kinase C (PKC), which lead to activation of NF-κB, phosphatidylinositol 3-kinase (PI3K) and ERK. Tonic BCR is an antigen-independent process that maintains B cell survival through PI3K-AKT-mTOR signaling rather than NF-κB. BLNK, B-cell linker; ERK, extracellular signal-regulated kinase; IgH, immunoglobulin heavy chain; IgL, immunoglobulin light chain; mTOR, mammalian target of rapamycin; P, phosphorylation.

include chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), marginal zone lymphoma (MZL), and activated B cell (ABC) DLBCL. CLL and MCL have a non-genetic mechanism of BCR signaling where stereotyped BCRs and biased usage of unmutated immunoglobulin heavy-chain variable-region gene (IGHV) support the presence of cognate self-antigen leading to chronic BCR activation (8, 9). MZL also has a non-genetic mechanism of chronic BCR activation and is often associated with hepatitis C virus or *Helicobacter pylori* infection which provides a chronic antigenic stimulus to the BCR (10). ABC-DLBCL is characterized by genomic alterations of the BCR pathway and its downstream components (i.e. CD79A/B, CARD11) (11–13). Such mutations or amplification of genes in the BCR signaling pathway have not been reported in CLL (14) or MZL (1), and are rare in other indolent lymphomas (15, 16). Follicular lymphoma (FL) appears to have heterogeneous mechanisms of BCR activation. Although FL utilizes nonstereotyped BCR (17), studies have demonstrated antigen-mediated BCR signaling in a subset, not all, of FL (18, 19).

Of several approaches available for targeting the BCR pathway, BTK inhibition is the most popular and advanced in drug development. The U.S. Food and Drug Administration (FDA) has approved three BTKis for the treatment of B cell malignancies and graft-versus-host disease (GVHD): ibrutinib, acalabrutinib, and zanubrutinib. PI3K inhibition is another important BCR targeting strategy with four FDA-approved agents for the treatment of B cell malignancies (idelalisib, duvelisib, copanlisib, umbralisib). Isoform selectivity and potency of individual PI3K inhibitors contribute to observed differences in immune-mediated adverse events. The rates and severity of inflammatory toxicity, typically presenting as enteritis, hepatotoxicity, or pneumonitis, are relatively high with idelalisib. The rationale behind this observation is that idelalisib selectively inactivates the PI3K p110δ isoform in regulatory T cells thereby activating immune response (20, 21). Immune-mediated toxicity can be improved by less potent PI3Kδ inhibition *via* umbralisib, by targeting additional PI3K isoforms *via* copanlisib (pan-PI3K inhibitor), or in the relapsed/refractory setting where the immune effector function is downregulated (22). MALT1 inhibition is in the early stages of clinical development with no FDA-approved agent to date (23). We focus our discussion here on the successes and challenges of BTK inhibition in CLL, a malignant B cell disease with the most abundant data available on this topic.

COVALENT BTK INHIBITORS

Ibrutinib is the first-in-class, orally bioavailable, covalent BTKi. Ibrutinib binds to the cysteine 481 (C481) residue of BTK and irreversibly blocks phosphorylation of downstream kinases in the BCR signaling pathway (24). Since its discovery in 2007 (25), ibrutinib underwent rapid drug development leading to its initial FDA approval for the treatment of MCL in November 2013,

followed by expansion of approved indications to include CLL in 2014 and other hematologic diseases thereafter (**Table 1**). The approved doses of ibrutinib are 420mg once daily for CLL, Waldenström's macroglobulinemia (WM), and chronic GVHD, and 560mg for MCL and MZL. Both of these doses achieve sustained and complete BTK occupancy (>95%) (26). While ibrutinib is most potent against BTK (half maximal inhibitory concentration [IC_{50}] 0.5nM), it can also inhibit other targets at lower potency such as EGFR (IC_{50} 5.6nM), ErbB2 (IC_{50} 9.4nM), ITK (IC_{50} 10.7nM), and TEC (IC_{50} 78nM) (24). Many of these unintended targets of ibrutinib have a conserved cysteine residue aligning with the C481 in BTK. Their inhibition is a proposed mechanism of ibrutinib toxicity. In particular, bleeding and cardiac arrhythmia are among the important side effects of ibrutinib, which are thought to be related to inhibition of TEC family kinases involved in platelet activation (27) and inhibition of C-terminal Src kinase expressed in cardiac tissue (28).

To reduce toxicity and improve the tolerability of BTKis, alternative agents with more selective kinase inhibition profiles have been investigated. Acalabrutinib is a covalent BTKi with less potent inhibition of TEC compared to ibrutinib (IC_{50} 93nM for acalabrutinib vs. 7nM for ibrutinib in a comparative recombinant kinase assay) and no EGFR or ITK inhibition (IC_{50} >1,000nM for both targets) (29). Acalabrutinib is approved for the treatment of CLL and MCL in the United States. For both diseases, acalabrutinib is dosed at 100mg PO twice daily. Studies have shown that the twice-daily dosing of acalabrutinib achieves the least variability at steady-state trough (29) and higher BTK occupancy (30) compared to 200mg given once daily.

