Targeting leukemia on the DOT

A combination of chemical and genetic approaches has established a proof of concept in mouse models—with strong mechanistic underpinning—indicating that targeting the aberrantly recruited histone methyltransferase activity of DOT1L has therapeutic potential in aggressive leukemias driven by MLL fusion genes.

Jon Travers, Julian Blagg & Paul Workman

Cell type–specific licensing of gene transcription is critical for developmental processes in multicellular organisms. Thus, these processes are entirely dependent on the factors that regulate such licensing at the molecular level, particularly those that post-translationally modify chromatin at DNA sequence–specified genomic sites

Lyophilic nucleophilic methyltransferase targets in cells. Such chemical tools have proved incredibly powerful in many fields as they are valuable for exploratory biology, target validation, druggability analysis and the demonstration of mechanistic and therapeutic proof of concept—acting as pathfinder probe molecules as well as providing potential starting points for drug discovery. Moreover, chemical tools are especially valuable in the case of histone methyltransferases and other proteins that function in macromolecular, multifunctional super-complexes because, in contrast to genetic knockdown, they facilitate discrimination between scaffolding and catalytic functional effects on phenotype. So far, high-quality chemical tools for histone methyltransferases have proved hard to find despite the druggable S-(5′-adenosyl)-L-methionine (SAM) binding site in the catalytic domain of human DOT1L

This difficulty may be partly ascribed to the need for an appropriate methylation substrate and a suitable assay format for optimal high-throughput screens.

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not only identify a promising tool compound for DOT1L but also, together with Bernt et al., exemplify the utility of combining complementary genetic and chemical approaches by demonstrating, in in vivo mouse models as well as in cell culture, the dependence of leukemic cell subtypes that carry MLL fusions on the histone H3 Lys79 (H3K79) methyltransferase activity of DOT1L. AF-family MLL fusions—exemplified by MLL-AF4 and MLL-AF9—that are present in around 80% of MLL fusion–bearing leukemias cause the inappropriate recruitment of DOT1L to leukemogenic MLL-fusion target genes; DOT1L recruitment is associated with increased H3K79 methylation specifically at those sites. However, precise mechanistic understanding of the dependence of leukemogenesis and malignant progression on this DOT1L recruitment have until now remained obscure.

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use a rational design approach, based on the universal histone methyltransferase substrate SAM, to discover a potent SAM–competitive DOT1L inhibitor EPZ004777 ($K_c = 0.3 \text{nM}$, Fig. 1) with surprisingly exquisite selectivity (>1,000-fold) compared to other methyltransferases tested, as measured biochemically in vitro and in cells. Using model cell lines derived from leukemias with a variety of genetic backgrounds, they demonstrate the highly selective antiproliferative, differentiating and apoptotic activities of EPZ004777 specifically toward those cells harboring MLL fusions, which is a differential response that correlates with transcriptional repression of the key leukemogenic MLL fusion target genes HOXA9 and MEIS1. Leukemic cells lacking MLL fusions are less sensitive by a factor of approximately 100. Furthermore, this in vitro selectivity translates to the targeting of leukemic cells in mouse models of mixed-lineage leukemia, which results in prolonged survival. This finding complements the results of Bernt et al., which demonstrate that conditional knockout of DOT1L blocks radiation-induced leukemogenesis and induces apoptosis in MLL-fusion-bearing leukemic cells with minimal consequences for normal hematopoiesis. Thus, the findings from the
chemical probe and genetic knockdown experiments are in agreement, thereby increasing confidence in the potential therapeutic effectiveness of targeting DOT1L-dependent gene activation in MLL fusion–addicted leukemias.

Complementary application of the small-molecule-inhibitor and genetic approaches has also been mechanistically revealing for MLL fusion-target gene activation. Whereas in non-malignant cells, the amount of H3K79 methylation tracks with H3K4 and H3K36 methylation, aberrant recruitment of DOT1L to the MLL-fusion core target genes caused elevated H3K79 methylation independent from the other activating chromatin marks. Thus, increased H3K79 methylation could functionally substitute for the other modifications in this context, and leukemogenic target genes become aberrantly and pathogenically dependent on H3K79 methylation. Inhibition of DOT1L catalytic activity with the chemical probe EPZ004777 supports the finding that profound target inhibition is required to reverse pathogenic histone methylation–dependent effects on gene activity. Abrogation of global H3K79 methylation by DOT1L inhibition with a small-molecule inhibitor is likely to require excellent pharmacokinetics, slow offset kinetics from DOT1L, or both. This provides a challenging optimization hurdle for the promising EPZ004777 chemical tool (Fig. 1) that will need to be overcome for optimal therapeutic impact, including more prolonged survival benefit.

The work of Daigle et al. and Bernt et al. illustrates a pharmacological synthetic lethality between MLL fusions and DOT1L inhibition, with profound clinical implications for the personalized treatment of an aggressive form of leukemia. Importantly, these studies also represent a comprehensive example of mechanism-to-phenotype chemical biology in the exciting area of epigenetics. This is a significant advance in the histone methyltransferase field, which hitherto has not benefited from fully fit-for-purpose chemical tools—with the exception of the potent and selective G9a enzyme inhibitor UNC0638 (ref.13), which, in contrast to EPZ00477, has a peptide-competitive mechanism of action but likewise has suboptimal pharmacokinetic properties. In addition to opening up a new era of epigenetic therapies for cancer and other diseases, such chemical tools will provide chromatin scientists with a powerful set of reagents to tackle questions that abound in this highly complex and translationally relevant area of biology. In particular, further mechanistic elucidation and therapeutic attack on the ~100-member histone methyltransferase class—including other genetically well-validated targets such as EZH2—is now imminent, with many pharmaceutical and biotech companies investing substantially in this target class.

![Figure 1](image-url)
Cancer Research UK Cancer Therapeutics Unit, Division of Cancer Therapeutics, The Institute of Cancer Research, Sutton, UK. Julian Blagg is in the Medicinal Chemistry Team, Cancer Research UK Cancer Therapeutics Unit, Division of Cancer Therapeutics, The Institute of Cancer Research, Sutton, UK.
e-mail: paul.workman@icr.ac.uk

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Competing financial interests
The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturechemicalbiology/.

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