



New players in the Bruton's tyrosine kinase vs. ibrutinib match

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The Bruton's tyrosine kinase, also known as BTK, is a member of the Tec family kinases with a well-characterized role in B-cell receptor (BCR) signaling and B-cell activation. BTK is able to transmit and amplify several signals that are involved in the complex crosstalk between tumor cells and microenvironment. These signals include cytokines and growth factors, chemokine receptors, antigen receptors as BCR and integrins (1). Chronic lymphocytic leukemia (CLL) cells show an increased expression and activation of BTK that with a chain reaction modulates signaling pathways essential for CLL-cells survival, as the protein kinase AKT, extracellular signal-regulated kinase (ERK) and nuclear factor kappa light-chain enhancer of activated B cells (NF- κ B) pathways (2). In addition, BTK is involved in chemokine-mediated migration, homing and adhesion of CLL cells (3). Given the known importance of BCR signaling in CLL and the central role of BTK in this pathway, in the recent years a new drug called ibrutinib has been used in clinical practice to induce inhibition of this kinase. Ibrutinib is a relatively selective, irreversible inhibitor of BTK binding covalently the Cys-481 in the ATP binding domain of the kinase. Inhibition of BTK in CLL cells leads to an impressive delocalization of leukemic cells from the protective tissue microenvironment to the periphery interfering with pathogenic mechanism of recirculation and homing. Several studies demonstrate the considerable clinical success of ibrutinib showing a good safety profile determining an improvement of the quality of life of CLL patients (4).

However, it has been immediately clear the limited capacity of this agent to induce a complete eradication of

neoplastic clone, with the detection of persistent disease in blood and tissues of patients after years of single-agent therapy. Moreover, some patients lose the response and progress during treatment and, although infrequent, fail to respond, developing a significant resistance to treatment (5). These unwanted effects may be related, in part, to a wide spectrum of ibrutinib off-targets since its molecular effect is not completely restricted to the CLL clone but also regulates key functions in other cellular elements as NK cells, T cells, macrophages and osteoclasts (6-8). Moreover, ibrutinib resistance is known to be explained in the majority of cases by the acquired mutation in BTK at its binding site with a cysteine to serine substitution (C481S) and others involving a gain-of-function mutation of the phospholipase C γ 2, an important molecule downstream of BTK. The C481S mutation switches the irreversible inhibition of ibrutinib to a reversible binding to BTK leading to an impairment of inhibition of BTK autophosphorylation and downstream signaling (9,10). The possibility to manage the onset of mutant BTK clone combining ibrutinib with other agents is a therapeutic need for CLL patients. In order to dissect the complex and essential role of BTK and ibrutinib in the pathobiology of CLL, Bottoni and colleagues (11) in this issue of *Blood*, described for the first time the possibility to regulate BTK expression through the modulation of miRNAs by the use of small molecule inhibitors of histone deacetylase (HDAC). miRNAs are small non-coding RNA molecules that are involved in the regulation of gene expression binding target mRNA to silence protein production. For this reason, dysregulation

of miRNA expression is involved in tumor initiation and progression (12). The comparison of miRNA profile between normal B cells and malignant CLL lymphocytes shows substantial differences that are linked to initial progression and drug resistance (13). Bottoni *et al.* with their elegant experiments identified a peculiar signature of miRNAs expressed in CLL cells that are able to target BTK. Of interest, the putative BTK-targeting miRNAs are expressed at lower levels in CLL cells compared to normal B cells, implying the existence of a regulation mechanism in leukemic cells. Since in CLL it has been shown that overexpression of HDAC and chromatin-modulating enzymes mediates the epigenetic silencing of miRNA-15A, miRNA-16 and miRNA-29d (14), the authors proposed and validated a causal mechanism in which the recruitment of HDAC to the promoters for the BTK-targeting miRNAs is able to silence their expression. On this scenario, the possibility to revert this repression may lead to target BTK indirectly by inhibiting HDAC activity which in turn may overexpress BTK-targeting miRNAs with the consequent downregulation of its target genes. For this reason, the authors decided to use first in an *in vitro* model and then *in vivo*, two different HDAC inhibitors abexinostat and panobinostat. As expected, the HDAC inhibitors induced a significant increased expression of BTK-targeting miRNAs and the consequent reduction of BTK expression. Functionally, this result influences the BTK downstream pathways with reduction of pPLC γ 2, pERK, pAKT and the consequent induction of CLL cells death. At this point, the possibility to combine the reduction of BTK expression by HDAC inhibitors and inhibition of its kinase activity by ibrutinib is appealing. *In vitro* and *in vivo* E μ -TCL-1 mouse model, abexinostat and ibrutinib showed an interesting synergistic activity with cytotoxicity in CLL cells and a reduced lymphocytosis. These important data put forward the basis for the possibility to use HDAC inhibitors to target mutated BTK cells since *in vitro* a reduction of BTK signaling pathway and CLL cell death has seen. In conclusion, the study of Bottoni *et al.* provides new interesting data on the potential targeting of BTK by using HDAC inhibitors that are able to silence BTK-targeting miRNAs leading to the reduction of BTK expression. Although the possibility to influence BTK expression by modulating an epigenetic silencing is potentially relevant, what is not known is how these HDAC inhibitors, alone or in combination with ibrutinib, could be use in the clinical practice. All HDAC inhibitors evaluated in phase I–II trials for the treatment of CLL patients showed the development

of significant unwanted adverse effects limiting the possibility to continue therapy (15,16). A further concern is mainly related to the CLL patients with a mutant BTK, because is not yet completely known if mutations are present pre-treatment or if are acquired during treatment for induction of drug pressure. These open questions are to be addressed to optimize the use of ibrutinib with other agents, as HDAC inhibitors.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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