

Treatment Refractoriness to Pathway Inhibitors in Chronic Lymphocytic Leukemia

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ABSTRACT

Therapeutic options for Chronic Lymphocytic Leukemia (CLL) rely on pathway inhibitors targeting Bruton Tyrosine Kinase (BTK) or B Cell Lymphoma 2 (BCL2), while the use of Chemo Immunotherapy (CIT) is limited to contexts where pathway inhibitors are not available or accessible. Biomarkers of treatment refractoriness to CIT include the unmutated status of immunoglobulin heavy chain variable genes and genetic alterations of *TP53*, *NOTCH1*, and *BIRC3*. Acquired mutations of *BTK* and *PLCG2* genes represent the most common resistance mechanisms in patients receiving covalent BTK inhibitors (BTKi)-based therapy. The most frequent *BTK* mutations are C481S and C481R and impair binding of the drug to its target. Mutations of *PLCG2* promote B cell receptor signaling despite BTK inhibition. Recently, several *BTK* point mutations causing acquired resistance to non-covalent BTKi have been reported, including *T474I*, *L528W*, *A428D*, *M437R*, and *V416L*. Concerning BCL2 inhibitors (BCL2i), multiple mutations affecting the *BCL2* gene reduce or hinder the binding affinity between the BCL2i venetoclax and the anti-apoptotic BCL2 protein. The G101V substitution is the most common *BCL2* mutation and reduces the clinical response to venetoclax. Several additional *BCL2* mutations have been detected among CLL patients resistant to venetoclax. Interestingly, acquired 8p loss and 1q gain occur in a fraction of venetoclax-resistant patients and deregulate the expression of the anti-apoptotic *MCL1* gene. Leukemic cells characterized by 8p loss and 1q gain display increased resistance to venetoclax and exhibit an augmented sensitivity to *MCL1* inhibition. Knowledge of the molecular mechanisms of resistance to pathway inhibitors may help to better understand the sequencing and cross-resistance of available drugs and may contribute to the design of a precision medicine algorithm to be applied to the individual CLL patient.

Keywords: Chronic lymphocytic leukemia; Treatment refractoriness; Predictive biomarkers; Chemo immunotherapy; Pathway inhibitors

INTRODUCTION

Chronic lymphocytic leukemia, the most common leukemia in the adult population, is defined by the clonal proliferation of mature B cells that express CD19, CD5, and CD23 on their surface and accumulate in the blood and lymphatic tissues [1]. Although CLL displays a 5-year survival rate of approximately 90%, complete eradication of CLL is only achievable with allogeneic hematopoietic stem cell transplantation, a therapeutic

approach that is still gravated by a significant rate of morbidity and mortality [2].

For these reasons, therapeutic options for CLL rely on pathway inhibitors targeting Bruton Tyrosine Kinase (BTK) or B Cell Lymphoma 2 (BCL2), while the use of Chemo Immunotherapy (CIT) is limited to contexts where pathway inhibitors are not available or accessible [3]. Currently, available therapies have proven to be effective, but a fraction of patients are refractory to treatment ab initio or acquire secondary resistance during the

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clinical course [4]. Additionally, in the context of CLL, up to 10% of the cases develop Richter Syndrome (RS), an aggressive lymphoma characterized by histology consistent with diffuse large B cell lymphoma and by frequent refractoriness to CIT [5].

In CLL, several biomarkers of treatment resistance to CIT have been identified and have been recently reviewed in detail by our group [4]. In brief, the main predictors of treatment failure include the unmutated status of Immunoglobulin Heavy Chain Variable (IGHV) genes and genetic alterations of *TP53* [4]. Importantly, because of its role as “guardian of the genome”, *TP53* exerts a pro-apoptotic function after cellular exposure to DNA-damaging compounds, including CIT. Consequently, *TP53* disruption by deletion or mutation increases the resistance of CLL cells to CIT [4]. Genetic alterations of *NOTCH1* and *BIRC3* also play a role in defining refractoriness to CIT regimens with different mechanisms that, in the case of *NOTCH1* mutations, involve reduced levels of CD20 expression and inferior response to some anti-CD20 monoclonal antibodies [4].

