

express high levels of CD37 RNA, and it is likely that antibodies recognizing different epitopes of CD37, which, as already mentioned, undergoes heavy posttranslational modification (eg, glycosylation), might give different results.

On the basis of an expression pattern largely overlapping CD20, CD37 was one of the first proteins explored as therapeutic target in non-Hodgkin lymphomas in the late 1980s with the <sup>131</sup>I MB-1 radioimmunoconjugate. A renewed interest in this target has supported the development of new therapeutic agents targeting CD37, with naked monoclonal antibodies (BI836826), small modular immunopharmaceuticals (the single-chain, homodimeric therapeutic protein Orlertuzumab/TRU-016), antibody drug conjugates (IMGN529 and AGS67E, conjugated to the maytansinoid DM1 or to the monomethyl auristatin E, respectively), and novel radioimmunoconjugates (<sup>177</sup>Lu Betalutin) currently in clinical investigation.<sup>6-10</sup> Clinical activity has been observed in chronic lymphocytic leukemia, indolent and DLBCL, and some of these agents have now moved to combination studies with rituximab, chemotherapy, or targeted agents (see the following studies at ClinicalTrials.gov: NCT00614042, NCT01188681, NCT01317901, NCT01534715, NCT01403948, NCT01403948, NCT01644253, NCT01796171, NCT02175433, NCT02538614, NCT02564744, NCT02624492, NCT02658968, NCT02759016). CD37 staining is not routinely performed in the clinical practice. There is preclinical evidence suggesting CD37 expression levels do not correlate with therapeutic benefit<sup>5,7</sup>; however, this will need validation in clinical studies.

Xu-Monette et al have provided interesting new insights on the biologic role of CD37 in human DLBCL in relation to its expression across a very large number of homogeneously treated patients. Further studies should be performed to properly address the relationship between CD37 and sensitivity to the anti-CD20 monoclonal antibody rituximab and to harmonize staining protocols before including CD37 staining as a novel prognostic marker in the diagnostic workup for patients with DLBCL.

*Conflict-of-interest disclosure:* The authors have received institutional research grants from Immunogen (F.B., A.S.) and Nordic Nanovector (F.B.). ■

## REFERENCES

- Xu-Monette ZY, Li L, Byrd JC, et al. Assessment of CD37 B-cell antigen and cell of origin significantly improves risk prediction in diffuse large B-cell lymphoma. *Blood*. 2016;128(26):3083-3100.
- van Spruiel AB, de Keijzer S, van der Schaaf A, et al. The tetraspanin CD37 orchestrates the  $\alpha(4)\beta(1)$  integrin-Akt signaling axis and supports long-lived plasma cell survival. *Sci Signal*. 2012;5(250):ra82.
- Lapalombella R, Yeh YY, Wang L, et al. Tetraspanin CD37 directly mediates transduction of survival and apoptotic signals. *Cancer Cell*. 2012;21(5):694-708.
- de Winde CM, Veenbergen S, Young KH, et al. Tetraspanin CD37 protects against the development of B cell lymphoma. *J Clin Invest*. 2016;126(2):653-666.
- Gaudio E, Tarantelli C, Arribas A, et al. Identification of anti-lymphoma biomarkers of response to the anti-CD37 antibody drug conjugate (ADC) IMGN529. *Blood*. 2016;126(23):1548.
- Pereira DS, Guevara CI, Jin L, et al. AGS67E, an Anti-CD37 monomethyl auristatin E antibody-drug conjugate as a potential therapeutic for B/T-cell malignancies and AML: A new role for CD37 in AML. *Mol Cancer Ther*. 2015;14(7):1650-1660.
- Deckert J, Park PU, Chicklas S, et al. A novel anti-CD37 antibody-drug conjugate with multiple anti-tumor mechanisms for the treatment of B-cell malignancies. *Blood*. 2013;122(20):3500-3510.
- Stathis A, Freedman AS, Flinn IW, et al. A phase I study of IMGN529, an antibody-Drug Conjugate (ADC) Targeting CD37, in adult patients with relapsed or refractory B-cell non-Hodgkin's lymphoma (NHL). *Blood*. 2014;124(21):1760.
- Pagel JM, Spurgeon SE, Byrd JC, et al. Orlertuzumab (TRU-016), an anti-CD37 monospecific ADAPTIR<sup>TM</sup> therapeutic protein, for relapsed or refractory NHL patients. *Br J Haematol*. 2015;168(1):38-45.
- Repetto-Llamazares AH, Larsen RH, Patzke S, et al. Targeted cancer therapy with a novel anti-CD37 beta-particle emitting radioimmunoconjugate for treatment of non-Hodgkin lymphoma. *PLoS One*. 2015;10(6):e0128816.

