Mechanisms of resistance to targeted therapies

Romain Guièze, Service d'hématologie clinique et de thérapie cellulaire, CHU de Clermont-Ferrand
Équipe Chelter, Université Clermont Auvergne

Correspondence : R. Guièze
rguieze@chu-clermontferrand.fr

Mécanismes de résistance aux thérapies ciblées

Resistance, ibrutinib, venetoclax, chronic lymphocytic leukaemia

Abstract

The therapeutic landscape for patients with chronic lymphocytic leukaemia is growing with the advent of BCR inhibitors and the BCL2 inhibitor, venetoclax. Cases of resistance are emerging, and the underlying mechanisms, such as mutations affecting the therapeutic target, are highlighted and presented in this review.

Résumé

L'arsenal thérapeutique de la leucémie lymphoïde chronique s'est considérablement enrichi avec le développement des inhibiteurs de la signalisation du récepteur B à l'antigène et d'un l'inhibiteur de BCL2 (pour B-cell lymphoma 2): le vénétoclax. Des cas de résistances à chacune de ces molécules émergent et les mécanismes sous-jacents impliquant en partie des mutations de la cible thérapeutique sont en train d'être élucidés. Ils sont présentés dans cette revue.

In addition to immunochemotherapy, the therapeutic arsenal for chronic lymphocytic leukaemia (CLL) has been considerably enriched with the development of inhibitors of B-cell receptor (BCR) signalling, which include Bruton's tyrosine kinase (BTK) inhibitors and phospho-inositide-3-kinase 5 (P13Kδ). Even more recently, an inhibitor of B-cell lymphoma 2 (BCL2), venetoclax, has opened up new perspectives. Despite such a wide range of therapies, resistance to these targeted therapies is becoming increasingly frequent. Identifying underlying mechanisms offers the opportunity to optimise the use of these new approaches. Resistance may be naturally linked to a mutation in the therapeutic target, but other mutational or non-mutational mechanisms are also involved.

Resistance to inhibition of Bruton's tyrosine kinase

Ibrutinib is the first covalent irreversible BTK inhibitor. According to the marketing authorisation, it is indicated for use during relapse as well as a first-line treatment and is administered until progression or unacceptable toxicity. However, state reimbursement of the treatment is currently only permitted for relapse or first-line treatment in the case of alteration of *TP53.* Resistance to ibrutinib can sometimes be observed at the outset: approximately 7–14% of patients [1, 2] will fail to respond to first-line treatment and 14–35% will fail to respond during relapse [3, 4]. Resistance also occurs with ibrutinib and concerns 35% of relapsed patients at five years [5]. Approximately two thirds of patients will progress to CLL on ibrutinib, although one third develop Richter syndrome [6]. Isotopic and/or histological investigation in case of progression may be warranted prior to initiation of a new line of therapy. Other BTK inhibitors, such as acalabrutinib and zanubrutinib, are under development and the first cases of resistance are also emerging.

Mutations in the target, Bruton's tyrosine kinase, and the downstream effector, 1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase γ2

Investigation of the first resistant patients (n = 6) by exome sequencing rapidly revealed that resistance could naturally be linked to a mutation in the kinase domain of BTK affecting its binding domain and affinity to ibrutinib

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(C481; cysteine mutation) [7]. This observation was a new example of mutational resistance by alteration of the target, as generally observed with targeted therapies and, in particular, in haematology regarding resistance to tyrosine kinase inhibitors linked to *BCR-ABL* mutations in chronic myeloid leukaemia. This same study also revealed the role of mutations in 1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase γ2 (*PLCG2*), a downstream effector of BCR signalling, in resistance to ibrutinib. In a study of BCR stimulation-dependent calcium signalling, the authors showed that mutations in *PLCG2* (R665W and L845F) induced constitutive signalling that was not inhibited by ibrutinib.

Larger studies (46 patients) [8] revealed that mutations in the C481 residue of *BTK* are found in approximately 80% of patients relapsing on ibrutinib and that mutations in *PLCG2* are found in 20% of patients (usually in patients who also carry *BTK* mutations). Using highly sensitive techniques, these mutations are detectable in 85% of patients, approximately nine months (three to 18 months) before full clinical relapse. Analysis of a cohort of patients responding to ibrutinib for three years, with no evidence of relapse, showed that mutations in *BTK* were in fact detectable in 57% of patients (including four *PLCG2* mutations) [9]. The presence of these mutations was significantly associated with a higher risk of relapse. Rarer mutations in other BTK residues may also affect sensitivity to ibrutinib. This is the case for other mutations in the kinase domain, the gatekeeper T474 [10] and the SH2 domain (T316A) [11].

