HHS Public Access

Author manuscript

Br J Haematol. Author manuscript; available in PMC 2023 January 14.

Published in final edited form as:

Br J Haematol. 2023 January ; 200(2): 137–149. doi:10.1111/bjh.18418.

Resistance to BTK inhibition in CLL and non-Hodgkin lymphoma

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Abstract

Bruton tyrosine kinase inhibitors (BTKi) have transformed the therapeutic landscape of chronic lymphocytic leukemia (CLL) and Non-Hodgkin lymphoma (NHL). However, primary and acquired resistance to BTKi can be seen due to a variety of mechanisms including tumor intrinsic and extrinsic mechanisms such as gene mutations, activation of bypass signaling pathways, and tumor microenvironment. Herein, we provide an updated review of the key clinical data of BTKi treatment in CLL, mantle cell lymphoma (MCL), and diffuse large B-cell lymphoma (DLBCL). We incorporate the most recent findings regarding mechanisms of resistance to covalent and non-covalent inhibitors, including ibrutinib, acalabrutinib, zanubrutinib and pirtobrutinib. We also cover the clinical sensitivity of certain molecular subtypes of DLBCL to an ibrutinib-containing regimen. Lastly, we summarize ongoing clinical investigations aimed at overcoming resistance via use of BTKi-containing combination therapies or the novel non-covalent BTK inhibitors. The review article targets an audience of clinical practitioners, clinical investigators, and translational researchers.

Short Summary

BTK targeted therapy in B cell lymphomas is a fast-moving field. Despite the remarkable efficacy of BTKi, several mechanisms of resistance have been identified that lead to lack of or shortened duration of response. In this review, we will summarize key clinical trials of BTKi in CLL, MCL, and DLBCL as well as the unique mechanisms of resistance within these histological subtypes. We also discuss efforts to overcome resistance via combination targeted therapies and use of novel non-covalent BTKi therapy.

Keywords

B-cell receptor; BTK; ibrutinib; CLL; non-Hodgkin lymphoma

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Author Contribution

SN and YLW reviewed and compiled the literature, wrote the paper, and designed the tables. YLW and AV made the graphic designs for figures.

Competing Interest

The authors do not have any financial conflicts of interest to disclose.

Introduction

B cell non-Hodgkin lymphomas and leukemias make up ~3.5% of all cancer diagnoses in the United States and consist of a heterogeneous group of malignancies. Historically, the backbone of treatment has been cytotoxic chemotherapy, but in recent decades, targeted therapies have been incorporated into earlier line settings. The B-cell receptor (BCR) signaling pathway is a key driver of B cell malignancies (Fig. 1) and agents disrupting this pathway have changed the landscape of management in both the frontline and relapsed/refractory (R/R) settings. A key target is the Bruton tyrosine kinase (BTK), a component of early BCR signaling pathway. In normal B-cells, BTK activation, reflected by its phosphorylation, triggers downstream events and ultimately, the activation of nuclear factor kappa B (NFκB) pathways enabling increased B-cell survival, proliferation, differentiation into plasma cells, and subsequent antibody production [1, 2] (Fig. 1). In this review, we will discuss the use of Bruton tyrosine kinase inhibitors in B cell malignancies and cover the mechanisms of resistance. These understandings not only help improve care and survival of patients treated with BTK inhibitors but also help direct BCR-targeted therapeutic strategies for future clinical trial design.

The first in class BTK inhibitor is ibrutinib, an orally available small molecular inhibitor of the kinase. At the molecular level, the drug binds covalently to the cysteine 481 at the ATP binding site of BTK (Fig. 2) to inhibit its activity and downstream signaling cascade [3, 4]. At the cellular level, the consequence of BCR inhibition is primarily cell proliferation deceleration rather than direct cell killing [5, 6]. Aside from cell proliferation, ibrutinib has demonstrated inhibitory effects on cell adhesion and migration of malignant cells as well as on the tumor microenvironment including T cells and mesenchymal stromal cells [7–11]. On the systemic level, inhibition of cell adhesion results in dislodging of CLL cells from the lymph node and their relocation (or release) to the periphery. Inhibition of cell migration, on the other hand, prevents homing of the circulating CLL cells back into the lymph nodes leading to lymphocytosis [12].

Ibrutinib has been FDA approved for use in various B cell malignancies including MCL, CLL, lymphoplasmacytic lymphoma/ Waldenström's macroglobulinemia and marginal zone lymphoma (Table 1 summarizes the trials that led to the approval). Ibrutinib was first approved in MCL based on phase II data revealing an overall response rate (ORR) of 68% in the relapsed/refractory setting. This response rate is close to that of highly myelosuppressive salvage chemotherapy but with a much more favorable toxicity profile [13, 14]. In CLL, ibrutinib demonstrated an ORR of 71% in a phase I/II study of heavily pre-treated patients. Phase III data comparing ibrutinib to chemotherapy confirmed this superior rate and durability of response, particularly for those patients with del(17p) and other high-risk genetic features such as presence of del(11q) and unmutated immunoglobulin heavy chain (UM-IGHV) [15, 16]. Notably, ibrutinib does not produce high rates of complete response (CR), probably related to its inability to produce significant apoptotic activity as a monotherapy [5, 7].