DOI 10.1182/blood-2016-11-748137

© 2016 by The American Society of Hematology

## ● ● ● LYMPHOID NEOPLASIA

Comment on Bottoni et al, page 3101

# Releasing the brakes on BTK-targeting miRNA

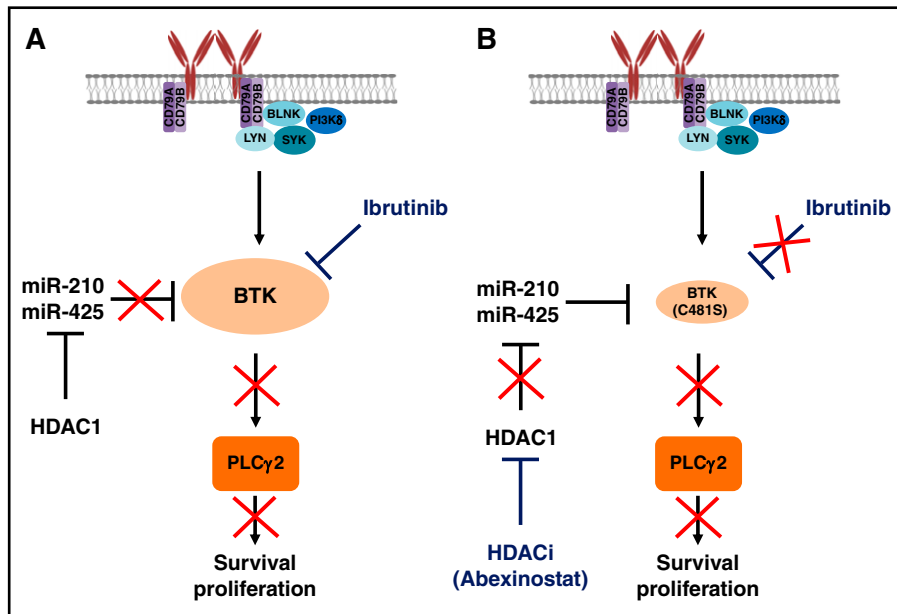
Matthew D. Blunt and Andrew J. Steele UNIVERSITY OF SOUTHAMPTON

In this issue of *Blood*, Bottoni et al demonstrate that histone deacetylase (HDAC) inhibition promotes the upregulation of microRNAs (miRNAs or miRs) which target Bruton tyrosine kinase (BTK), subsequently suppressing prosurvival signaling in chronic lymphocytic leukemia (CLL) samples, and highlight a rationale for HDAC inhibitors in combination with ibrutinib to treat patients.<sup>1</sup>

**B**-cell receptor (BCR) signaling is instrumental in the biology of CLL, and kinase inhibitors (KIs) such as ibrutinib and idelalisib, which target BTK and phosphatidylinositol 3-kinase  $\delta$  (PI3K $\delta$ ), respectively, are revolutionizing treatment of this disease. However, these agents are not curative, are taken indefinitely, and resistance and intolerance to these agents is already emerging. Ibrutinib discontinuation is associated with extremely poor survival and represents an important unmet clinical need. The primary resistance mechanism for ibrutinib is through mutations in BTK (C481S) or phospholipase C $\gamma$ 2 (PLC $\gamma$ 2; R665W and L845F),<sup>2</sup> however, microenvironmental signals may also influence sensitivity to this drug.<sup>3</sup>

miRNAs are small double-stranded RNA molecules of ~19 to 23 nucleotides, identified

as a mechanism that cells use to regulate posttranscriptional expression of various genes and subsequently protein production. They do this by reducing messenger RNA (mRNA) stability, blocking mRNA translation, or promoting mRNA degradation,<sup>4</sup> and numerous publications have described important roles for miRNA in CLL pathogenesis and BCR signaling.<sup>5,6</sup> HDACs regulate deacetylation and demethylation of lysine residues on histones resulting in the silencing of various genes. In CLL, HDACs are known to inhibit expression of miRNA, particularly miR15a, miR16, and miR29b, which are associated with the suppression of Bcl-2 and Mcl-1.<sup>7</sup> However, CLL samples treated in vitro with HDAC inhibitors (HDACi's) upregulated these miRNAs and consequently suppressed Mcl-1 and Bcl-2 protein expression.<sup>7</sup>



Pharmacological inhibition of HDAC relieves epigenetic suppression of BTK-targeting microRNA. (A) HDAC repressor complex inhibits the function of miR-210 and miR-425, causing an upregulation of BTK. BCR ligation induces activation of BTK, promoting CLL cell survival and proliferation. Ibrutinib covalently binds C481 on BTK to inhibit BTK activity and hence inhibit survival and proliferation of CLL cells. (B) Mutation of BTK C481S prevents covalent binding of ibrutinib to BTK and inhibits ibrutinib activity. The HDACi abexinostat releases HDAC1-mediated suppression of miR-210 and miR-425, leading to a decrease in BTK protein expression and suppression of downstream signaling, even in the presence of C481S mutation. Drugs are highlighted in blue and red crosses indicate the pathway is inhibited. BLNK, B-cell linker; LYN, tyrosine-protein kinase Lyn; SYK, spleen tyrosine kinase.