Second-generation BTK inhibitors offer the hope of potentially distinct resistance profiles. Acalabrutinib is a more specific irreversible BTK inhibitor than ibrutinib. The mechanisms of resistance to acalabrutinib appear to be similar to those for ibrutinib. Mutations in BTK residue 481 were found in 11 of 16 acalabrutinib-resistant patients [12]. Two of these patients also had mutations in *PLCG2*. Zanubrutinib is also an irreversible BTK inhibitor. One recent analysis found a mutation in the kinase domain of BTK (Leu528Trp) in all patients who received zanubrutinib (n = 4) [13]. This mutation affects zanubrutinib and ATP binding and is thought to be fairly specific to zanubrutinib treatment. Finally, compounds acting through non-covalent linkages, and therefore theoretically less dependent on residue 481, such as GDC-0853 fenetbrutinib, are also under development [14].

BTK mutations are clearly associated with the vast majority of cases of resistance. The existence of specific mutations associated with each inhibitor may justify a search for these mutations before a therapeutic choice is made; before patients are exposed to such inhibitors

Other mechanisms

The gene mutations of *BTK* and *PLCG2* have been explored by sensitive next-generation sequencing techniques and ultimately affect only a limited, sometimes tiny, proportion of the tumour population. In some cases, a very large proportion of leukaemia cells that cause relapse do not carry such mutations. These observations support the involvement of other resistance mechanisms associated with these mutations, which may ultimately be a biomarker of more complex resistance. Another hypothesis could be a paracrine protective role of mutated *BTK* cells on wild-type *BTK* cells [15]. The study of clonal evolution under ibrutinib revealed that in some cases, subclones carrying the 8p deletion were selected by BTK inhibition [16]. One explanation could be related to the loss of expression of the receptor for tumour necrosis factor-related apoptosis inducing ligand (TRAIL), located on chromosome 8. One effect of ibrutinib is to induce TRAIL-dependent apoptosis, which occurs particularly in peripheral blood. Loss of the TRAIL receptor is thought to render leukaemia cells resistant to ibrutinib.

The implication of compensation through alternative signalling pathways is also possible. One team has investigated the characteristics of persistent tumour cells

under ibrutinib that appear to maintain competence in BCR signalling via phosphoinositide 3-kinase (P13K) / protein kinase B (Akt) / extracellular signal-regulated kinases (ERK) pathways [17]. The involvement of the NF-kB pathway has also been suggested.

The microenvironment has a fundamental role in the survival of the leukaemic clone as well as in therapeutic resistance. It may also induce resistance to ibrutinib. Chiorazzi's team suggests the involvement of interleukin 4 (IL-4), as resistant cells are able to resume production and respond to IL-4 [18]. Resistance could also be induced by IL-10, CD40L or CpG-oligodeoxynucleotides [19].

Finally, general phenomena of reduced sensitivity to treatment such as genetic complexity, reflected in a complex karyotype, or alterations in *TP53*, also seem to play a role, as evidenced by their negative prognostic impact under ibrutinib [20].

Transformation into Richter syndrome

Transformation into Richter syndrome is another `escape' route from ibrutinib and may account for about a third of relapsed progressions. It occurs within two years of treatment initiation and does not appear to follow the same evolutionary trajectories as progression to CLL [21]. Some evidence suggests that the role of *BTK* mutations is more marginal in Richter syndrome than in CLL progression [22]. Indeed, only a half of the cases have mutations in *BTK,* and other mutations are more frequent and could be involved (*SF3B1, TP53*). In one case, further investigation showed simultaneous progression of CLL associated with expansion of a BTK clone and Richter syndrome that was associated with a *PLCG2* mutation rather than *BTK* mutation [23]. A case of progression to histiocytic sarcoma further illustrates the polymorphism of resistance under ibrutinib [16].