Despite its remarkable clinical efficacy, resistance to ibrutinib does develop. Conceptually, resistance is classified as primary resistance and secondary (acquired resistance) from a

clinical perspective. Primary resistance is seen in patients who fail to respond to ibrutinib upfront whereas secondary resistance is seen in patients who initially respond but then relapse. Meanwhile, predisposition to resistance refers to pathological parameters present at baseline. Although they are not associated with upfront failure to respond, rather, they increase the risk of later disease progression while patients are on treatment. From the biological point of view, the mechanisms of resistance can be intrinsic or extrinsic to the tumor cells. Intrinsic mechanisms include mutations and activation of bypass pathways leading to restoration of tumor cell survival and proliferation. Extrinsic factors include protection of tumor cells through cytokines or cell-cell contact with the tumor microenvironment. In this article, we primarily focus on intrinsic mechanisms.

Resistance to ibrutinib may be overcome by other newer BTK inhibitors. As a prototype, ibrutinib is followed by an expanding class of BTK inhibitors with either irreversible (covalent) or reversible (non-covalent) BTK binding mechanism [17]. Second generation covalent BTKi, acalabrutinib and zanubrutinib, have generally more favorable toxicity profiles than ibrutinib but are susceptible to similar resistance mechanisms as ibrutinib [18, 19]. Meanwhile, novel noncovalent BTKi including pirtobrutinib (LOXO-305) and ARQ-531 (MK-1026) do not form a covalent bond with BTK molecule and have demonstrated activity in ibrutinib resistant disease [20, 21].

In this article, we will cover B-cell malignancies including CLL, MCL and DLBCL. We will first review ibrutinib clinical data, then the primary and secondary resistance mechanism. We will summarize results of the clinical trials involving combination therapies and newer BTK inhibitors. The most recent developments in the understanding of resistance to newer BTK inhibitors will be discussed.

Chronic Lymphocytic Leukemia

CLL and small lymphocytic lymphoma (SLL) are a spectrum of a single mature B cell malignancy presenting with predominantly lymphocytosis and/or nodal disease, respectively. Constitutive activation of the BCR signaling pathway and upregulation of the BCL2 anti-apoptotic protein leads to increased cell survival and proliferation. The advent of ibrutinib, has drastically changed the landscape of CLL management in recent years. In addition to inhibiting the BCR pathway, the drug also promotes mobilization of malignant cells out of the protective nodal niche of nodal or bone marrow microenvironment into circulation, leading to decreased lymphadenopathy [22].

Clinical data with ibrutinib

Historically, chemoimmunotherapy (CIT) had been the backbone of treatment in symptomatic patients with CLL. While fixed duration CIT demonstrates a 90% response rate, more than half of patients have disease progression at 5 years on this regimen and treatment carries significant toxicity risk [23].Ibrutinib has demonstrated superior duration of response over CIT, particularly in those with the high-risk features such as del(17p)/TP53, in addition to a more tolerable side effect profile [24, 25] (Table 2). Additionally, high rates of response are seen with BTKi even in patients with multiple prior lines of chemotherapy

[25, 26]. However, ibrutinib monotherapy fails to achieve deep responses in 71–90% of patients with CLL due to absence of direct killing [27].

Predisposition to future relapse

Primary resistance to ibrutinib develop in 10–16% of cases and the mechanisms are mostly unknown. Predominant baseline CLL mutations in treatment naïve patients are mostly unrelated to the BCR pathway, including mutations in ATM, BIRC3, NOTCH, SF3B1, and TP53 [28, 29]. However, more is known about what genetic characteristics predispose to future relapse. Baseline molecular and cytogenetic features including del(17p)/TP53 and complex karyotype (≥3 chromosomal abnormalities) increase the risk of disease progression in CLL patients treated with ibrutinib [30–32]. TP53 abnormality, in particular, is the only independent molecular factor that was included in a four-factor model that predicts inferior overall survival (OS) and progression-free survival (PFS) on ibrutinib treatment [33]. Thus, patients with del(17p)/TP53 aberrations continue to have worse prognosis even in the era of targeted therapies. In addition, del(18p), occurs in 2–7% of cases in untreated population, was found at a particularly high frequency in 56% (5 of 9 patients) of ibrutinib-relapsed patients and is also associated with the development of BTK mutation [30].

Secondary resistance to ibrutinib

Disease relapse/progression develop in 10–18% of CLL patients [32, 34–36]. In ~70 % of cases, this is due to mutations at C481 residue of BTK which disrupts the covalent binding of ibrutinib to the BTK kinase domain [30, 37–41] (Fig. 2A and major mutation mechanisms summarized in Table 4). The cysteine residue is most commonly mutated to serine (C481S), but mutations to other amino acid residues are also seen in practice, including C481Y/R/F/G (tyrosine arginine/phenylalanine/glycine, approximately in the order of decreasing frequencies) [32, 36, 42, 43].

C481 and other mutations are clustered in the tyrosine kinase domain of the BTK protein (Fig. 2B). T316A, however, is located at the Src homology 2 domain. Cells carrying BTK T316A showed resistance to ibrutinib at both cellular and molecular levels to a similar extent as BTK C481S [44]. The functional impacts of other mutations have not yet been demonstrated (Fig. 2B).

The next most common mechanism is gain of function PLCG2 missense mutations, seen in 11% of ibrutinib resistant cases (Fig. 1 and Kadri S et al 2017). PLCG2 is the enzymatic substrate of BTK, its activation enables CLL cell proliferation independent of BTK control [30, 36, 38, 41]. Interestingly, PLCG2 mutations often co-exist with the BTK mutations [30, 36, 38, 41, 45]. In the remaining ~20% of relapsed/progression cases, del (8p) leading to loss of TRAIL-R was reported in 3 of 5 ibrutinib resistant patients [46]. Besides the genetic mechanisms, epigenetic changes have been shown to play a role in conferring the CLL resistance [47].