In 2019, the FDA granted accelerated approval of zanubrutinib for the treatment of MCL. Zanubrutinib has less potent ITK and EGFR inhibition and a more favorable pharmacokinetic profile than ibrutinib (31). The twice daily dosing of zanubrutinib achieves 8-fold higher plasma drug exposure than ibrutinib and a longer half-life than acalabrutinib (4 vs. 1 hour), which effectively blocks the function of newly synthesized BTK protein as well as preexisting BTK irreversibly bound by zanubrutinib. A phase 1 study did show higher BTK occupancy with twice daily (>95%) than once daily dosing (89%) of zanubrutinib in lymphoid tissue (31). The same study, however, showed uniformly high BTK occupancy in blood and no difference in clinical outcome across once- and twice-daily dosing groups. Both doses of zanubrutinib are being marketed. Taken together, the positive correlation between pharmacokinetics and BTK inhibition in lymphoid tissue provides a theoretical basis for zanubrutinib being a better covalent BTKi than others. Longer follow-up coupled with correlative studies are needed to determine whether more effective blockade of BTK resynthesis translates to deeper and more durable response to zanubrutinib.

Spebrutinib and tirabrutinib are covalent BTKis without regulatory approval in the United States. Spebrutinib is less selective than other BTKis and was withdrawn from development due to lack of efficacy (32). Tirabrutinib is a

TABLE 1 | Covalent BTK inhibitors.

	Ibrutinib (PCI-32765)	Acalabrutinib (ACP-196)	Zanubrutinib (BGB-3111)	Spebrutinib (CC-292)	Tirabrutinib (ONO-4059)
Half-life	4-6 hours	1 hour	2-4 hours	1.9 hour	4-7 hours
IC_{50}*	0.5nM	5.1nM	1.8nM	<0.5nM	6.8nM
Biochemical IC_{50} (Selectivity)*	BTK 0.5nM (1x)	BTK 5.1nM (1x)	BTK 1.8nM (1x)	BTK 9.2nM (1x)	BTK 6.8nM (1x)
	ITK 10.7nM (20x)	ITK >1,000nM (>1,000x)	ITK 3,277nM (>1,000x)	ITK 10,50nM (110x)	ITK >20,000nM, (>1,000x)
	EGFR 5.6nM (10x)	EGFR >1,000nM (>1,000x)	EGFR 606nM (336x)	EGFR >20,000nM (>1,000x)	EGFR 3,020nM (>400x)
	TEC 78nM (156x)	TEC 93nM (>19x)	TEC 1.9nM (1x)	TEC 8.4nM (1x)	TEC 48nM (7x)
Comments	First-in-class BTKi	No ITK or EGFR inhibition	No ITK inhibition Does have TEC inhibition	Withdrawn from development	Approved in Japan for the treatment of PCNSL, WM and LPL
Approved dose	420mg QD	100mg BID	160mg BID	Not approved	480mg QD#
	560mg QD		320mg QD		
Approved indications	CLL/SLL	CLL/SLL	MCL	Not approved	PCNSL#
	MCL				WM#
	MZL	MCL			LPL#
	WM				
Clinical trials	Phase 1, 2, 3	Phase 1, 2, 3	Phase 1, 2, 3	Phase 1, 2	Phase 1, 2

*IC_{50} and fold selectivity over BTK within each BTK inhibitor; not for comparison across BTK inhibitors.
#Approved doses and treatment indications in Japan. Tirabrutinib has not been approved in the United States.
BID, twice daily; BTK, Bruton's tyrosine kinase; CLL, chronic lymphocytic leukemia; EGFR, epidermal growth factor receptor; IC_{50}, half maximal inhibitory concentration; ITK, Interleukin-2-Inducible T-cell Kinase; LPL, lymphoplasmacytic lymphoma; LYN, LYN tyrosine kinase; MCL, mantle cell lymphoma; MZL, marginal zone lymphoma; PCNSL, primary CNS lymphoma; QD, once daily; SLL, small lymphocytic lymphoma; SYK, spleen tyrosine kinase; TEC, TEC kinase; WM, Waldenström's macroglobulinemia.

selective BTKi approved in Japan for the treatment of primary CNS lymphoma, WM, and lymphoplasmacytic lymphoma (33, 34).

Despite the limited amount of high-quality evidence, emerging data indicate that selective BTKis may be safer and potentially more efficacious than ibrutinib. In the phase 3 ASPEN study for WM, the zanubrutinib arm achieved numerically higher rates of complete or very good partial responses (28%) than the ibrutinib arm (19%) without reaching statistical significance for the difference (35). Zanubrutinib was associated with a lower frequency and severity of bleeding and cardiovascular toxicities. The observed rate of atrial fibrillation, for instance, was 0.1 per 100 person-months with zanubrutinib compared to 1 per 100 person-months with ibrutinib. Indirect, prospective evidence further supports improved tolerability of second-generation BTKis. An open-label study of 33 CLL patients with ibrutinib intolerance reported 72% of the patients had tolerated acalabrutinib without having reoccurrences of ibrutinib-related adverse events (36). Two randomized studies ongoing in relapsed/refractory (R/R) CLL are expected to provide a direct comparison of first- and second-generation BTKis (ibrutinib vs. acalabrutinib, NCT02477696; ibrutinib vs. zanubrutinib, NCT03734016). Further research is needed to determine the comparative safety and efficacy of different BTKis including investigations in treatment-naïve (TN) CLL, long-term follow-up, and pharmacodynamic assessments of BTK and other targets.

