Comment on Göbel et al, page 1802

Dual targeting of EZH2 and DOT1L in DLBCL

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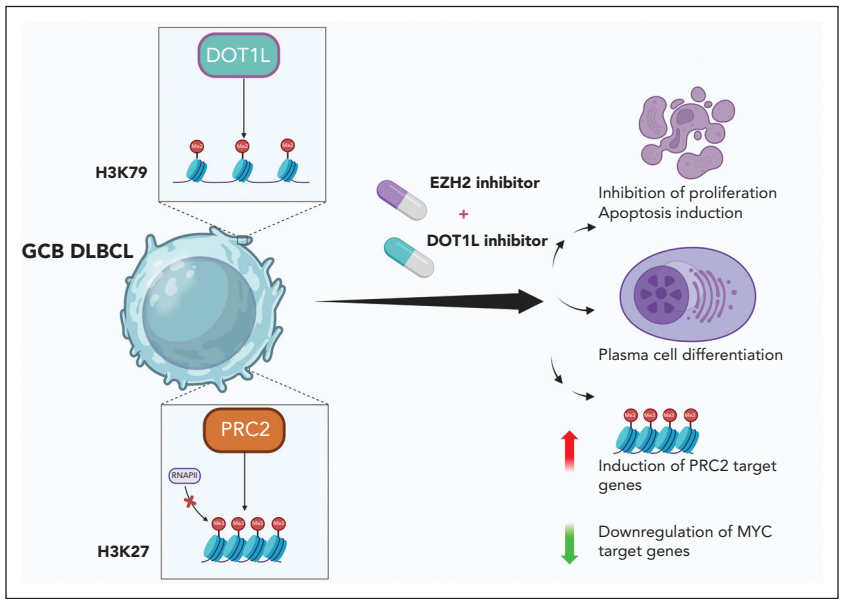
In this issue of *Blood*, Göbel et al[1] show that the germinal center (GC) diffuse large B-cell lymphoma (DLBCL) cells depend on EZH2 and DOT1L epigenetic writers. In vitro treatment with the combination of DOT1L and EZH2 inhibitors results in synergistic activity associated with loss of proliferation and subsequent plasma cell differentiation.

DLBCL is the most common lymphoid malignancy in adults. EZH2, the catalytic subunit of the polycomb PRC2 complex, represses gene transcription through trimethylation of lysine 27 of histone H3 (H3K27me3). EZH2 is a key regulator in the germinal center reaction repressing genes involved in cell cycle regulation, response to immune signaling, and plasma cell differentiation.[2-4] DOT1L is a histone methyltransferase catalyzing mono-, di-, and trimethylation of the lysine 79 of histone H3 (H3K79). EZH2 gain-of-function mutations were identified as driver mutations in DLBCL and follicular lymphoma.[5,6] DOT1L is required in the development of B-cell germinal centers and humoral immune response.[7,8]

Since GC B cells depend on DOT1L and EZH2 epigenetic enzymes, Göbel et al aimed to decipher the role of DOT1L and EZH2 in the pathophysiology of GC DLBCL. The authors reported the coexpression of DOT1L and EZH2 in human GC B cells.[9] Using a collection of DLBCL cell lines, the authors identified that DOT1L inhibitor induces cell cycle defects in a subset of the GCB DLBCL cell lines, and those cell lines were further investigated for their association with H3K79 demethylation. In the DepMap database, the authors identified a strong codependency of EZH2 and SUZ12 polycomb PRC2 subunits with DOT1L more specifically in DLBCL cell lines compared with other cell lines. However, the codependency between EZH2 and DOT1L was also present in other cancers overexpressing EZH2. Based on the role of EZH2 and DOT1L in GC B cells, the authors investigated the synergistic toxicity DOT1L and EZH2 inhibitors in GCB DLBCL cell lines. They reported a significant toxicity of the combination in 7 out of the 9 cell lines investigated, which was associated with induction of apoptosis. Moreover, DOT1L inhibition did not affect EZH2 expression and H3H27me3 levels.

Using H3K27me3 chromatin immunoprecipitation sequencing, the authors identified PRC2 target genes deregulated by DOT1L inhibition. They showed upregulation of PRC2 target genes including plasma cell transcription factors PRDM1 and IRF4 and genes associated with cellular morphogenesis. The authors investigated the effects of EZH2 and DOT1L dual inhibition on H3K27me3/H3K4me3 bivalent promoters. They demonstrated that combined inhibition of DOT1L and EZH2 increased median expression of both bivalent and H3K27me3-only genes. Gene set enrichment analysis showed that both bivalent and H3K27me3-only genes were derepressed under all treatment conditions with a stronger effect of the combination compared with EZH2 inhibitor alone. Specifically, bivalent genes are primarily involved in cell differentiation, cellular development, and cellular morphogenesis, suggesting that EZH2 and DOT1L work together to maintain the poised state of bivalent genes and the repression of H3K27me3-only genes to inhibit B to plasma cell differentiation. The authors identified that EZH2 and DOT1L inhibition results in induction of plasma cell transcriptional programs. This loss of GC B-cell identity was associated with deregulation of BCL6 and MYC target genes. Since BCL6 is a master transcription factor of GC B cells and MYC is indispensable for GC maintenance, EZH2 and DOT1L cooperate to maintain GC B-cell proproliferative state in DLBCL cells and inhibit plasma cell differentiation.

The authors then turned their attention to explore the therapeutic potential of EZH2 and DOT1L inhibitors in vivo. Using a xenograft murine model with the



Göbel et al defined that EZH2 and DOT1L cooperate to repress PRC2 target genes and support the proproliferative GC B-cell state in DLBCL. Dual inhibition of EZH2 and DOT1L represents a differentiation-based therapeutic strategy to target GCB DLBCL.

Oci-Ly7 cell line, the authors identified that the combination of EZH2 and DOT1L inhibitors prevented tumor growth in all mice. The use of either alone was less effective than in combination. With combined treatment, the authors found a significant decrease in H3K79me2 and H3K27me3 levels in peripheral blood mononuclear cells in vivo. Furthermore, the authors investigated the toxicity of the combination in mice. Even though the body weight remained mostly stable, a significant reduction in erythropoiesis and nephrotoxicity was found in some animals. No significant toxicity was identified in the gastrointestinal tract, heart, brain, lungs, or liver.

The authors conclude that DOT1L and EZH2 cooperates to epigenetically block the differentiation of GCB DLBCL cells and support proproliferative state (see figure).

Of interest, their findings underline the existence of a mechanism involving DOT1L in the silencing maintenance of PRC2 target genes, in GCB DLBCL cells, independently of EZH2.

The current work revealed the previously unrecognized therapeutic potential of EZH2 and DOT1L inhibitors, given in combination, as a differentiation-based therapeutic strategy in GCB DLBCL (see figure). Since both EZH2 and DOT1L inhibitors have been clinically tested, this should prompt clinical evaluation of the synergistic potential of this combination.

Conflict-of-interest disclosure: J.M. declares no competing financial interests. ■

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