Resistance to phospho-inositide-3-kinase inhibition δ

Like BTK inhibitors, the PI3Kδ inhibitor, idelalisib, acts through inactivation of BCR signalling and is indicated in relapsed CLL or when *TP53* is impaired [24]. Although its efficacy is satisfactory, several signals of intolerance (opportunistic infections, digestive toxicity) have led to a preference of other molecules with marketing authorisation. Because of its limited use, its resistance mechanisms remain poorly documented. No mutation of PI3Kδ or a downstream effector in resistant patients has been observed, according to one preliminary study [25]. In a mouse model, resistance results from activation of the insulin-like growth factor 1 receptor (IGFR1), leading to mitogen-activated protein kinase (MAPK) signalling [26].

Resistance to BCL2 inhibition

The BCL2 protein is classically hyper-expressed in CLL, notably through the 13q14 deletion that carries miR-15a/16-1, negative regulators of BCL2 expression [27, 28]. BCL2 has therefore long been a rational therapeutic target. The BCL2 protein is one of the members of the BCL2 family that acts to govern mitochondrial apoptosis. These members share similarities in structure but have opposite, pro- or anti-apoptotic roles. In particular, the anti-apoptotic protein, BCL2, acts by sequestering activating members (BIM, BID) that are unavailable to induce effectors involved in the permeabilisation of the mitochondrial membrane prior to apoptosis (BAX, BAK). Myeloid cell leukemia-1 (MCL1) and BCL-extra-large (BCL-XL) are other anti-apoptotic members of the BCL2 family.

Venetoclax was the first BCL2 inhibitor to be marketed. It has marketing authorisation and is reimbursed by the state in cases of relapsed CLL or *TP53* alterations when the patient is ineligible for a BCR inhibitor [29]. It acts by binding to BCL2, thereby releasing activators of mitochondrial apoptosis. In monotherapy, it allows an overall response of 80%, including situations in which the reference immuno-chemotherapy is inactive, such as patients with altered *TP53* [30].

Despite this efficacy, cases of venetoclax-resistant patients are emerging and approximately 30% of patients progress to a situation of relapse at 15 months [29].

Mutation of the target

As with ibrutinib, resistance to venetoclax can naturally result from mutations in its target. A point mutation in BCL2 (Gly101Val) was initially found in seven patients in a cohort of 15 patients relapsing after venetoclax [31]. Another team also reported the same mutation in three of the four patients investigated [32]. This mutation, which has not been described in patients not exposed to venetoclax, clearly affects the binding of venetoclax *in vitro.* It is detected after 19–42 months of exposure and may precede clinical progression by up to 25 months.

Other mutations in *BCL2* have since been reported [32, 33]. Resistant patients were shown to carry several mutations of *BCL2,* with a median of three mutations (between one and seven) per patient and these different *BCL2* mutations could be present in different subclones [33]. These observations justify the characterisation of BCL2-venetoclax interactions, as recently reported [34]. In particular, the Asp103 residue, located in the P4 pocket of BCL2, is frequently affected (six of the 11 patients investigated). Other alterations such as the Val156Asp mutation located in the P2 pocket, or the Arg107_Arg110dup insertion affecting the sequence separating the α2 and α3 helices, have also been described in resistant patients. Finally, mutations in *BCL2* (Ala113Gly and Arg129Leu) can be found but are also encountered in B lymphoid haemopathies not treated with venetoclax. These data are fundamental to developing BCL2 inhibitors for this type of mutation.

As observed for ibrutinib, only a small proportion of resistant leukaemia cells are ultimately affected by *BCL2* mutations (allelic frequency between 0.01 and 32% for Gly101Val [31]). Other resistance mechanisms not related to *BCL2* mutations are involved.

Compensation mechanisms

The BCL2 family includes several proteins with redundant roles. Resistance to BCL2 inhibition may also depend on compensation by other antiapoptotic members.