CLINICAL ACTIVITY

BTKis pioneered the major shift in therapeutic approaches for CLL from chemoimmunotherapy to targeted therapy (**Table 2**). In randomized studies, single-agent ibrutinib demonstrated superior progression-free survival (PFS) and overall survival (OS) compared to conventional single-agent chemo- or immunotherapy in TN (55) and R/R CLL (42). In the TN setting, BTKi-containing regimens outperformed doublet or triplet chemoimmunotherapy regimens by improving PFS in four randomized studies (37–39, 49) and OS in one of the four studies (38).

Patients who were classically considered to have high-risk characteristics benefit the most from BTKis (56, 57). *TP53* aberration, referring to a mutation of the *TP53* tumor suppressor gene or deletion of chromosome 17p where *TP53* is encoded, is a strong negative prognostic marker in CLL (58). First-line treatment with an intensive chemoimmunotherapy regimen of fludarabine, cyclophosphamide, and rituximab (FCR) reported median PFS of 15 months for patients with *TP53* aberration as opposed to nearly 5 years for those without the aberration (48, 54). Treatment with BTKis substantially extended the survival of patients with *TP53* aberration (48, 59) with the observed rates of 5-year PFS and OS being 70% and 85%, respectively, when ibrutinib was first-line therapy (47). Patients with unmutated IGHV, another high-risk genetic marker in CLL, also achieved superior PFS with BTKi-based therapy compared to chemoimmunotherapy (37–39, 49). However, such PFS benefit was not observed in the subgroup with mutated IGHV. Outcome data based on the IGHV mutation status should be interpreted with caution because of relatively short follow-up and small numbers of events at the time of analyses. Equally critical to consider is long-lasting remission—and possibly cure—observed after treatment with FCR in a subset of patients with mutated IGHV (60, 61). Chemoimmunotherapy remains an option for young and fit CLL patients with mutated IGHV who prefer a defined duration of treatment to avoid concerns of long-term toxicity, treatment adherence, and financial burden related to continuous BTKi therapy.

Although covalent BTKis provide an excellent disease control in most patients, BTK inhibition alone is insufficient to eradicate CLL or achieve deep responses. Undetectable minimal residual disease (U-MRD) with fewer than 1 CLL cell per 10,000 leukocytes is rarely observed with ibrutinib (46, 59) or acalabrutinib alone (<7%) (49). Depth of response marginally improves with prolonged therapy (33% reduction of circulating CLL cells with each additional year on ibrutinib) (59). To improve the efficacy of BTKis, multiple trials have tested the combination of BTKis with the following chemoimmunotherapy regimens: anti-CD20 monoclonal antibodies (mAbs) (37–39, 46, 49, 53, 62), fludarabine (63), bendamustine (64), FCR (65), fludarabine, cyclophosphamide plus obinutuzumab (66, 67), and bendamustine plus rituximab (BR) (44, 68). A few exceptional studies used conventional agents for an abbreviated period as part of sequential therapy (64, 66), debulking (63), or MRD clearance (67). The vast majority of the combination studies adopted up to six cycles of conventional therapy. Combination approaches did improve the rate of U-MRD up to 80-90% by adding triplet chemoimmunotherapy to ibrutinib (65–67), and a range of 5-35% by adding an anti-CD20 to a BTKi in TN CLL (37–39, 46, 49, 53, 62). Despite high rates of U-MRD, cytotoxic agents have fallen out of favor because of safety concerns related to hematologic toxicities and secondary myeloid neoplasms observed in 2-5% of long-term survivors treated with FCR (61, 69).

The combination of an anti-CD20 mAb and a BTKi is generally well tolerated, and its use is supported by U.S. prescriber information for ibrutinib and acalabrutinib. Nevertheless, it is unclear if mAbs add clinically meaningful benefit to BTK inhibition. Several thoughtfully designed randomized trials tackled this question and arrived at different conclusions. A randomized phase 2 study enrolling mostly R/R CLL patients showed no difference in PFS with or without the addition of rituximab to ibrutinib (46). A randomized phase 3 study in elderly TN CLL reached a similar conclusion (37). Nevertheless, trials testing newer generations of BTKis and anti-CD20 mAbs challenge previous observations. The GENUINE study showed significant PFS benefit with the addition of ublituximab to ibrutinib, and the PFS benefit was largely driven by high-risk patients with R/R disease and *TP53* aberration (70). The ELEVATE-TN study also reported 50% reduction in risk of progression or death in patients treated with acalabrutinib plus obinutuzumab compared to those receiving

TABLE 2 | Selected clinical trials testing covalent BTK inhibitors in CLL.

