disease at high risk for relapse. However, although biomarkers can be useful clinically without an understanding of the underlying biology, further exploration of the functional role of UCH-L1 in the pathogenesis of DLBCL is needed.

Recently, an inhibitor of UCH-L1 deubiquitinase activity, LDN-57444, was used successfully to show that UCH-L1 is a prometastatic molecule in a murine model of pulmonary metastasis.[8] This is the first indication that blockage of UCH-L1 might be beneficial for anticancer treatment in vivo. The results of Bedekovics et al pave the way for further studies on the physiological roles of UCH-L1 to be carried out to clarify whether this deubiquitinating enzyme is a therapeutic target in aggressive B-cell lymphomas.

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

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Comment on Schulze et al, page 1575

Ectopic DNMT3B expression delays leukemogenesis

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In this issue of *Blood*, Schulze et al use a tetracycline-inducible *Dnmt3b* knock-in mouse model to investigate how DNMT3B-mediated DNA methylation affects leukemogenesis. Increased DNMT3B expression prolonged survival in retrovirally induced *Myc-Bcl2*– or *MLL-AF9*–driven leukemia, and acute myeloid leukemia (AML) patients with high expression of DNMT3B target genes showed inferior overall survival.[1]

DNA methylation plays a crucial role in control of chromatin structure and gene expression during human development, stem cell differentiation, and carcinogenesis. DNA methyltransferases (DNMTs) regulate methylation at position 5 of cytosine (5-methylcytosine), which occurs primarily in the context of cytosine guanine dinucleotides. This process is regulated by both DNA methylating and DNA demethylating proteins. Of the 5 mammalian DNMT family proteins (DNMT1, DNMT2, DNMT3A, DNMT3B, and DNMT3L), only DNMT1, DNMT3A, and DNMT3B are functional DNA methyltransferases. DNMT1 is the major maintenance methyltransferase, responsible for maintaining existing DNA methylation patterns, whereas DNMT3A and DNMT3B function primarily as de novo DNA methyltransferases that establish novel DNA methylation marks.[2]

Recently, changes in DNA methylation patterns and abnormal expression of de novo methyltransferases have been detected during human hematopoietic stem cell (HSC) differentiation and in hematologic malignancies and correlate with overall survival of AML patients.[3,4] The identification of *DNMT3A* mutations in 12% to 20% of AML cases, 8% of myelodysplastic syndrome cases, and 7% to 15% of patients with myeloproliferative neoplasms, implicate this methyltransferase as a key epigenetic factor in myeloid malignancy.[5] Interestingly, few mutations of the structurally similar *DNMT3B* methyltransferase have been identified in hematologic disease[6]; however, several recent studies of *DNMT3B* expression in leukemic bulk cells from AML patients revealed a decrease in overall survival in patients with high *DNMT3B* expression.[7]

Although there have been significant functional and mechanistic studies of DNMT3A in murine HSCs, there have been relatively few studies of DNMT3B in the context of hematopoiesis. Conditional deletion of DNMT3B results in a mild hematopoietic phenotype with minimal effects on HSC function, and combined deletion of both DNMT3A and DNMT3B leads to a block in differentiation and diminished self-renewal potential.[8] DNMT3B gain-of-function studies suggest an oncogenic role for DNMT3B in APC_{min} mice prone to gastrointestinal tumors.[9] Paradoxically, DNMT3B may act as a tumor suppressor in *Eμ-Myc* lymphomas,[10] suggesting dual context-dependent roles of DNMT3B in cancer. The precise role of DNMT3B in the context of hematopoiesis and myeloid malignancies has not been adequately studied, and the function of increased DNA methylation is not fully understood.

