immunotherapies and powerful triplet and quadruplet regimens in earlier lines of treatment, with the real prospect of operational cure. The time frame to demonstrate statistically significant PFS superiority of new therapies over existing ones will be too long for timely regulatory approval of new drugs.

The exciting challenge in the years ahead will be to unravel the appropriate use of MRD status to guide therapeutic decisions. For example, can we escalate therapy in the presence of MRD positivity or de-escalate therapy upon achieving MRD negativity, particularly in the context of maintenance? What duration of sustained MRD negativity is needed to consider de-escalation/cessation of continuous treatment? Will salvage therapy upon loss of MRD negativity be necessary in our quest for operational cures in MM? These MRD-driven therapeutic strategies are being explored in an increasing number of clinical trials (see table) and are gaining momentum. Undoubtedly, MRD assessment will serve as a gateway to better therapeutic strategies in the quest for an operational cure in MM. The data presented by Cavo et al are a firm step toward this quest, and at the very least, indicate that the use of MRD status as a surrogate end point for PFS in MM is ready for prime time.

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HEMATOPOIESIS AND STEM CELLS

Comment on Yang et al, page 845

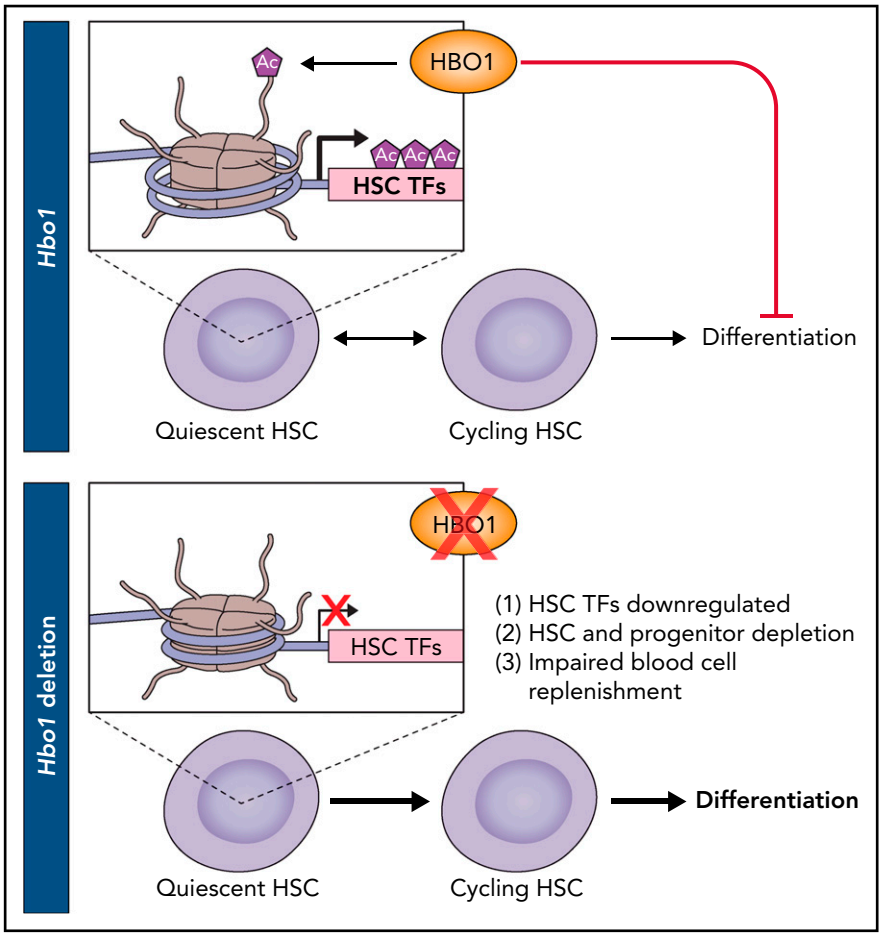
Renewing your HBO1 subscription

Fadi J. Najm[1] and Peter van Galen[2] | [1]Broad Institute of MIT and Harvard and [2]Brigham and Women's Hospital

In this issue of *Blood*, Yang et al[1] show that HBO1 (KAT7) promotes hematopoietic stem cell (HSC) quiescence and self-renewal through histone acetylation and transcription of HSC genes, revealing an important role for HBO1 in maintaining the blood system.