References	Phase	Patient population	Median FU	Primary endpoint	BTKi arm N, therapy	BTKi arm PFS	Control arm N, therapy	Control arm PFS	Comments
Ibrutinib									
Alliance 041702 (37)	3	TN CLL with age ≥65Y	38M	PFS	180, Ibru 181, Ibru-R	87% at 2Y 88% at 2Y	176, BR	74% at 2Y	3 treatment arms, no PFS difference between two BTKi containing arms
ECOG-ACRIN 1912 (38)	3	TN CLL with age ≤70Y	34M	PFS	354, Ibru-R	91% at 3Y	175, FCR	63% at 3Y	Excluded del17p, improved OS with BTKi (P<0.001)
iLLUMINATE (39)	3	TN CLL with age ≥65Y or comorbidities	31M	PFS	113, Ibru-G	79% at 30M	116, Chlb-G	31% at 30M	Less infusion-related reactions with Ibr-G (25%) than Chlb-G (58%)
RESONATE-2 (40, 41)	3	TN CLL with age ≥65Y	60M	PFS	136, Ibru	70% at 5Y	133, Chlb	12% at 5Y	Excluded del17p
RESONATE (42, 43)	3	RR CLL	65M	PFS	195, Ibru	40% at 5Y	196, G	3% at 5Y	
HELIOS (44, 45)	3	RR CLL	35M	PFS	289, Ibru-BR	68% at 3Y	289, BR	14% at 3Y	Excluded del17p, OS benefit with Ibru-BR despite crossover
Burger et al. (46)	2	RR, or TN CLL with *TP53* aberration	36M	PFS	104, Ibru 104, Ibru-R	86% at 3Y 87% at 3Y	–	–	No PFS or OS difference between two Ibr arms
Ahn et al. (47)	2	TN CLL with TP53 aberration	78M	Overall response	34, Ibru	85% at 5Y	–	–	TN subset of a phase 2 study
RESONATE-17 (48)	2	RR CLL with *TP53* aberration	28M	Overall response	145, Ibru	63% at 2Y	–	–	
Acalabrutinib									
ELEVATE-TN (49)	3	TN CLL Age ≥65Y or with comorbidities	28M	PFS	179, Acala 179, Acala-G	87% at 2Y 93% at 2Y	177, Chlb-G	47% at 2Y	3 treatment arms, PFS difference between two Acala arms
ASCEND (50)	3	RR CLL	16M	PFS	155, Acala	88% at 1Y	155, Idela-R or BR	68% at 1Y	Most (77%) patients in the control arm received Idela-R rather than BR (23%).
Byrd et al. (51, 52)	1/2	RR CLL with *TP53* aberration	41M	Safety, efficacy	27, Acala	36M median	–	–	*TP53* aberration subset of a phase 1/2 study
Zanubrutinib									
Tam et al. (53)	1b	RR CLL	29M	Safety	45, Zanu	91% at 2Y*	–	–	CLL cohort of the phase 1 study
SEQUOIA Arm C (54)	3**	TN CLL with TP53 aberration	18M	Efficacy	109, Zanu	89% at 18M	–	–	

**Duration of response at 2 years, PFS was not reported.*

***Non-randomized arm of the phase 3 study.*

Acala, acalabrutinib; BR, bendamustine and rituximab; BTKi, Bruton's tyrosine kinase inhibitor; Chlb, chlorambucil; del17p, deletion 17p; FCR, fludarabine, cyclophosphamide, and rituximab; FU, follow-up; G, obinutuzumab; Ibru, ibrutinib; Idela, idelalisib; M, months; OS, overall survival; PFS, progression-free survival; R, rituximab; R/R, relapsed or refractory CLL; TN, treatment-naïve CLL; TP53 aberrations, deletion 17p or TP53 mutation; Y, years; Zanu, zanubrutinib.

acalabrutinib alone (49). Across four randomized trials testing ibrutinib- or acalabrutinib-based combinations, there was a greater risk reduction in studies adding obinutuzumab to a BTKi (hazard ratio [HR] for progression or death 0.08-0.15) (39, 49) than those adding rituximab to a BTKi (HR 0.26-0.51) (37, 38) in patients with unmutated IGHV. Although these data are limited as they are often exploratory or subgroup analyses of trials, nonetheless they raise an important question of whether BTK inhibition can be optimized by using specific drug combinations in selected patient populations.

Why did randomized trials testing the combination of anti-CD20 mAbs and BTKis draw contradicting conclusions? This is because effector mechanisms of mAbs vary by molecules and by BTKis used in combination. The newer generation of anti-CD20 mAbs exhibits improved effector function compared to rituximab and has been shown to circumvent mechanisms of rituximab failure *in vitro*. Obinutuzumab, a glycoengineered type 2 antibody, induces greater direct cell killing, greater antibody-dependent cellular cytotoxicity (ADCC), and less intra- and trans-cellular loss of target antigens compared to rituximab (71). Ublituximab is a glycoengineered type 1 antibody that targets a unique epitope of CD20 and has shown greater ADCC than rituximab *in vitro* (72). In support of these findings, two randomized studies conducted in context of chemoimmunotherapy favored obinutuzumab to be more efficacious than rituximab for the treatment of TN CLL (73, 74) and FL (75). These effector mechanisms of mAbs can be hampered by off target effects of ibrutinib but preserved with the use of more selective BTKis. In a preclinical study, a wide range of doses of ibrutinib, but not acalabrutinib, inhibited antibody-dependent cellular phagocytosis, a key therapeutic mechanism of any anti-CD20 mAb (76). Ibrutinib also downregulates CD20 expression *via* decreased NF-κB activity (77) and by reducing supportive chemokines from the microenvironment that are necessary for CD20 upregulation (78). No data specific to the dynamics of CD20 expression during treatment with second-generation BTKis have yet been generated.