Secondary resistance to newer covalent BTK inhibitors

Second generation covalent BTK inhibitors have been developed including acalabrutinib and zanubrutinib. These newer BTKi demonstrated similar or improved efficacy and superior

toxicity profiles in phase III comparison to ibrutinib in the R/R setting [48, 49]. Resistance to these BTKi is also driven by mutations in the BTK and, to a much less degree, in the downstream enzyme PLCG2. For patients treated with acalabrutinib, the most frequent mutation is BTK C481 [19] (Fig.2B). However, different from ibrutinib, in cases of zanubrutinib, BTK L528W seems to be the more predominant mutation that occurs in more cases and at a much higher variant allele frequency than the C481 mutation [18, 50] (Fig.2 A&B). Interestingly, in this small cohort, L528W may be present in the same cells as the C481 substitutions [50]. Finding multiple BTK mutations in the same patient samples has been reported previously in ibrutinib-relapsed cases as well [30].

Non-covalent BTKi therapies

As mentioned in the Introduction, one way of overcoming resistance mediated by BTK C481 mutation is the use of reversible, noncovalent BTKi such as Pirtobrutinib [20, 21]. Pirtobrutinib is highly selective for BTK and significantly inhibits BTK phosphorylation, cell proliferation and tumor growth in mice [51, 52]. It binds to BTK but does not depend on C481. Therefore, this agent is predicted to overcome ibrutinib resistance. Both pirtobrutinib and ARQ531, another non-covalent BTK inhibitor, are active in CLL/SLL with either C481 mutated or unmutated BTK enzyme [20, 21]. The ORR in multiple relapsed/refractory CLL is 63%, of which the majority had prior BTKi exposure [21]. Ongoing trials evaluating this agent in the front line setting and as part of combination regimens are underway (NCT05023980 and NCT04965493).

Secondary resistance to non-covalent BTKi

Acquired resistance to pirtobrutinib has emerged. Several non-C481mutations were newly acquired in patients with progressive disease. These include V416L, A428D, M437R, T474I, and L528W that are clustered in the tyrosine kinase domain (Fig.2B). Of note, L528W was commonly found in patients resistant to the covalent inhibitor ibrutinib, zanubrutinib, and non-covalent pirtobrutinib. Together with A428D, L528W is predicted by the in vitro assay to be universally resistant to covalent and non-covalent BTK inhibitors as well [53]. Apparently, positions of BTK mutations may be different for different BTK inhibitors. Therefore, sequencing the entire BTK gene rather than hot spot locations may help reveal less frequent mutations, accumulate data for further understanding and foster ongoing research in addressing next line treatment.

Overcoming resistance with combination therapies

Deepening response by achieving undetectable minimal residual disease (uMRD) would perhaps reduce the emergence of resistance. uMRD correlates with improved survival in CLL treated with CIT and venetoclax-based regimens, particularly in patients with high-risk disease such as those with del(17p)/TP53 or del(11q) [54, 55]. While BTKi monotherapy fails to achieve high rates of deep response, this can be achieved with BTKi combination therapies.

Addition of anti-CD20 monoclonal antibody to ibrutinib induces higher rates of undetectable MRD, although it did not demonstrate statistically significant improvements in response or PFS [56]. Similarly, addition of obinutuzumab achieved slightly higher rates of complete

response in post hoc analysis of acalabrutinib based treatments which correlated with improvement in PFS [57]. BCL2 inhibitor venetoclax delivers direct killing by enhancing rate of apoptosis of the resting subpopulation of CLL cells [6]. When combined with ibrutinib, the regimen improved rate of uMRD from <10% to 55–75% in the frontline setting [58–60]. While long term survival data is not yet available, patients who achieved uMRD in this study showed a promising 30 month PFS of ≥ 95% [60]. However, it remains to be seen if combination therapies may overcome resistance in patients who progress on BTKi monotherapy. Clinical efficacy of BTKi containing combination therapies is summarized in Table 2 which bring promises for long-term disease control and a potential cure [58–60].

Mantle Cell Lymphoma

MCL is a mature B cell neoplasm characterized by a t(11;14) translocation involving the cyclin D1 (CCND1) cell cycle regulator gene and immunoglobulin heavy chain locus (IGH) leading to overexpression of cyclin D1 [61, 62]. However, presence of other mutations are frequently seen in treatment naïve MCL contributing to the clonal proliferation and resistance mechanisms of this disease [62]. Activation of BCR signaling and overexpression of BTK has been observed in MCL cells that served as the main rationale for use of Ibrutinib [63, 64].

Clinical data

Patients with R/R MCL after standard of care frontline intensive chemotherapy therapy historically have had poor prognosis. Those with TP53-mutated disease have particularly poor outcomes, with a median overall survival (OS) of less than 2 years compared to 10.2 years in those with unmutated wild-type TP53 [62, 65]. While ibrutinib monotherapy has shown efficacy in the R/R setting, 30% of patients fail to respond and 60% achieve only a partial response [66]. Moreover, outcomes after ibrutinib failure are dismal [67]. The second generation covalent irreversible BTKi did not seem to improve outcomes which can be predicted from the chemical binding mechanism [68].

Primary resistance

Primary resistance to ibrutinib has been reported in 32% of patients with MCL [13]. Compared to CLL, patients with MCL have higher rates of high-risk mutations at baseline that predispose to treatment resistance and relapse with CIT. These include the ATM and TP53 gene mutations, seen in 44% and 27% of patients, respectively, according to a meta-analysis of 2045 samples [62]. Several gene mutations are associated with primary ibrutinib resistance in cell lines including CARD11 and CCND1 [69, 70] (Fig.1).