To assess the consequences of increased de novo DNMT3B activity in normal and malignant hematopoiesis, Schulze et al used a tetracycline-inducible *Dnmt3b* knock-in mouse model in which the tetracycline-inducible *Dnmt3b* transgene was targeted to the 3′ untranslated region of the collagen I gene, and the tetracycline-responsive transactivator (M2-rtTA) was inserted into the *ROSA26* locus. Analysis of steady-state hematopoiesis of *Dnmt3b* knock-in mice showed an increase in the total number of progenitor cells at the level of the phenotypic lymphoid-primed multipotent progenitor population; however,

no overt hematopoietic disease phenotype was detected. To study the role of DNMT3B in the context of leukemic initiation, Schulze et al isolated lineage negative bone marrow cells from *Dnmt3b* knock-in mice and retrovirally induced *Myc-Bcl2*– or *MLL-AF9*–driven leukemia in a transplantation model. High expression of DNMT3B significantly delayed leukemogenesis in both models of disease. These findings combined with results from serial transplantation assays suggested that high DNMT3B expression impairs the leukemic potential of leukemia stem cells (LSCs).

Methylation and gene expression studies performed on wild-type or DNMT3B leukemic cells revealed that increased expression of DNMT3B induced global DNA hypermethylation in *Myc-Bcl2*–induced leukemias, preferentially at gene bodies; however, *MLL-AF9* leukemias had more modest DNA hypermethylation. High DNMT3B-expressing leukemic cells from both models showed overlap of DNA hypermethylated regions, in addition to loss of expression of genes associated with stem cell function. There was little correlation between gene expression and methylation changes; however, several candidate genes that showed hypermethylation and reduced gene expression were associated with better overall survival when expressed at low levels in a clinical cohort of AML patients.

The findings by Schulze et al highlight the importance of DNMT3B expression in normal hematopoiesis and leukemogenesis. Interestingly, despite a negative correlation between *DNMT3B* expression and overall survival in human AML patients, Schulze et al find that increased expression of DNMT3B in LSCs delays leukemogenesis and that expression of target genes dysregulated by DNMT3B-mediated DNA methylation has prognostic value in AML patients. This discrepancy may be due to context- and/or cell-specific effects of DNMT3B, ie, this study was performed in LSCs, but the analyzed published AML patient data are generated from unsorted leukemic bulk cells. Nonetheless, additional studies of the expression of *DNMT3B* in different AML subtypes, and ideally at the stem cell level, would further our understanding of DNMT3B-mediated DNA methylation and leukemogenesis.

In summary, through a series of genetic and molecular approaches, Schulze et al provide a novel study of the effects of increased DNMT3B expression on normal and malignant hematopoiesis. This work establishes increased DNMT3B expression as an antagonist of leukemogenesis and highlights a critical role for DNMT3B-mediated DNA methylation in leukemia development and maintenance of LSC function. The finding that increased expression of DNMT3B in LSCs delays leukemogenesis and the potential relevance of DNMT3B-target genes in AML patients provides a strong rationale for further investigation of DNMT3B in other AML mouse models and in patients with myeloid malignancies.

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

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● ● ● PLATELETS AND THROMBOPOIESIS

Comment on Hou et al, page 1587

Move over Tregs, MDSCs are here

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In this issue of *Blood*, Hou et al show the involvement of myeloid-derived suppressor cells (MDSCs) in the pathogenesis of immune thrombocytopenia (ITP) and identify a novel mechanism by which high-dose dexamethasone (HD-DXM) promotes MDSC expansion and function and correlates with increased platelet counts.[1]

Autoimmune disease can occur when the natural immunosuppressive cells that keep autoreactive lymphocytes in check are lost or are suppressed themselves. For example, impaired $CD4^+CD25^+Foxp3^{high}$ T regulatory cells (Tregs) have emerged as significant contributors to the pathogenesis of various autoimmune disorders including ITP. Other immunosuppressive cells called MDSCs are a morphologically and functionally heterogeneous population of myeloid progenitor cells that are also potent regulators of adaptive immunity.[2] The most striking feature of MDSCs is their ability to inhibit T-cell proliferation through depleting nutrients required for the functional enzymatic activity of arginase-1 (Arg-1) and/or nitric oxide (NO) production.[3,4] Although initially described in malignancies, MDSCs are also present in inflammatory and autoimmune settings. Several culture conditions for the in vitro generation of MDSCs have been developed using a combination of cytokines and colony-stimulating factors (CSFs). The effects of common immunosuppressive therapies, such as glucocorticoids (GCs), on MDSC development are unknown.