To overcome resistance to CIT, BTK inhibitors (BTKi) and BCL2 inhibitors (BCL2i)-based therapies have now become the mainstay of CLL therapy. Remarkably, continuous treatment with the BTKi ibrutinib, acalabrutinib, zanubrutinib have obtained similar results in both IGHV mutated and unmutated CLL, documenting that BTKi have the potential to overcome refractoriness due to IGHV unmutated status. Also, *TP53* disrupted CLL treated with continuous treatment with BTKi displayed improved outcomes [4]. Conversely, fixed-duration therapies with BCL2i have shown suboptimal results in *TP53*-disrupted patients, most likely due to the time limit in exposure to the drug. Because of the clinical impact of *TP53* alterations and IGHV unmutated status, the international guidelines from the International Workshop on Chronic Lymphocytic Leukemia (iwCLL) and European Research Initiative on CLL (ERIC), as well as other guidelines, recommend testing these biomarkers at the time of treatment requirement [6,7].

The precise understanding of the mechanisms of resistance to pathway inhibitors in CLL is still under scrutiny, although several concepts have been progressively clarified. On these grounds, the aim of this mini-review is to focus on the molecular mechanisms and clinical implications of refractoriness to CLL pathway inhibitors, in order to provide updates on the latest findings and input for further research on this topic. Mechanisms of refractoriness to both BTK inhibitors (BTKi) and BCL2 inhibitors (BCL2i) will be addressed.

LITERATURE REVIEW

Refractoriness to BTK inhibitors

BTK is a crucial component of the B Cell Receptor (BCR) signaling pathway, which regulates B cell function, proliferation, and survival (Figure 1) [4]. BTKi are a class of drugs that inhibit BTK and thereby have an anti-proliferative and pro-apoptotic effect on CLL cells. BTKi are divided into covalent and non-covalent inhibitors according to the mechanism of action. Covalent inhibitors include ibrutinib, acalabrutinib, and

zanubrutinib and bind *via* a covalent irreversible bond to a cysteine residue at position 481 (Cys481) in the ATP binding pocket abrogating downstream signaling activity. Non-covalent inhibitors (e.g. pirtobrutinib) bind to the ATP-binding domain or a specific H3 pocket of BTK independent of C481 through reversible weak interactions, such as hydrophobic interactions or hydrogen bonding.