Acetylation, one of the oldest described histone modifications, involves the transfer of an acetyl group from acetyl-CoA to the ε-amino group of a lysine residue by histone acetyltransferases.[2] Histone acetylation acts to unfold chromatin to facilitate DNA access for replication or transcription. Histone H3 lysine 14 acetylation (H3K14ac) is found throughout gene bodies and promoters[3] and promotes processivity of RNA polymerase. H3K14ac is mainly established by HBO1, a conserved, widely expressed member of the MYST acetyltransferase family.[4] The MYST family includes 4 additional acetyltransferases: KAT6A, KAT6B, KAT8 (MOF), and KAT5 (TIP60). HBO1 acts as a core catalytic subunit in multimeric complexes along with a host of cofactors that includes MEAF6, ING4, ING5, BRPF1, BRPF2, BRPF3 (H3K14), JADE1, JADE2, and JADE3 (H4). HBO1 and its cofactors work in concert with other epigenetic regulators in various complexes, such as MLL, that promote epigenetic or transcriptional activation.[5]

Yang et al provide important new insights into the role of HBO1 in normal HSC function and blood replenishment (see figure). The investigators produced 2 mouse models that enabled inducible deletion of exon 1 of *Hbo1*. Deletion led to HSC loss and significantly decreased production of blood cells of all lineages, suggesting HSC exhaustion as a potential cause. Further experimentation suggested that depletions were apparent in the HSC compartment before losses in the differentiated populations. The authors strengthened these findings through experiments with competitive transplantation into lethally irradiated mice. *Hbo1*$^{fl/+}$*;CreER* (heterozygous) donor cells contributed 56% to 86% of peripheral blood cells in bone marrow chimeras whereas *Hbo1*$^{fl/fl}$*;CreER* (deletion) donor cells made no detectable contribution. Furthermore, lower



Model of HSC quiescence and self-renewal promoted by HBO1-mediated H3K14 acetylation (Ac). Deletion of *Hbo1* results in downregulation of transcription factors (TFs) that are important for HSC maintenance, leading to increased differentiation. Professional illustration by Patrick Lane, ScEYEnce Studios.

proportions of HSCs and multipotent progenitors were found in G_0 and approximately twofold more were found in S and G_2-M, indicating a depletion of the HSC quiescent state, which is often linked to loss of self-renewal.[6] Indeed, the fate of HSCs with deletion of *Hbo1* was shifted toward differentiation at the expense of symmetric self-renewal, indicating that *Hbo1* is required for HSC self-renewal. Mechanistically, H3K14ac CUT&Tag experiments in *Hbo1*-deleted HSCs revealed a widespread loss of this epigenetic mark across gene bodies, promoters, and downstream of transcription end sites. In $Lin^{-}Sca1^{+}Kit^{+}$ cells with *Hbo1* deletion, strong reductions in H3K14ac were found at canonical HSC self-renewal genes. These genes, including *Hoxa9*, *Meis1*, and *Erg*, were consequently downregulated. The comprehensive analysis of hematopoiesis in the absence of HBO1 thus yields a coherent picture of H3K14ac loss and downregulation of HSC genes, increased stem cell proliferation and differentiation, and reduced replenishment of mature blood cells.

Given that histone acetylation maintains cellular homeostasis, acetyltransferases play complex and differential roles in disease. Germline mutations in acetyltransferases lead to severe developmental defects, and mutations in adults can lead to malignancy.[5] Translocations and mutations in CBP, p300, TIP60, KAT6A, and KAT6B are often found in acute myeloid and lymphoid leukemias, as well as numerous solid tumors. Unsurprisingly, inhibitors to acetyltransferases have been developed. For example, KAT6A and KAT6B inhibitors arrest lymphoma progression by inducing senescence, CBP and p300 inhibition can relieve oncogene-induced differentiation block in leukemia, and TIP60 inhibitors induce apoptosis of breast cancer cells.[5,7] Various inhibitors of acetyltransferase family members have been discovered but relatively few have entered clinical trials. Recent studies put forth an HBO1 inhibitor candidate, WM-3538, which broadly inhibits MYST family members as a competitive analog of acetyl-CoA.[8] Acetyltransferase inhibition has diverse effects in different cell types, complicating their suitability for therapeutic treatment. For example, genetic targeting has revealed that HBO1 has important functions in promoting senescence,[9] survival of MLL-rearranged leukemia stem cells,[8,10] and now maintenance of normal HSCs. These various effects should be taken into account when designing therapeutic regimens.

The role of histone acetyltransferases such as HBO1 in regulating cell fate and self-renewal highlights the importance of understanding the functions of the acetyltransferase family of epigenetic enzymes. The fundamental knowledge established by the work of Yang et al will inspire the development of new epigenetic modulators for therapeutic purposes.