Primary resistance is also mediated by upregulation of other oncogenic pathways, including activation of the alternative NFκB pathway with associated mutations in MAP3K14, TRAF2, TRAF3, BIRC3 [71] (Fig. 1). Other bypass pathways including PI3K/AKT, MEK/ERK, canonical NFκB activation have also been described as a mechanism of primary resistance, as well as ROR1 overexpression and MYC activation mainly based on cell line studies [72–74]. Resistance may also be mediated by BCR-induced upregulation of RAC2,

a master control of the cell adhesion program, counteracting ibrutinib's ability to dislodge malignant cells from nodal stroma [75].

Secondary resistance

With single agent ibrutinib in the R/R setting, 69% of patients progress within 2 years of therapy [14]. Unlike CLL, mutations in BTK are rarely detected at disease progression after ibrutinib [67, 76, 77]. Instead, newly acquired recurrent mutations were found in the cyclin D1 (CCND1) gene and CDKN2A/MTAP genes, closely located on 9p, in a study of small number of ibrutinib resistant patients.

Secondary resistance, similar to primary resistance, mainly involve bypass pathways of the B cell receptor signaling. Activation of PI3K/AKT/mTOR pathway (Fig.1) was consistently identified by several studies in cell line models [64, 78, 79] as well as in a patient-derived xenograft model [78]. In the xenograft model, it is further demonstrated that adding PI3K or mTOR inhibitors to ibrutinib significantly slows down the growth of the ibrutinib-resistant tumor [78]. Moreover, in clinical samples collected from patients, in addition to pathway changes (including mTOR), downstream metabolic reprogramming towards oxidative phosphorylation and glutaminolysis was identified as one of the main changes in ibr-resistant samples versus ibr-sensitive ones [77].

Overcoming ibrutinib resistance with non-covalent BTK inhibitors and combination therapies

Despite the rare occurrence of acquired BTK mutations in MCL, non-covalent BTKi pirtobrutinib has also demonstrated activity in ibrutinib-resistant MCL with an ORR of 52% in R/R cases [21, 80]. This may have to do with a higher target binding and a longer exposure of BTK to the drug [80]. Head-to-head ~~to~~ comparison of this agent versus early generation BTKi in BTK-naïve patients with MCL is ongoing (NCT04662255) (Table 3).

Much like CLL, combination therapies can deepen response with ibrutinib. Combination with venetoclax or rituximab +/− lenalidomide have demonstrated deeper responses than single agent BTKi, with higher CR rate of 44–71% compared to 27% with single agent ibrutinib. U-MRD is as high as 67–68% [66, 80–82]. Median PFS was not reached in the dose finding cohort of venetoclax/ibrutinib combination and phase III investigation is ongoing [83] (Table 3). Combination of ibrutinib with rituximab +/− lenalidomide demonstrated 12-month PFS to 57–75% in the R/R setting [82, 84]. Combination of ibrutinib with novel anti-ROR1 monoclonal antibody, cirmtuzumab is also promising in R/R MCL with ORR of 80% and CR of 35% based on preliminary phase I/II data [85]. CDK4/6 inhibitor palbociclib, which prolongs the G1 cell cycle arrest has also been investigated in combination with ibrutinib and demonstrated an ORR of 67% and CR of 37% in patients with R/R MCL [86] (Summarized in Table 3).

Lastly, incorporating BTKi into the frontline setting has shown promising activity as either part of a chemotherapy-free regimen for elderly/frail patients or induction therapy prior to consolidative CIT in medically fit patients [87, 88] (Table 3). In a single center phase II study of older patients with newly diagnosed classic MCL (excluding histologically aggressive variants, Ki67<50%), first line treatment with ibrutinib and

rituximab demonstrated a 3 year PFS of 87% [88]. In a dose finding phase I/II study evaluating frontline ibrutinib combined with obinutuzumab and venetoclax, 87% and 100% patients achieved CR and uMRD, respectively, with a 1 year PFS of 93% [89]. In a large phase III study of 523 patients aged ≥65 years old, ibrutinib was combined with lower intensity chemotherapy regimen bendamustine/rituximab in the upfront setting. Median PFS was improved by a remarkable 2.3 years with fewer patients requiring subsequent therapy (20% vs. 41%, respectively) at 7-year follow up [90]. These and other ongoing studies of novel BTK agents, combination therapies, and incorporation of targeted therapies in earlier line settings will likely change the treatment paradigm of MCL and lower occurrence of BTK resistance.

Diffuse Large B-cell Lymphoma

Molecular subclassification of DLBCL

Diffuse large B cell lymphoma represents a heterogeneous disease derived from germinal or post germinal center B cells. Gene expression profiling has been used to separate these into distinct subgroups based on the cell of origin, those related to germinal center B cell (GCB) and those related to activated B cell (ABC) [91, 92]. ABC-subtype defined by gene expression profiling is closely related to, but not equivalent to the non-GCB subtype defined by immunohistochemistry [93]. The ABC subtype is characterized by chronically active NFκB signaling downstream of the BCR pathway (Fig. 1) which has served as the rationale for investigational BTKi therapy in this subtype [94–96]. In contrast, GCB-DLBCL is more dependent on PI3K and BCL2 signaling pathways.