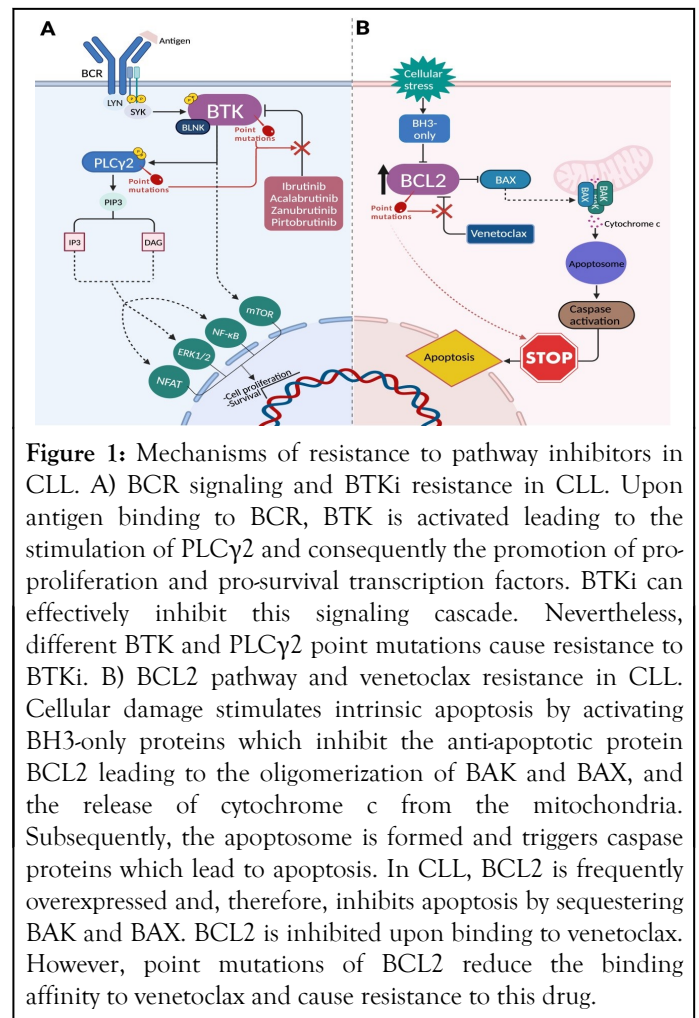


Figure 1: Mechanisms of resistance to pathway inhibitors in CLL. A) BCR signaling and BTKi resistance in CLL. Upon antigen binding to BCR, BTK is activated leading to the stimulation of PLCγ2 and consequently the promotion of pro-proliferation and pro-survival transcription factors. BTKi can effectively inhibit this signaling cascade. Nevertheless, different BTK and PLCγ2 point mutations cause resistance to BTKi. B) BCL2 pathway and venetoclax resistance in CLL. Cellular damage stimulates intrinsic apoptosis by activating BH3-only proteins which inhibit the anti-apoptotic protein BCL2 leading to the oligomerization of BAK and BAX, and the release of cytochrome c from the mitochondria. Subsequently, the apoptosome is formed and triggers caspase proteins which lead to apoptosis. In CLL, BCL2 is frequently overexpressed and, therefore, inhibits apoptosis by sequestering BAK and BAX. BCL2 is inhibited upon binding to venetoclax. However, point mutations of BCL2 reduce the binding affinity to venetoclax and cause resistance to this drug.

BTKi have been practice-changing in both first-line and Relapsed/Refractory (R/R CLL) [4]. Despite their high efficacy and safety, 60% of patients eventually relapse. Different primary and acquired mechanisms have been described to cause resistance to both covalent and non-covalent BTKi. Baseline characteristics, such as del(17p), *TP53* mutation, and complex karyotype, carry a higher risk of progression in ibrutinib-treated CLL [8]. Acquired mechanisms of resistance have been determined during treatment with BTKi or at relapse [9]. In this respect, acquired mutations of the BTK and phospholipase-C-gamma-2 (*PLCG2*) genes represent the most reported resistance mechanisms in patients receiving covalent BTKi-based therapy (Figure 1). Mutations occur frequently at cysteine residue 481 of BTK, resulting in the replacement of cysteine by other amino acids such as serine (C481S) or arginine (C481R). Mutations at this site lead to the abrogation of covalent binding of ibrutinib, with only transient inhibition of the mutant protein. In contrast, multiple, albeit less frequent, point mutations (i.e. R665W) of the downstream signaling molecule PLCγ2 generally

lead to gain-of-function and promote BCR signaling despite BTK inhibition [9].

Using Next Generation Sequencing (NGS), a multicenter international retrospective study by Bonfiglio, et al. showed that only 65% of CLL relapsing on ibrutinib harbor mutations of *BTK* and/or *PLCG2*, suggesting the existence of alternative mechanisms of resistance [10]. Interestingly, enrichment of 8p loss has been reported in relapsing cases, leading to haplo insufficiency of TRAIL receptor (TRAIL-R) that causes resistance of leukemic cells to TRAIL-induced apoptosis, independent of the mutational status of *BTK* and/or *PLCG2*. Moreover, *BIRC3* and *NFKBIE* mutations were detected exclusively in the *BTK* wild-type ibrutinib relapsing patients, suggesting aberrant activation of the canonical/non canonical NF- κ B pathway as a possible mechanism of drug evasion [10]. Longer follow-up is needed to determine whether the presence of these mutations is associated with subsequent resistance to ibrutinib treatment. Other genetic alterations may complement *BTK* mutations in inducing BTKi resistance. For example, the transcription factor *EGR2* was found to be almost exclusively mutated in relapsed patients carrying *BTK* mutations [10]. *EGR2* is activated by ERK phosphorylation upon BCR stimulation, suggesting that *EGR2* mutations may lead to constitutively dysregulated BCR signaling that, together with the existing *BTK/PLCG2* mutations, results in ibrutinib resistance.