Venetoclax, a BCL2 inhibitor, is one of the preferred partners to BTKis. Single-agent venetoclax can achieve U-MRD in bone marrow in 16% of R/R CLL (79). The rate of U-MRD in bone marrow increases to ~60% in TN CLL by combining venetoclax with either obinutuzumab (80) or ibrutinib (81). Early data from single-arm trials testing triplet therapy with a BTKi, venetoclax, and obinutuzumab reported further improvement in the proportion of patients achieving deep responses. In TN CLL, the rate of U-MRD in bone marrow was 67% with ibrutinib, venetoclax and obinutuzumab (82), 78% with acalabrutinib, venetoclax and obinutuzumab (83), and 84% with zanubrutinib, venetoclax and obinutuzumab (84). Simultaneous targeting of BCL2 and BTK with or without an anti-CD20 mAb has several advantages over single-agent approaches. Targeted combinations can achieve U-MRD in a substantial proportion of patients, enabling treatment cessation after a fixed period (NCT04608318, NCT03701282, NCT02950051) or based on MRD status (NCT04639362). Such modifications in treatment duration, which are being actively investigated in clinical trials, can potentially reduce long-term toxicities linked to continuous therapy. Moreover, a lead-in period with a BTKi and/or obinutuzumab can reduce risk of tumor lysis syndrome (TLS), a potentially fatal toxicity of venetoclax (82). BTKis can additionally reduce the rate and the severity of infusion-related reactions (IRR) associated with mAbs. The reported rate of IRR was 44% in patients treated with venetoclax and obinutuzumab (80) and 20% in a different study testing acalabrutinib, venetoclax and obinutuzumab (85). Similar findings were reported from a randomized study for WM, which demonstrated a significantly lower rate of IRR in patients receiving ibrutinib and rituximab (1%) than in those treated with ibrutinib alone (16%) (86).

SAFETY

Safety profiles of individual BTKis have shared features and differences depending on relative selectivity to BTK. Because ibrutinib has the largest and longest safety data available among BTKis, our discussion focuses on key non-hematologic toxicities originally identified from ibrutinib and highlights differences in safety profiles of BTKis.

BTKis increase the risk of bleeding by inhibiting platelet aggregation and adhesion (87). BTK has been experimentally shown to be necessary for collagen-induced and von Willebrand factor-dependent thrombus formation (88). Further, both BTK and TEC are independently involved in platelet activation through glycoprotein (GP) VI signaling (89). Treatment with ibrutinib can block the downstream GP VI signaling and subsequent platelet aggregation, although there is substantial inter-patient variability in the effects of ibrutinib on platelet functions (90, 91). Compared with ibrutinib, acalabrutinib is less effective at inhibiting GP VI signaling *in vitro* and overall weaker at inhibiting collagen-mediated platelet aggregation *ex vivo* (92). Nevertheless, acalabrutinib still impacts platelet aggregation in certain settings such as in the presence of concurrent anti-platelet therapy and samples with known sensitivity to ibrutinib in terms of platelet functions (92). In clinical trials, most bleeding was low grade presenting as contusion or petechiae. Major bleeding is uncommon with both ibrutinib (2-5%) (93) and acalabrutinib (2-5%) (49, 51). Concurrent administration of warfarin is generally avoided during treatment with BTKis because warfarin was an exclusion criterion for most trials after four patients on a phase 2 study developed subdural hematoma while taking ibrutinib and warfarin or aspirin (94). Direct oral anticoagulants, anti-platelet agents including aspirin and clopidogrel, and low molecular weight heparins can be used during treatment with BTKis (95). Dual anti-platelet therapy is generally avoided given very limited data on safety. Patients undergoing elective surgical procedures are recommended to interrupt BTKis for 3 to 7 days before and after the procedure to minimize the risk of post-operative bleeding.

Hypertension and atrial fibrillation (Afib) are the two most common cardiovascular toxicities of BTKis. In a retrospective analysis of 562 patients treated with ibrutinib, new or worsening hypertension affected 78% of the patients, occurred early in the treatment course (50% of the events occurred within 2 months of

treatment initiation), and was associated with major cardiovascular events including Afib (96). Emergence of Afib during treatment with BTKi poses a particular challenge to clinicians. The complexity of care increases with the diagnosis of Afib as it requires cardiology consultation, assessment of the need for anticoagulation, and rate- and/or rhythm-controlling interventions. Reported incidences of Afib range from 7-13% in studies of ibrutinib (48, 93, 96) and 3-7% for acalabrutinib (49, 51, 97). This difference in incidences of Afib can be explained by the fact that ibrutinib, but not acalabrutinib, inhibits C-terminal Src kinase (CSK) expressed in cardiac tissue (28). In support of this hypothesis, CSK knock-out mice, as well as ibrutinib-treated mice with wild-type CSK, developed increased Afib, recapitulating observations from patients treated with ibrutinib. Results from ongoing randomized studies are eagerly awaited to determine the safety of second-generation BTKis in comparison with ibrutinib (NCT02477696, NCT03734016).

Opportunistic infection (OI) is an uncommon, yet important side effect of BTKis. Although variations in the use of antimicrobial prophylaxis make it difficult to determine the true risk of infection, a study by Rogers et al. reported the OI incidence rate of 1.9 per 100 person-years in a retrospective analysis of over 500 patients treated with BTKis (98). Invasive aspergillosis was the most common pathogen identified from this study (2% of the cohort), while others reported *Pneumocystis jirovecii* pneumonia in up to 3% of patients not on prophylaxis during BTKi therapy (99, 100). Other rare pathogens observed during BTKi therapy include atypical *Mycobacterium* spp., JC virus, and toxoplasmosis (98). Impaired immune surveillance during treatment with a BTKi is linked to the known role of BTK in macrophage toll-like receptor 9 activation (101). BTK deficient mice are unable to mount an immune response to fungus, indicating that BTKis control innate immunity (102).