In the era of emerging precision medicine, many efforts have been made to sub-classify DLBCL by genetic and biological features which are potentially amenable to targeted therapeutic agents. Hundreds of DLBCL tumors have been characterized using multi-omic technologies interrogating point mutations, indels, chromosomal structural alterations and gene expression profiles [97–99]. In the recent LymphGen algorithm developed by Staudt's group, DLBCL is subclassified into 7 groups with 37% of tumors unclassifiable [100]. Among molecular subgroups is MCD, which is enriched with gain of function of MYD88 L265P and/or CD79B mutations (Fig. 1). MYD88 is a key molecule mediating Toll-like receptor signaling while CD79B is part of the B-cell receptor complex that plays a role in maintaining the cell surface expression of the receptor [95]. Other subgroups include BN2 defined by BCL6 fusion and NOTCH2 mutations, EZB enriched for EZH2 mutations and BCL2 translocations and N1 characterized by NOTCH1 mutations.

In a separate study from Shipp's group, the consensus clustering algorithm subclassified the tumors into C1-C5 clusters [98]. There are substantial molecular and biological similarity and overlaps between LymphGen and the consensus clusters categories. For example, MCD is related with C5, BN2 with C1, and EZB with C3.

Finally, to reduce the molecular classification into routine clinical practice, a UK network subclassified DLBCL into 6 molecular groups based mostly on mutations detected in FFPE tissues by targeted sequencing of ~300 genes instead of the multiomic whole genome sequencing. The subgroups are named after the major genetic features. Grossly, the MYD88

group is the counterpart of MCD/C5 group, NOTCH2 group corresponds to BN2/C1 while BCL2 group is similar to EZB/C3.

While these various molecular subgroup classifications are not yet widely available for routine clinical practice, use of NGS testing as a correlative in clinical trials is rapidly increasing in order to determine which particular subtypes benefit from therapies targeted to the underlying molecular pathology (Also see below).

Clinical data

Patients with ABC DLBCL have significantly inferior survival with standard R-CHOP based treatment in comparison to those with GCB subtype [96]. Since ABC lymphomas are characterized by chronic active BCR signaling, clinical investigations were started with correlative subtype determination of DLBCL. In the initial trial, ibrutinib has demonstrated activity predominantly in the ABC subtype with ORR of 37% versus only 5% in those with GCB subtype [101].

Encouraged by these initial results, the phase III PHOENIX trial evaluated ibrutinib vs. placebo in combination with frontline R-CHOP in patients with non-GCB subtype disease and failed to show an overall survival benefit of ibrutinib plus R-CHOP. However, it did demonstrate event free survival (EFS), PFS, and OS benefit in the <60-year-old population [102]. This lack of benefit in older patient population was attributed in part to increased toxicity with combination therapy thereby limiting optimal CIT dosing. Later, a more in-depth subgroup analysis in the younger patients revealed that ABC lymphomas of MCD and N1 subtype had a 3-year EFS and OS of 100% with ibrutinib plus R-CHOP compared to 42.9 and 50% in the R-CHOP alone arm. While this study was not powered to assess differences in response or survival among these subgroups, it is hypothesis generating and warrants larger studies to identify which genetic subgroups will have better outcomes on BTKi-containing therapy [103].

Primary sensitivity and resistance

Notably, the MCD and N1 subgroups only make up 14% and 2.8% of the total of 574 DLBCL tumors, respectively [97, 98, 100]. Therefore, only about 1/5 of patients seem to benefit from ibrutinib-containing CIT regimen and a significant number of ABC tumors did not respond well to ibrutinib. In the GCB subtype, ibrutinib is not as active as in ABC DLBCL as demonstrated in vitro and in clinical studies [101, 104] (107, 110, 109). GCB DLBCL is featured by activation of the PI3K/AKT pathway enabling cell survival and proliferation [105, 106] (Fig.1).

Regarding molecular mechanisms of primary resistance, activating CARD11 and inactivating mutations in TNFAIP3 (aka A20), a negative regulator of NFκB, are associated with primary ibrutinib resistance in a phase I/II clinical trial [95, 107, 108] (Fig.1). These genes act downstream of BTK, promoting NFκB activity with no regards to the upstream BTK activity. Mutations in KLHL14 gene is also associated with primary resistance [109] (Fig.1). KLHL14 is a negative regulator of the BCR signaling. It promotes the ubiquitination and degradation of the BCR subunits IgM, CD79A, and CD79B. KLHL14 inactivating

mutations is present in ~11% of DLBCL tumors, especially in the MYD88/CD79B double mutant (MCD) genetic subtype of ABC DLBCL.

Regarding sensitivity to other BTK inhibitors, in a phase II clinical trial evaluating zanubrutinib in R/R DLBCL, the ORR to zanubrutinib was 46.2% (6/13) and 28.6% (6/21) in patients with or without CD79B mutations, and 40% (4/10) and 33.3% (8/24) in patients with or without MYD88 L265P mutations. Notably, these differences in ORR was not statistically significant [110]. Larger studies will be needed to investigate the association of genetic biomarkers with response to zanubrutinib and other BTK inhibitors.

Secondary resistance

Clinical experience of DLBCL treated with BTKi is limited, and long-term follow-up is still lacking. Thus, secondary resistance to ibrutinib in DLBCL is mostly studied in cell line models. In DLBCL cell lines cultivated to become resistant to ibrutinib, BTK and PLCG2 mutations were not identified, however, PI3K/AKT/mTOR signaling is upregulated leading to increased tumor cell survival [111, 112] (Fig. 1). In addition to pathway alterations, the role of epigenetic mechanisms was recently revealed by a study using ABC DLBCL cell lines [47]. Interestingly, RAC2, the small GTPase, was identified as the mediator of the epigenetic ibrutinib resistance. As mentioned above, RAC2 is also involved in primary ibrutinib resistance in MCL, but through enhanced cell adhesion [75]. This molecular commonality between DLBCL and MCL may worth further investigation. Long term follow-up on patients treated with BTKi-containing regimens is still in progress. Mechanisms of secondary resistance in patients who relapse after initial BTKi response remain to be seen.