In this issue, Bottoni et al hypothesized that treatment of CLL cells with HDACi's may also regulate miRNA that target BTK. Indeed, they demonstrated that BTK protein expression was targeted and reduced most strongly by miR-210 and miR-425 in CLL, and that miR-210 and miR-425 were expressed at lower levels in primary CLL samples compared with normal B cells. They suggested this reduced expression in CLL cells was caused by silencing of BTK-targeting miRNAs via the HDAC repressor complex, indicating a novel outcome of epigenetic silencing, as well as providing a potential explanation for the overexpression of BTK in CLL. Furthermore, pharmacological or small interfering RNA-mediated targeting of HDAC1 activity increased the expression of BTK-targeting miRNAs and consequently reduced BTK expression and downstream signaling (see figure). Importantly, these reductions were evident in BTK(C481S) mutated samples, indicating this approach may hold potential for the treatment of patients who become resistant to ibrutinib. Indeed, the authors demonstrated the therapeutic potential of combining ibrutinib and HDACi's in vitro on primary CLL cells and in vivo using the Eμ-TCL-1 mouse model, whereby responses were

superior to either agent alone. Importantly, computational biology also predicts that preexisting drug-resistant clones may exist in patients prior to ibrutinib treatment.<sup>8</sup> This indicates that the combination of HDACi's with ibrutinib at least at therapy initiation may prevent/inhibit expansion of resistant clones during ibrutinib therapy, perhaps resulting in longer-term progression-free survival (PFS). However, there are some potential concerns associated with targeting HDACs in CLL. First, HDACi's have known toxicity in CLL patients<sup>9</sup> which may limit the usefulness of this approach. In particular, 1 side effect from treatment with HDACi's is thrombocytopenia; therefore, how this drug combination will affect bleeding-related events, particularly if long-term usage in combination with ibrutinib is necessary, consequently requires further investigation. As a result, can HDACi's be used at reduced doses to reduce toxicity to patients, while retaining their biological effect on miRNA expression? Can we use HDACi's for only a short period of time and only if resistance to ibrutinib emerges? Or is HDACi treatment required prior to ibrutinib treatment? Indeed, Mato et al suggested an alternative KI may prove effective after resistance has emerged, although in that study overall response rate to

a subsequent KI of 50% and a median PFS of 11.9 months suggested alternative salvage therapies still require investigation.<sup>10</sup> In addition to the BTK(C481S) mutation, progressive disease in CLL while on ibrutinib can alternatively be attributed to PLCγ2 mutations (R665W and L845F) which promote BCR-downstream signaling independently of BTK. But how HDACi treatment will affect signaling/resistance associated with PLCγ2 mutations and whether this will select for PLCγ2 mutations in patients while on treatment still remains to be evaluated.

In conclusion, despite these unresolved queries, this pioneering study identifies that epigenetic silencing of BTK-targeting miRNAs may contribute to BTK overexpression in CLL and demonstrates the possibility of using HDACi's to remove/reduce the emergence of ibrutinib-resistant clones. This study highlights the need to initiate clinical trials to assess this combination, given the urgent and unmet clinical need of kinase-resistant CLL patients at present.

*Conflict-of-interest disclosure:* The authors declare no competing financial interests. ■

## REFERENCES

1. Bottoni A, Rizzotto L, Lai T-H, et al. Targeting BTK through microRNA in chronic lymphocytic leukemia. *Blood*. 2016;128(26):3101-3112.
2. Woyach JA, Furman RR, Liu TM, et al. Resistance mechanisms for the Bruton's tyrosine kinase inhibitor ibrutinib. *N Engl J Med*. 2014;370(24):2286-2294.
3. Aguilar-Hernandez MM, Blunt MD, Dobson R, et al. IL-4 enhances expression and function of surface IgM in CLL cells. *Blood*. 2016;127(24):3015-3025.
4. Lindsay MA. microRNAs and the immune response. *Trends Immunol*. 2008;29(7):343-351.
5. Balatti V, Pekarky Y, Croce CM. Role of microRNA in chronic lymphocytic leukemia onset and progression. *J Hematol Oncol*. 2015;8:12.
6. Mraz M, Kippis TJ. MicroRNAs and B cell receptor signaling in chronic lymphocytic leukemia. *Leuk Lymphoma*. 2013;54(8):1836-1839.
7. Sampath D, Liu C, Vasan K, et al. Histone deacetylases mediate the silencing of miR-15a, miR-16, and miR-29b in chronic lymphocytic leukemia. *Blood*. 2012;119(5):1162-1172.
8. Komarova NL, Burger JA, Wodarz D. Evolution of ibrutinib resistance in chronic lymphocytic leukemia (CLL). *Proc Natl Acad Sci USA*. 2014;111(38):13906-13911.
9. Blum KA, Advani A, Fernandez L, et al. Phase II study of the histone deacetylase inhibitor MGCD0103 in patients with previously treated chronic lymphocytic leukaemia. *Br J Haematol*. 2009;147(4):507-514.
10. Mato AR, Nabhan C, Barr PM, et al. Outcomes of CLL patients treated with sequential kinase inhibitor therapy: a real world experience. *Blood*. 2016;128(18):2199-2205.

DOI 10.1182/blood-2016-11-748145

© 2016 by The American Society of Hematology