DRUG RESISTANCE

Disease progression remains among the most common reasons for BTKi discontinuation in CLL. Long-term follow-up of patients treated with ibrutinib monotherapy reported 5-year PFS of 70-92% for first-line treatment with ibrutinib (40, 103), and 40-44% for relapsed CLL (43, 103). In addition to prior treatment status, risk of progression increases in the presence of high-risk genetic or biochemical markers at pre-treatment such as *TP53* aberrations (56, 59), complex karyotype (104), increased ß-2 microglobulin, and elevated lactate dehydrogenase (105).

There are two types of disease progression on BTKis. First, CLL can histologically transform into a more aggressive type of lymphoma, a phenomenon termed Richter's transformation (RT). Atypical B cells found in RT commonly have DLBCL-like immunophenotypes and less often present as Hodgkin-like lesions with Reed-Sternberg cells (106). Although mechanisms of histologic transformation are unclear, studies have identified enrichment of several notable molecular events in RT. There is a high prevalence of stereotyped BCR (70%) and biased usage of *IGHV4-39* in patients with RT (107). Mutations of known driver genes in CLL (*TP53, NOTCH1*) and the *CDKN2A/B* cell cycle regulator are found more frequently in RT than CLL (108). RT is further characterized by complex copy number changes including 17p loss leading to *TP53* aberration, gain/amplification of *MYC* on 8q, 9q loss resulting in haploinsufficiency of *CDKN2A/2B*, and 18p loss without a candidate gene (109). However, these genetic lesions are not unique to RT, raising the possibility of an additional non-genetic inducer of aggressive transformation. Akt-mediated transcriptional control and subsequent NOTCH activation have been recently proposed to have a role in RT, which is supported by increased AKT activation in primary RT samples and an accelerated lymphoma phenotype observed in the Eμ-TCL1 mouse model with constitutive Akt activation (110). Once RT develops during treatment with BTKis, most patients relapse shortly after or become refractory to alternative therapy including immune checkpoint inhibitors (111) and anthracycline-based chemoimmunotherapy (112, 113). Allogeneic stem cell transplant (114) and CD19 chimeric antigen receptor modified T-cell infusion (CAR T) (115) can offer durable remission in a minority of patients. Unfortunately, many patients with RT are deemed unsuitable for cell therapy because of comorbidities, low rates of remission after initial therapy, lack of stem cell donors, or limited access to cellular products.

The second and more commonly observed type of progression is CLL with secondary resistance to BTKis. Up to 80% of the patients with BTKi-resistant CLL carry *BTK* and/or *PLCG2* mutations at the time of progression (**Figure 2**) (116–118). *BTK* mutations substitute the C481 residue with an alternative amino acid, most commonly serine, leading to the loss of a covalent bond between the drug and the kinase. The vast majority of *PLCG2* mutations identified to date affect the N-terminal SH2 domain with an autoinhibitory function. Functional studies in CLL and autoimmune diseases demonstrated that a mutation or deletion of the SH2 domain

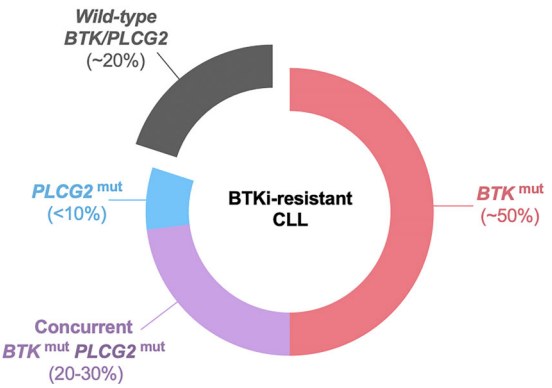


FIGURE 2 | *BTK* and *PLCG2* mutations in BTKi-resistant CLL. Approximately 20% of patients do not have detectable *BTK* or *PLCG2* mutation at progression. *BTK* mutation is the most common mutation, found in half the patients as *BTK* mutation alone and in an additional 20-30% with coexisting *PLCG2* mutation. Less than 10% of the patients have *PLCG2* mutation alone. BTKi, Bruton's tyrosine kinase inhibitor; mut, mutation.

can activate PLCγ2 and downstream BCR signaling (119–121). Acquisition of *BTK* and *PLCG2* mutations can occur any time in the clinical course. Preexisting *BTK* or *PLCG2* mutation is exceedingly rare and comprises a minor fraction of CLL, if any [allele frequency of 0.0002% in a report by Burger et al. (122)]. Under selective pressure of BTK inhibition, CLL undergoes linear or branching evolution with the latter giving rise to a multiclonal disease (**Figure 3**) (118). Multiclonality of BTKi-resistant CLL was clearly demonstrated by a single-cell analysis of a patient with four different *PLCG2* mutations, which presented as distinct clonal subpopulations rather than coexisting mutations within the same cells (122).

Several theoretical approaches can be considered to prevent the emergence of BTKi resistance. The simplest method is to stop BTKis after a defined period. Another approach is to combine multiple targeted agents with non-overlapping mechanisms of action as discussed previously. Several ongoing trials investigate time-limited, targeted agent-based combination therapy in CLL (NCT04608318, NCT03701282, NCT02950051). Critical to these approaches is the ability to monitor clonal evolution during and after treatment cessation in both investigational and control arms of these trials.