Non-covalent BTKi are a therapeutic alternative for patients who have failed covalent BTKi [4]. Several mutations causing acquired resistance to non-covalent BTKi have recently been reported, among which point mutations in the tyrosine kinase domain of BTK including *T474I*, *L528W*, *A428D*, *M437R*, and *V416L* (Figure 1) [4,11]. A recent study has shown that both gatekeeper and alternative-site mutations in BTK result in resistance to non-covalent BTKi [12]. The BTK gatekeeper mutation *T474I* has been observed to emerge during pirtobrutinib therapy, replacing the previous C481S clone that had been selected by covalent BTKi. The *T474I* mutation leads to clinical resistance to non-covalent BTKi. In addition, the *T474I* clone may also carry an additional *BTK* mutation (*M477I*) at a very low frequency which proved to be disabling and was acquired upon progression on pirtobrutinib. In addition to *T474I*, a previously unreported *T474L* mutation also resulted in an active BTK [12]. The prevalence of these mutations suggests convergent evolution driven by strong selection pressure, particularly in patients progressing after previous treatment with covalent BTKi. This notion highlights the possible role of reactivation of the proximal component of the BCR pathway as a mechanism of resistance to non-covalent BTKi [11].

An arising problem with covalent and non-covalent BTKi pertains to the emergence of cross-resistance, whereby a single mutation has the potential to confer resistance to both types of inhibitors. This phenomenon significantly complicates the judicious selection of therapeutic interventions. In particular, the *BTK L528W* mutation demonstrated an increased prevalence in patients who experienced disease progression on treatment with zanubrutinib in comparison to ibrutinib [13]. The same mutation was also detected in several patients progressing during the administration of pirtobrutinib.

Interestingly, this mutation also interferes with the binding of adenosine triphosphate, resulting in a protein with reduced kinase activity, rendering it "kinase-dead" but still capable of activating PLC γ 2 [13].

Refractoriness to BCL2 inhibitors

BCL2, an anti-apoptotic protein, is overexpressed in CLL allowing cancer cells to evade apoptosis by sequestering pro-apoptotic proteins (Figure 1) [4]. Venetoclax selectively binds to BCL2, displacing pro-apoptotic proteins and triggering apoptosis, and has significantly improved the therapeutic landscape of CLL in combination with anti-CD20 Monoclonal Antibodies (mAbs). Nevertheless, a subset of patients experiences a decline in efficacy and develops secondary resistance to venetoclax [4]. This phenomenon holds true for treatment regimens of both continuous and fixed durations. Specific features of the microenvironment, namely dysregulated expression of IL-10 and CD40L signaling, play a role in venetoclax resistance by triggering Toll-Like Receptor 9 (TLR9) activation, which subsequently initiates NF- κ B signaling [14]. It is noteworthy that stimulation of the NF- κ B transcription factor is of central importance because it leads to increased levels of the anti-apoptotic proteins BCL-xL and MCL-1 [14]. Baseline genetic lesions also affect venetoclax outcome. Among high-risk

CLL patients with TP53 abnormalities, ~50% relapses after 2 years on venetoclax as monotherapy [15]. Also, when venetoclax is combined with the anti-CD20 mAb obinutuzumab, TP53 disruption predicts an inferior outcome most likely due to the fixed duration of the venetoclax-obinutuzumab regimen [16]. In addition, 1q23 amplification, as described by Gui  ze, et al. can lead to abnormal activation of adenosine monophosphate-activated protein kinase (AMPK)/protein kinase A (PKA), disrupting the release of cytochrome c release and ultimately leading to venetoclax resistance [17].