Trials with newer BTK inhibitors and other targeted therapies

While certain DLBCL genetic subgroups appear to have increased ibrutinib sensitivity, the role of BTKi in the frontline and R/R setting remains unclear. Investigation of 2nd generation covalent BTKi combined with CIT, such as the ESCALADE study (NCT04529772) is still ongoing.

Additionally, reversible non-covalent BTKi provide an alternative to patients who are intolerant to covalent BTKi or who develop progressive diseases during therapies [21]. Compared to covalent BTKi, pirtobrutinib has demonstrated higher BTK selectivity and more durable target inhibition over 24 hours on pharmacokinetic studies [51]. This suggests a potential role for this agent in more proliferative B cell malignancies like DLBCL where earlier generation BTKi have been less efficacious. In a phase I/II evaluation, 24% of patients with R/R DLBCL responded to pirtobrutinib [21].

As mentioned above, upregulation of AKT/PI3K serves as a bypass pathway for tumor survival. PI3Ki demonstrated in vitro activity in BTKi resistant cells lines [112]. However, in an early clinical trial investigation, durability of response to umbralisib was poor and the study was prematurely closed [113]. It remains to be seen if other PI3Ki would have clinical activities in the BTKi-resistant patient population.

Conclusion

Targeting the B cell receptor signaling pathway via BTK inhibition has played a pivotal role in treatment of B cell malignancies. In this review, we summarized the clinical data on using ibrutinib in CLL, MCL and DLBCL. The mutational mechanisms of primary and secondary resistance to ibrutinib and novel covalent and non-covalent BTK inhibitors are summarized in Table 4 and Fig. 1 and 2. We also reviewed the current clinical investigations on overcoming such resistance with either newer BTK inhibitors or combination therapies. Due to its success as a class, several more BTK inhibitors as well as BTK degraders are being developed. Since single agent therapy is unlikely to completely eliminate the malignant B-cell clones, the combination therapies either with other class of targeted therapies or with antibody-based therapies would stand a better chance for a durable remission.

Additional clinical assessments of non-covalent BTKi in upfront setting and as part of combination regimens are still underway. Optimal sequencing of covalent and non-covalent BTKi and combination regimens has yet to be determined. Looking forward, understanding the resistance mechanisms and sensitive detection of emergent resistant clones during therapies would help guide new therapeutic development, therapeutic sequencing, and rational drug combination in the future.

Acknowledgement

This work is supported by NCI R21CA263415 to YLW. We apologize that we have to leave out some important publications to meet the limitations of number of cited references.

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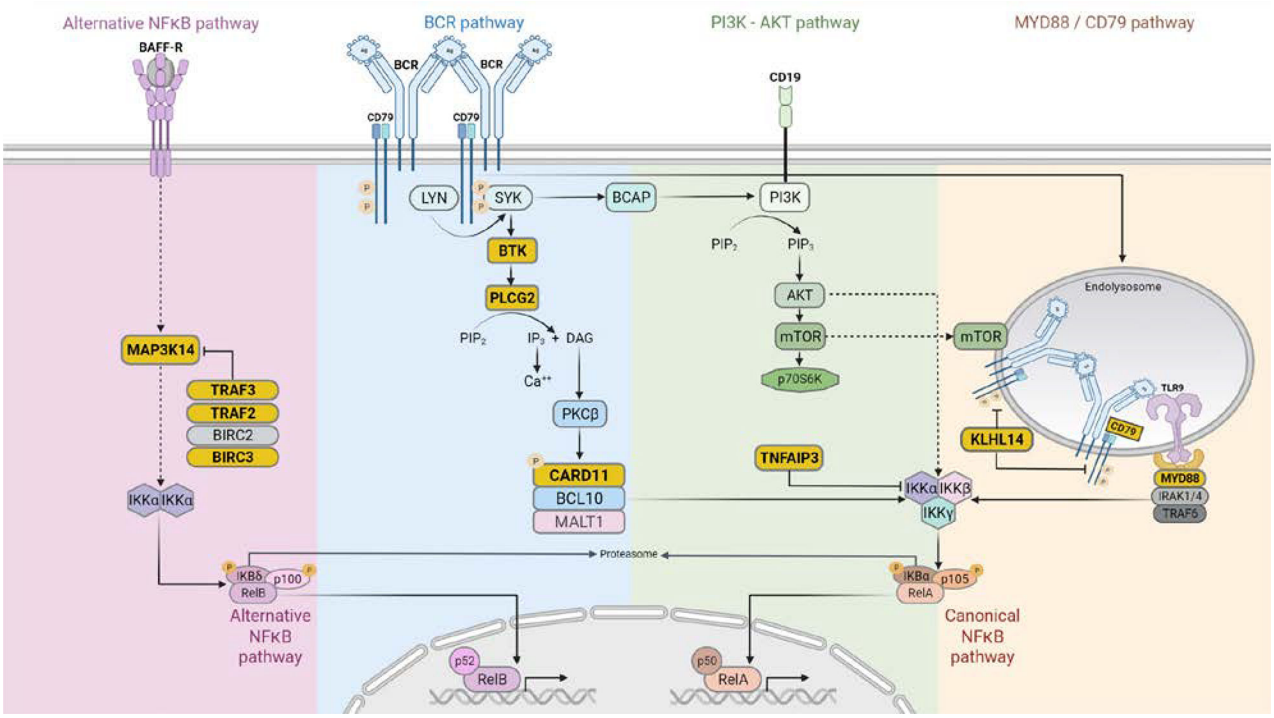

Fig. 1. Resistance-Relevant mutations and signaling pathways in CLL, MCL and DLBCL.