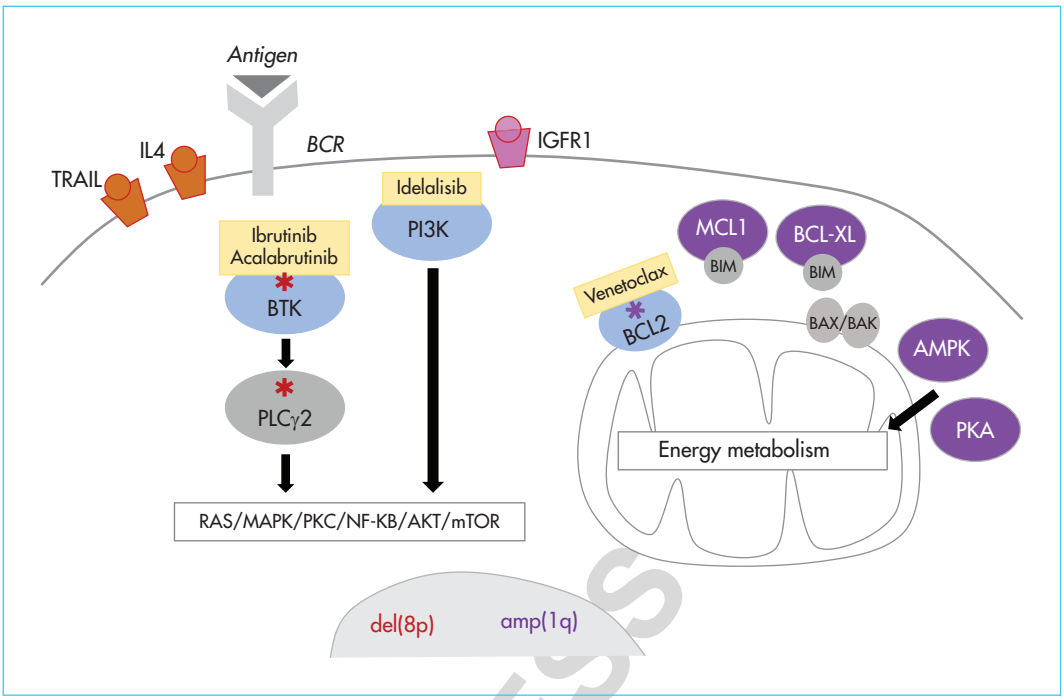
The MCL1 protein has been described to compensate for the inhibition of BCL2 *in vitro* by sequestering pro-apoptotic proteins, such as BIM [35, 36]. Combined genome-wide screening and analysis of resistant cell lines confirmed a major role for MCL1 in venetoclax resistance *in vitro* [37]. Analysis of a cohort of nine patients revealed higher MCL1 expression in relapse compared to pre-treatment in six patients. This hyperexpression of MCL1 may be related to an amplification of the *MCL1* locus (amp1q) observed in three patients and in a resistant lymphoid cell line [37]. The amplification of the *MCL1* locus was recently observed by another team [33]. MCL1 inhibitors are being evaluated and could be very useful in patients relapsing on venetoclax if the toxicity profile proves acceptable [38].

Hyperexpression of BCL-XL, another anti-apoptotic protein of the BCL2 family, has also been reported [31]. In particular, it was found in a patient with a *BCL2* mutation in another subclone. A previous study noted that the lymph node microenvironment protected against BCL2 inhibition-induced apoptosis by inducing hyperexpression of BCL-XL [39].

Involvement of energy metabolism

The mitochondrion is the organelle responsible for apoptosis but also for energy production. The above-mentioned study [37] showed that venetoclax induced a rapid disruption of mitochondrial metabolism and that resistance to venetoclax

Figure 1



Mechanisms of resistance to targeted therapies in chronic lymphocytic leukaemia. Therapeutic targets are shown in blue. Mechanisms of resistance to ibrutinib, idelalisib and venetoclax are shown in orange, pink and purple, respectively.

was mediated by changes in energy metabolism. In particular, an increase in oxidative phosphorylation, the mechanism by which ATP is produced at the inner membrane of mitochondria, was associated with resistance. The involvement of AMP-dependent signalling pathways (AMP-activated protein kinase [AMPK] and protein kinase A [PKA]) and repression of regulators of lymphoid transcription have been suggested. In patients, AMPK signalling assessed by immunohistochemistry was shown to be more prominent during relapse compared to before initiation of treatment in four of the nine patients examined. The involvement of metabolism in resistance has also been documented in acute myeloid leukaemia [40]. Metabolic modulators, some of which are used for other indications, are therefore an interesting avenue for restoring sensitivity to venetoclax.

Conclusion

We now have a better understanding of resistance to modern therapies for CLL, which offers hope for optimising the use of these approaches through combination or sequential administration. Target mutations, in *BTK* for ibrutinib and *BCL2* for venetoclax, are a common mechanism of resistance, but other compensatory mechanisms are also involved, sometimes in the same patient (summarised in *figure 1*). Oligoclonal resistance seems to be a new rule in haemopathies treated with targeted therapies [41].

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