DISCUSSION

Multiple mutations affecting the *BCL2* gene have been identified as factors contributing to venetoclax resistance by reducing or hindering the binding affinity between the drug and the anti-apoptotic BCL2 protein (Figure 1) [15]. The most common mutation is represented by a substitution of glycine to valine at position G101 (G101V). The presence of *BCL2* G101V reduces the cellular response to venetoclax *in vitro* by altering the BH3-binding domain of BCL2. This mutation is associated with clinical resistance in a maximum of 50% of patients who relapse or progress. In recent years, several additional *BCL2* mutations, such as D103Y, which directly disrupts the BH3 binding P4 pocket, have been detected in CLL patients receiving venetoclax [18]. *BCL2* D103Y was reported in a recent study to occur also independent of the presence of G101V, which may indicate its role as a driving resistance mechanism to venetoclax [19]. The fact that *BCL2* mutations are restricted to a subset of patients suggests the involvement of alternative mechanisms in the emergence of venetoclax resistance [19].

Recently, whole exome sequencing has revealed that certain venetoclax-resistant patients exhibited acquired 8p loss [20]. In tandem, the same patients demonstrated the presence of

1q21.2-21.3 gain which concurrently affected the anti-apoptotic *MCL1* gene. Notably, patients characterized by 8p loss displayed heightened resistance to venetoclax in comparison to those lacking such genomic alteration. Intriguingly, cells of patients harboring both 8p loss and 1q21.2-21.3 gain exhibited an augmented sensitivity to *MCL1* inhibition, highlighting a possibility for future combinations of *MCL1* inhibitors and venetoclax. Furthermore, venetoclax-resistant specimens showed upregulation of the MAPK pERK levels during progression across all patient samples examined [20]. This fact implicates a potentially significant role of MAPK signaling in the development of acquired venetoclax resistance in CLL, as observed in other hematological malignancies, including multiple myeloma and acute myeloid leukemia.

CONCLUSIONS AND PERSPECTIVES

The therapeutic landscape of CLL has been changing rapidly during the last decade thanks to the advent of pathway inhibitors and may continue to evolve in the near future. The availability of both BTKi and BCL2i for first-line treatment as well subsequent lines of therapy poses the question of the optimal sequencing of these medicines during the clinical course of the patient. In this respect, knowledge of the molecular mechanisms of resistance to pathway inhibitors may help to better understand the cross-resistance of available drugs and may in the future contribute to the design of a precision medicine algorithm to be applied to the individual patient. Emerging resistance to pathway inhibitors prompts the exploration of novel pharmaceutical agents, such as Proteolysis-Targeting Chimeras (PROTACS) including BTK degraders, bispecific mAbs and chimeric antigen receptor (CAR)-T cells, for the management of relapsed and refractory CLL. Also, the link between molecular resistance to pathway inhibitors and the development of RS needs to be clarified in more detail, in order to devise better treatment strategies for RS which is still a major unmet clinical need.