Key components of the signaling pathways including BCR, PI3K-AKT, MYD88/CD79, canonical NFκB and alternative NFκB are depicted. Mutated genes along these pathways associated with BTKi resistance are highlighted in yellow. Also see Table 4 for their relationship with the disease and resistance setting. Positive interactions are indicated by arrows, indirect interactions by dashed arrows, and inhibitory interactions by T-bars. The graph was generated with BioRender.

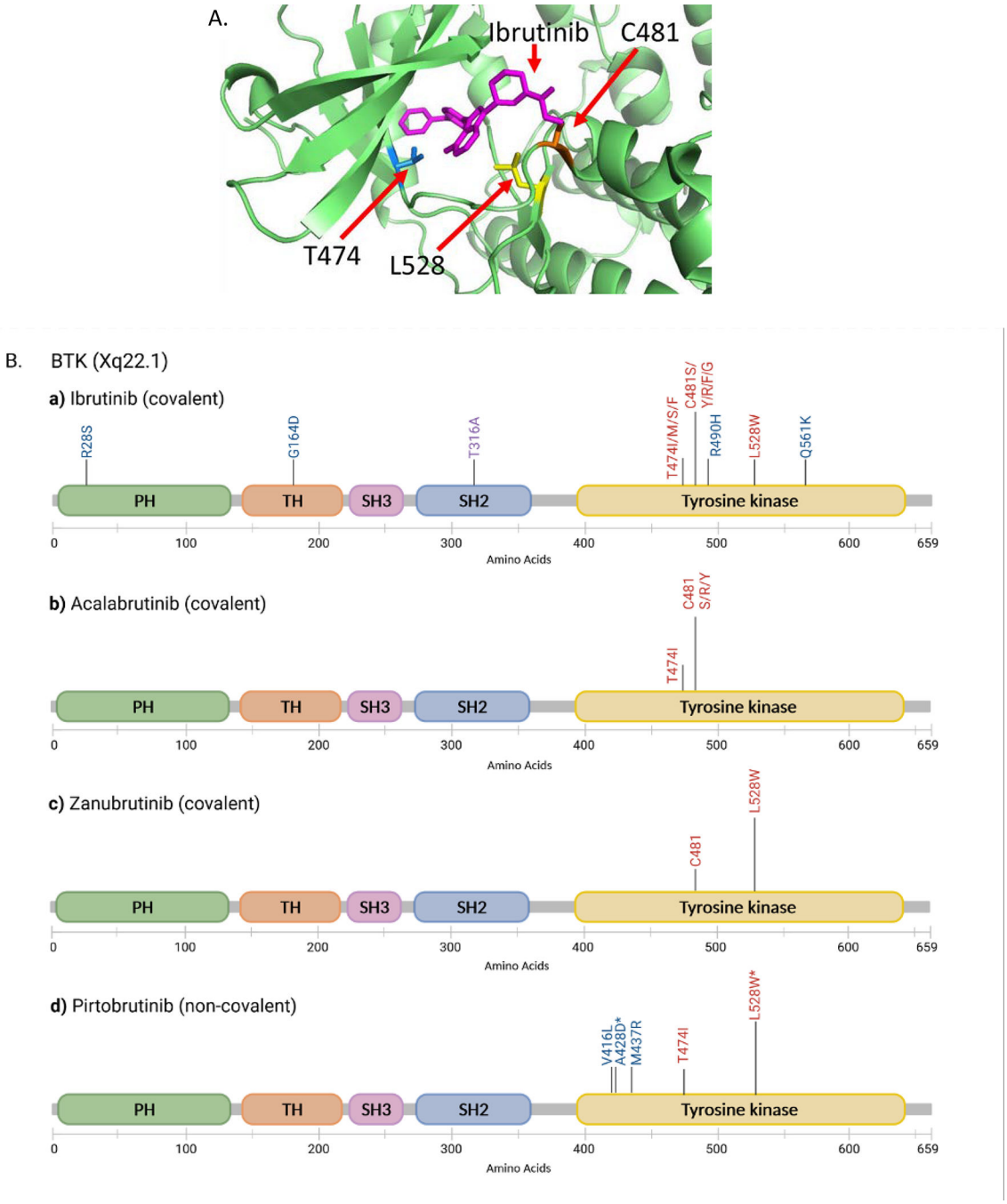

Fig.2. Map of clinically documented BTK mutations.

A) 3D mapping of common BTK missense mutations associated with resistance to multiple BTK inhibitors (previously published in Sharma et al, PMID: 27626698). **B)** Clinically reported BTK mutations in patients treated with ibrutinib, acalabrutinib, zanubrutinib and pirtobrutinib. Note that not all mapped mutations have been functionally validated. PH, pleckstrin homology domain; TH, Tec homology domain; SH, Src homology domain; Tyrosine kinase, tyrosine kinase domain. The height of the vertical bars represents the

relevant abundance of the particular variants. *Experimentally predicted to be resistant to multiple BTK inhibitors. The graph was generated with BioRender.

Table 1.