OVERCOMING RESISTANCE TO COVALENT BTK INHIBITORS

BTKi-resistant CLL demonstrates an aggressive clinical course in the absence of effective salvage therapy (123). Venetoclax can achieve initial responses (124). However, the responses are not durable, and most patients progress within 2 years. Novel combinations are being tested in BTKi-resistant CLL including

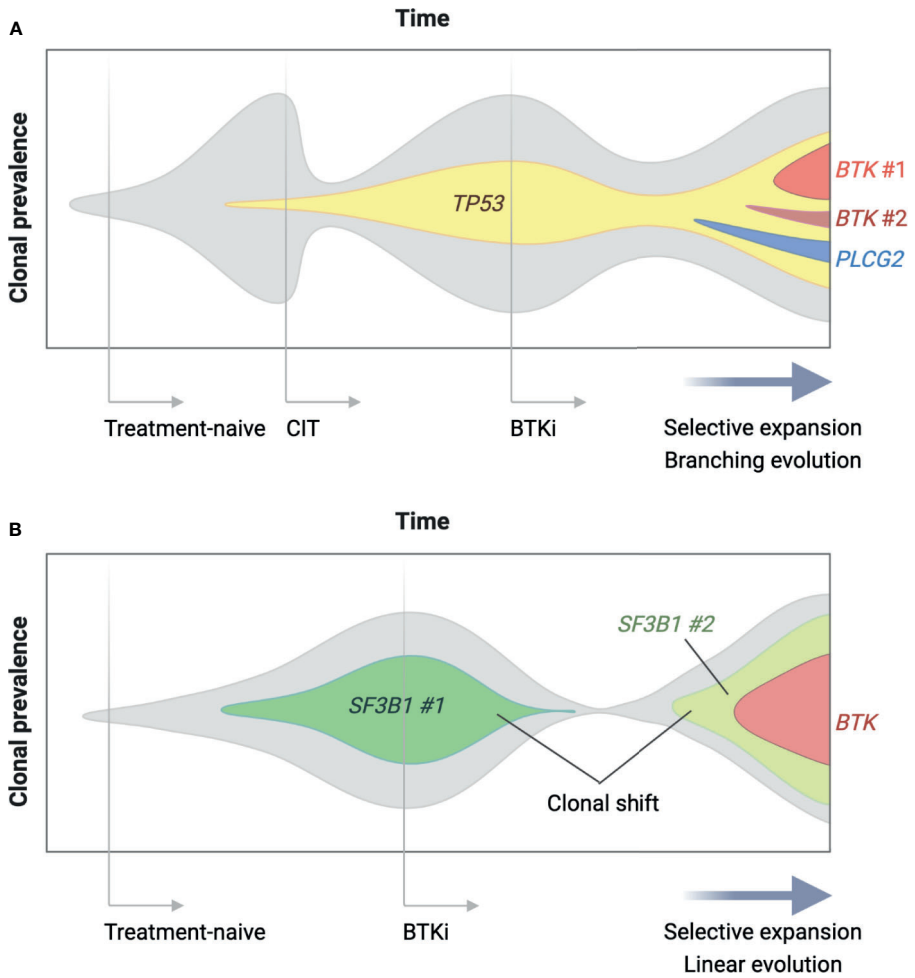


FIGURE 3 | Clonal evolution of BTKi-resistant CLL. Clonal architecture of CLL changes over time under the selective pressure of treatment and in the presence of driver gene mutations. Panel **(A)** is a schematic representation of a patient who was treated with chemoimmunotherapy (CIT) as first-line and a BTK inhibitor (BTKi) as second-line therapy for CLL. Two lines of therapy selectively expanded a parental clone with a driver gene mutation (*TP53* in this case), which became a parental clone of BTKi-resistant disease. Multiple *BTK* and *PLCG2* mutations arose after branching evolution and were detectable at relatively low allele frequency at the time of progression. Panel **(B)** shows a patient who underwent a major shift in clonal dominance from one clone (*SF3B1* mutation #1) to another (*SF3B1* mutation #2) during treatment with a BTKi. Linear evolution of the emerging clone led to a single dominant *BTK* mutation at progression detectable at high allele frequency.

ibrutinib plus venetoclax (NCT03943342, NCT03513562) and umbralisib plus ublituximab (NCT04149821). A phase 2 study of ibrutinib plus duvelisib in ibrutinib-resistant CLL was halted due to sudden death (NCT04209621). It will be of interest to determine the tolerability of novel combinations and the subsequent risk of clonal evolution in patients who became resistant to non-covalent BTKis.

Immune-directed therapy has an advantage over targeted agents as it can bypass resistance mechanisms intrinsic to tumor cells. In the modern era, allogeneic hematopoietic stem cell transplant has become safer and more accessible. Recent retrospective analyses of transplant outcomes in CLL reported 2-year PFS of 63% (125) and 3-year non-relapse mortality of 7% in patients who were previously treated with targeted agents (126). Treatment with CD19 CAR T can also achieve high rates of initial response in patients who failed ibrutinib (115). At present, none of the commercially available CD19 CAR T products are approved for the treatment of CLL. Further research is needed to define the durability of response to CAR T, minimize toxicities mediated by cytokine release, and improve access to adoptive cell therapy. Preclinical data indicate bispecific T-cell engager antibodies targeting CD3 and CD19 are efficacious against *BTK/PLCG2* mutant CLL cells *in vitro* and in patient-derived xenograft models (127). Several bispecific antibodies have entered clinical development to test dual targeting of CD3xCD19 (blinatumomab, NCT02568553), CD3xCD20 (REGN1979, NCT02290951), and CD3xCD22 (JNJ-75348780, NCT04540796).