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CONFLICTS OF INTEREST

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REFERENCES

1. Alaggio R, Amador C, Anagnostopoulos I, Attygalle AD, Araujo IB, Berti E, et al. The 5th edition of the World Health Organization classification of haematolymphoid tumours: Lymphoid neoplasms. *Leukemia*. 2022;36(7):1720-1748.
2. Shadman M, Maloney DG. Immune therapy for chronic lymphocytic leukemia: Allogeneic transplant, chimeric antigen receptor T-cell therapy, and beyond. *Hematol Oncol Clin North Am*. 2021;35(4):847-862.
3. Eichhorst B, Robak T, Montserrat E, Ghia P, Niemann CU, Kater AP, et al. Chronic lymphocytic leukaemia: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Annals of Oncology*. 2021;32(1):23-33.
4. Maher N, Mouhssine S, Matti BF, Alwan AF, Gaidano G. Treatment refractoriness in chronic lymphocytic leukemia: Old and new molecular biomarkers. *Int J Mol Sci*. 2023;24(12):10374.
5. Mouhssine S, Gaidano G. Richter syndrome: From molecular pathogenesis to druggable targets. *Cancers*. 2022;14(19):4644.
6. Hallek M, Cheson BD, Catovsky D, Caligaris-Cappio F, Dighiero G, Döhner H, et al. iwCLL guidelines for diagnosis, indications for treatment, response assessment, and supportive management of CLL. *Blood*. 2018;131(25):2745-2760.
7. Malcikova J, Tausch E, Rossi D, Sutton LA, Soussi T, Zenz T, et al. ERIC recommendations for TP53 mutation analysis in chronic lymphocytic leukemia-update on methodological approaches and results interpretation. *Leukemia*. 2018;32(5):1070-1080.
8. Ahn IE, Tian X, Ipe D, Cheng M, Albitar M, Tsao LC, et al. Prediction of outcome in patients with chronic lymphocytic leukemia treated with ibrutinib: development and validation of a four-factor prognostic model. *J Clin Oncol*. 2021;39:576-585.
9. Woyach JA, Furman RR, Liu TM, Ozer HG, Zaparka M, Ruppert AS, et al. Resistance mechanisms for the Bruton's tyrosine kinase inhibitor ibrutinib. *N Engl J Med*. 2014;370(24):2286-2294.
10. Bonfiglio S, Sutton LA, Ljungström V, Capasso A, Pandzic T, Weström S, et al. BTK and PLCG2 remain unmutated in one third of patients with CLL relapsing on ibrutinib. *Blood Advances*. 2023;2022008821.
11. Wang E, Mi X, Thompson MC, Montoya S, Notti RQ, Afaghani J, et al. Mechanisms of resistance to noncovalent Bruton's tyrosine kinase inhibitors. *N Engl J Med*. 2022;386(8):735-743.
12. Naeem A, Utro F, Wang Q, Cha J, Vihinen M, Martindale S, et al. Pirtobrutinib targets BTK C481S in ibrutinib-resistant CLL but second-site BTK mutations lead to resistance. *Blood Adv*. 2023;7(9):1929-1943.
13. Blombery P, Thompson ER, Lew TE, Tiong IS, Bennett R, Cheah CY, et al. Enrichment of BTK Leu528Trp mutations in patients with CLL on zanubrutinib: Potential for pirtobrutinib cross-resistance. *Blood Adv*. 2022;6(20):5589-5592.
14. Jayappa KD, Portell CA, Gordon VL, Capaldo BJ, Bekiranov S, Axelrod MJ, et al. Microenvironmental agonists generate *de novo* phenotypic resistance to combined ibrutinib plus venetoclax in CLL and MCL. *Blood Adv*. 2017;1(14):933-946.
15. Blombery P, Anderson MA, Gong JN, Thijssen R, Birkinshaw RW, Thompson ER, et al. Acquisition of the recurrent gly101val mutation in BCL2 confers resistance to venetoclax in patients with progressive chronic lymphocytic leukemia. *Cancer Discov*. 2019;9:342-353.
16. Tausch E, Schneider C, Robrecht S, Zhang C, Dolnik A, Bloehdorn J, et al. Prognostic and predictive impact of genetic markers in patients with CLL treated with obinutuzumab and venetoclax. *Blood*. 2020;135(26):2402-2412.

17. Gui  ze R, Liu VM, Rosebrock D, Jourdain AA, Hern  ndez-S  nchez M, Zurita AM, et al. Mitochondrial reprogramming underlies resistance to BCL-2 inhibition in lymphoid malignancies. *Cancer cell*. 2019;36(4):369-384.
18. Tausch E, Close W, Dolnik A, Bloehdorn J, Chyla B, Bullinger L, et al. Venetoclax resistance and acquired BCL2 mutations in chronic lymphocytic leukemia. *Haematologica*. 2019;104(9):e434.
19. Kotmayer L, L  szl   T, Mikala G, Kiss R, L  vay L, Hegyi LL, et al. Landscape of BCL2 resistance mutations in a real-world cohort of patients with relapsed/refractory chronic lymphocytic leukemia treated with venetoclax. *Int J Mol Sci*. 2023;24(6):5802.
20. Khalsa JK, Cha J, Utro F, Naeem A, Murali I, Kuang Y, et al. Genetic events associated with venetoclax resistance in CLL identified by whole-exome sequencing of patient samples. *Blood*. 2023;142(5):421-433.