FDA Approved Indications for BTKi Therapy

Clinical Trial that led to FDA Approval	BTKi	Date of approval	Disease Setting
Chronic Lymphocytic Leukemia			
Byrd 2013 [114]	Ibrutinib	2/12/14	Relapsed/Refractory disease
Byrd 2014 [15]	Ibrutinib	7/28/14	Del(17p) disease
Burger 2020 [16]	Ibrutinib	3/4/16	Treatment Naive
Sharman 2020 [57] Ghia 2020 [115] Byrd 2021 [48]	Acalabrutinib	11/21/19	Treatment Naïve and Relapsed/Refractory disease
Tam 2019 [116]	Zanubrutinib	Not FDA approved Included in NCCN guidelines	Treatment Naïve and Relapsed/Refractory disease
Mantle Cell Lymphoma			
Wang 2012 [13]	Ibrutinib	11/13/13	Relapsed/Refractory disease
Wang 2018 [68]	Acalabrutinib	10/31/17	Relapsed/Refractory disease
Tam 2021 [117]	Zanubrutinib	11/14/19	Relapsed/Refractory disease
DLBCL			
Wilson 2015 [118]	Ibrutinib	Not FDA approved Included in NCCN guidelines	Relapsed/Refractory non-GCB DLBCL
Lymphoplasmacytic Lymphoma			
Treon 2015 [119]	Ibrutinib	1/29/15	Relapsed/Refractory disease
Dimopoulos 2017 [120]	Ibrutinib	8/27/18	Treatment Naïve and Relapsed/Refractory disease, with rituximab
Tam 2020 [121]	Zanubrutinib	9/1/21	Treatment Naïve and Relapsed/Refractory disease
Marginal Zone Lymphoma			
Noy 2020 [122]	Ibrutinib	1/19/17	Relapsed/Refractory Disease
Opat 2020 [123]	Zanubrutinib	9/15/21	Relapsed/Refractory disease

Table 2.

Ibrutinib based Regimens in CLL in the Frontline Setting

Study	Regimen	N	Phase	PFS (median follow up in months)	uMRD in peripheral blood
Woyach, 2018 [124]	BR vs Ibr vs Ibr-R	208	II	74% vs 87% vs 88% (24)	8% vs 1% vs 4%
Moreno 2019 [125]	Ibr + O vs Chl + O	229	III	90% vs 31% (30)	35% vs 25%
Shanafelt 2019 [126]	Ibr+R vs FCR	529	III	PFS 89.4 vs 72.9% (36)	8.3 vs 59.2%
Tam 2019 [58] Wierda 2021 [60]	Ibr + Ven	164	II	95% (12) *	75%
Burger 2020 [16]	Ibr vs Chl	269	III	70% vs 12% (60)	N/A
Kater 2021 [59]	Ibr + Ven vs Chl-O	211	III	NR vs 21 **months** **	54.7% vs 39%

* end point of disease free survival

** median progression free survival

Ibr: Ibrutinib; R: Rituximab; FCR: Fludarabine/Cyclophosphamide/Rituximab; BR: Bendamustine/Rituximab; O: Obinutuzumab, Chl: Chlorambucil; Acal: acalabrutinib; Ven: Venetoclax; NR: not reached; uMRD: undetectable MRD

Table 3.

Ibrutinib Based Regimens in MCL in R/R and Frontline Settings

Study	Regimen	N	Phase	PFS (median follow-up in month)	ORR (CR)
R/R MCL Single Agent BTKi					
Wang 2013 [13, 14]	Ibr	111	II	21% (24)	67% (23%)
Dreyling 2016 [127]	Ibr vs. temsirolimus	280	III	14.6 vs. 6.2 m (20) *	81% (40%)
R/R MCL Combination Regimens					
Wang 2016 [84]	Ibr + R	50	II	69% (15) 75% (12)	88% (44%)
Tam 2018 [83]	Ibr + Ven	24	II	43 m (48)	71% (71%)
Jerkeman 2020 [82]	Ibr + Len + R	50	II	18 m (40) 56.9% (12)	76% (56%)
Lee 2021 [85]	Ibr + cirmtuzumab	20	I/II	NR (25)	90% (35%)
Frontline MCL					
Wang 2019 [87]	Ibr + R induction prior to CIT	131	II	82% (36)	100% (88%)
Jain 2021 [88]	Ibr + R in elderly	50	II	NR *	98% (60%)
Le Gouill 2021 [89]	Ibr + Ven + O	15	I	74.5% (12)	75% (67%)
Wang 2022 [90]	BR +/− Ibr	523	III	80.6 vs. 52.9 (85) *	

BR: Bendamustine/rituximab Ibr: Ibrutinib; R: rituximab; Ven: Venetoclax; Len: Lenalidomide; O: Obinutuzumab; NR: not reached; m: months

*median progression free survival

Table 4.

Major Mutation Mechanisms of Ibrutinib Resistance

	Primary Sensitivity	**Primary Resistance/Reduced Sensitivity**	**Predisposition for Later Progression**	**Secondary Resistance**
CLL	**UM-IGHV** *		TP53 del/mut, complex cytogenetics Del 18p	BTK PLCG2 Del 8p/TRAIL-R
MCL		CARD11 CCND1 TRAF2, TRAF3 & BIRC3 and MAP3K14		BTK CCND1 CDKN2A/MTAP
DLBCL	MCD or N1 molecular subtypes	CARD11 TNFAIP3 KLHL14 PIM1		

* Compared to CIT, patients with CLL who have UM-IGHV do better in terms of PFS with ibrutinib-containing therapy ([128, 129]