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LYMPHOID NEOPLASIA

Comment on Jebaraj et al, page 859

Overcoming resistance hurdles

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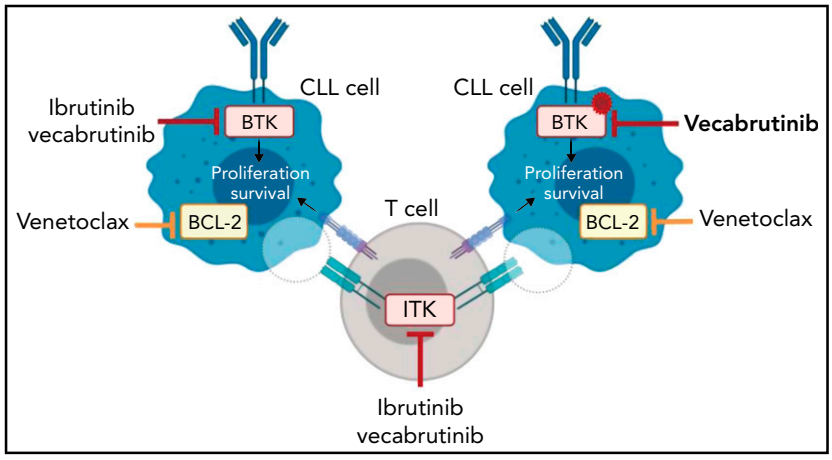
In this issue of *Blood*, Jebaraj et al[1] characterize a novel Bruton tyrosine kinase (BTK) inhibitor, vecabrutinib, that targets mutant BTK C481S and wild-type BTK in preclinical models. This is the first BTK inhibitor that also inhibits interleukin-2–inducible T-cell kinase (ITK) and can still bind to mutant BTK.

Clinical outcomes in chronic lymphocytic leukemia (CLL) have greatly improved over the last 2 decades with the development of regimens combining cytotoxic drugs with anti-CD20 monoclonal antibodies,[2] but outcomes vary significantly. For instance, patients in genetic high-risk groups, such as those harboring *TP53* mutations, continue to have suboptimal outcomes despite treatment with the best available therapy.[3] Similarly, patients who relapse early or are refractory to chemotherapy have an unfavorable outcome.[4] These areas of ongoing unmet clinical need lend impetus to developing truly novel targeted approaches to treating high-risk B-cell leukemias. Among the most promising classes of targeted therapies in CLL are inhibitors of BTK (BTKi's). BTK plays a prominent role in the BCR signaling pathway. Clinical activity of ibrutinib (the first US Food and Drug Administration–approved BTKi) is attributed to attenuated homing and retention of CLL cells to the microenvironment as a result of impaired BCR-controlled integrin-mediated adhesion and chemokine-controlled migration.[5]



The noncovalent BTK/ITK inhibitor vecabrutinib can block wild-type and C481S-mutant BTK in preclinical models. Vecabrutinib has immunomodulatory effects similar to those of ibrutinib in the murine Eμ-TCL1 model. Treatment with a combination of vecabrutinib and venetoclax leads to prolonged survival of Eμ-TCL1 mice. Illustration created with BioRender.com.

Microenvironmental crosstalk plays an important role in CLL pathogenesis and progression. CLL cells are strongly dependent on interactions with other immune cells, thus shaping a highly orchestrated network: the tumor microenvironment.[6] The inhibitory effects of ibrutinib on microenvironment homing and adhesion correlate with its clinical efficacy because ibrutinib treatment causes a rapid reduction of the lymph node size followed by a prolonged lymphocytosis.[7] This prolonged lymphocytosis resulting from treatment with kinase inhibitors seems to have no clinical disadvantage.[7] However, it could enhance the possibility of resistant clones accumulating. Acquired resistance to ibrutinib was reported in ~80% of patients as a result of mutations in BTK itself or the downstream kinase phospholipase Cγ2 (PLCγ2).[8] The BTK C481, most commonly serine, mutation confers resistance by preventing the covalent binding of ibrutinib to its target cysteine 481 in BTK (C481S).[8]

To date, 5 noncovalent BTKi's that can inhibit the kinase in the presence of a BTK C481 mutation[9] have entered clinical trials. In their article, Jebaraj et al characterized the noncovalent BTKi vecabrutinib (SNS-062) and demonstrated binding in the adenosine triphosphate binding pocket of BTK independent of the C481 residue (see figure). Vecabrutinib inhibited BCR signaling in wild-type and BTK C481S–mutant cells as measured by calcium flux. Furthermore, adoptive Eμ-TCL1 mice treated with vecabrutinib have increased survival compared with vehicle control (median survival, 35 vs 28 days).