Retargeting of BTK with agents with reversible covalent binding chemistry is an attractive strategy for the treatment of CLL. *BTK* mutation, found in ~80% of patients with ibrutinib resistance, validates the importance of this kinase in CLL. Reversible BTKis can inhibit the kinase in the presence of *BTK* C481 mutation (128). Five reversible BTKis have entered clinical trials to date (**Table 3**). Of these, pirtobrutinib is most advanced in development and most specific to BTK with little to no effect on other targets. In a phase 1/2 study, the overall response rate to pirtobrutinib was 71% for CLL patients with *BTK* C481 mutation and 66% for those with wild-type *BTK*, indicating that clinical activity was independent of *BTK* mutation status (129). MK-1026 (formerly ARQ-531) differs from other reversible BTKis for its ability to inhibit SYK and LYN and indirectly inhibit MEK1/ERK. Intriguingly, MK-1026 inhibited downstream signaling of DT40 cell lines transfected with *PLCG2* mutations, suggesting SYK/LYN inhibition has a role against *PLCG2* mutant clones (128). A phase 1 study of MK-1026 is expected to complete soon (130) and to be followed by a phase 2 study in hematologic malignancies including CLL (NCT04728893). CG-806 is a dual inhibitor of BTK and FMS-like tyrosine kinase 3 with internal tandem duplication, a mutation found in 30% of patients with acute myeloid leukemia (136). A phase 1 study of CG-806 is ongoing in CLL and non-Hodgkin lymphomas (NCT03893682) (136) besides two additional studies in myeloid diseases. Fenebrutinib (132) and vecabrutinib (135) showed acceptable tolerability in phase 1 studies, but were withdrawn from development in B cell malignancies. Fenebrutinib continues to be tested in multiple

TABLE 3 | Non-covalent BTK inhibitors.

	Pirtobrutinib (LOXO-305)	**MK-1026 (formerly ARQ-531)**	**CG-806**	**Fenebrutinib (GDC-0853)**	**Vecabrutinib (SNS-062)**
Company	Eli Lilly	Merck	Aptose	Genentech	Sunesis
IC_{50}*	3.15 nM (BTK WT) 1.42 nM (BTK C481)	0.85 nM (BTK WT) 0.39 nM (BTK C481)	8.4 nM (BTK WT) 2.5 nM (BTK C481) 0.8 nM (FLT3-ITD)	0.91 nM (BTK WT) 1.6 nM (BTK C481S) 1.3 nM (BTK C481R)	3 nM (BTK WT)#
Biochemical IC_{50} (Selectivity)*	BTK C481 1.42nM (1x) ITK 103nM (352 1x) EGFR >1,000nM (>700x) TEC 1,234nM (869x)	BTK C481 0.39nM (1x) ITK >10,000nM (>10,000x) TEC 5.8nM (14.9x) LYN 19nM (48.7x) SYK, not specified MEK1/ERK, indirect	BTK C481 2.5nM (1x) ITK 4.2nM (1.7x) EGFR >1,000nM (>400x) TEC >1,000nM (>400x)	BTK WT 2.3nM (1x) ITK >1,000nM (>400x) EGFR >1,000nM (>400x) TEC 1,000nM (>400x)	BTK WT 3nM (1x) ITK 14nM (5x) EGFR, not specified TEC 14nM (5x)
Clinical trials in CLL and B cell malignancies	Phase 1, 2	Phase 1, Phase 2 pending	Phase 1	Phase 1	Phase 1
Comment	Highly selective	Active against *PLCG2* mutation	Potent inhibitor of BTK and FLT3-ITD	Highly selective, Withdrawn from clinical development in B cell malignancies	Withdrawn from clinical development in B cell malignancies
References	(129)	(128, 130)	(131)	(132–134)	(135)

IC_{50} and fold selectivity over wild-type BTK (fenebrutinib and vecabrutinib) or mutant BTK (all others) within each BTK inhibitor; not for comparison across BTK inhibitors.

#IC_{50} *for BTK C481 has not been reported.*

BTK, Bruton's tyrosine kinase; EGFR, epidermal growth factor receptor; FLT3-ITD, FMS-like tyrosine kinase 3 with internal tandem duplication; IC_{50}, half maximal inhibitory concentration; ITK, Interleukin-2-Inducible T-cell Kinase; LYN, LYN tyrosine kinase; SYK, spleen tyrosine kinase; TEC, TEC kinase; WT, wild-type.

sclerosis (NCT04586023, NCT04586010). Vecabrutinib was withdrawn due to insufficient evidence of activity limiting its advancement to a phase 2 study (NCT03037645).

CONCLUSION

Data accumulated from clinical trials of covalent BTKis identified research questions critical for further optimization of the BTK targeting strategy. First, a better distinction of safety and efficacy profiles of individual BTKis is anticipated through ongoing randomized trials. Second, novel targeted combinations can achieve deep response and enable treatment cessation upon attainment of U-MRD in CLL. What remains to be addressed is durability of response and long-term safety of novel combinations, randomized comparisons with approved BTKi- or BCL-2-based regimens, and clonal dynamics traced with sequential genomic testing. Lastly, treatments capable of preventing or overcoming resistance to covalent BTKis are urgently needed. Several non-covalent BTKis with activity against BTKi-resistant disease are under investigation, highlighting the importance of BTK as a therapeutic target in CLL.

AUTHOR CONTRIBUTIONS

IA and JB performed literature review and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.