function and pathogen defense. *J Clin Invest*. 2017;127(9):3521-3526.

4. Claushuis TAM, de Stoppelaar SF, de Vos AF, et al. Nbeal2 deficiency increases organ damage but does not affect host defense during gram-negative pneumonia-derived sepsis. *Arterioscler Thromb Vasc Biol*. 2018; 38(8):1772-1784.

5. Rao DA, Wei K, Merola JF, et al. Myeloperoxidase-antineutrophil cytoplasmic antibodies (MPO-ANCA) and proteinase 3-ANCA without immunofluorescent ANCA found by routine clinical testing. *J Rheumatol*. 2015;42(5):847-852.

6. Nakazawa D, Tomaru U, Suzuki A, et al. Abnormal conformation and impaired degradation of propylthiouracil-induced neutrophil extracellular traps: implications of disordered neutrophil extracellular traps in a rat model of myeloperoxidase antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis Rheum*. 2012;64(11):3779-3787.

7. Cunin P, Bouslama R, Machlus KR, et al. Megakaryocyte emperipolesis mediates membrane transfer from intracytoplasmic neutrophils to platelets. *eLife*. 2019;8:e44031.

8. Gutierrez-Arcelus M, Rich SS, Raychaudhuri S. Autoimmune diseases–connecting risk alleles with molecular traits of the immune system. *Nat Rev Genet*. 2016;17(3):160-174.

9. Rao DA. T cells that help B cells in chronically inflamed tissues. *Front Immunol*. 2018;9:1924.

10. Ermann J, Rao DA, Teslovich NC, Brenner MB, Raychaudhuri S. Immune cell profiling to guide therapeutic decisions in rheumatic diseases. *Nat Rev Rheumatol*. 2015;11(9):541-551.

DOI 10.1182/blood.2020008196

MYELOID NEOPLASIA

Comment on Perner et al, page 1983

Rerouting DOT1L inhibitors in leukemia

Ross A. Dickins | Monash University

In this issue of *Blood*, Perner and colleagues show that 2 new DOT1L inhibitors with favorable administration routes and pharmacokinetic properties yield promising antileukemic activity in patient-derived xenograft (PDX) models of MLL-rearranged acute myeloid leukemia (AML).[1]

Acute leukemia arises when hematopoietic progenitor cells become locked into an immature state of perpetual proliferation. Several genetic alterations can impose this differentiation block, including chromosomal translocations affecting the *MLL* (*MLL1*, *KMT2A*) gene observed in ~10% of acute leukemias.[2,3] MLL rearrangements encode novel protein fusions of truncated MLL with a variety of partner proteins, resulting in aberrant transcriptional regulation.[2,3] MLL-fusion proteins have a bad reputation based in part on their apparent oncogenic autonomy: they are far more frequently found in infant and pediatric leukemia compared with adult disease, and MLL-fusion leukemias often have a paucity of additional genetic alterations.[2] In keeping with this, expression of MLL-fusions causes rapid, aggressive leukemia in mice.[2] The extraordinary oncogenicity of MLL-fusions is associated with poor prognosis[4]; hence, there is much interest in developing leukemia therapies targeting MLL-fusions or their cofactors.

One such cofactor is the enzyme DOT1L (KMT4), which methylates lysine 79 of histone H3 (H3K79).[4] As a component of MLL-fusion protein complexes, DOT1L facilitates aberrant transcription of MLL-fusion target genes.[4] Proof of principle for in vivo therapeutic targeting of DOT1L was originally established in 2011 in mouse models of MLL-fusion–driven leukemia, where genetic DOT1L deletion caused leukemia differentiation and apoptosis.[5-7] DOT1L ablation in mice compromised normal hematopoiesis but was relatively well tolerated, indicating a potential therapeutic window.[5-7] Soon after, Daigle et al described pinometostat (EPZ-5676), a selective inhibitor of DOT1L methyltransferase activity.[8] In rodents xenografted with a human MLL-rearranged AML cell line, pinometostat triggered sustained leukemia regression associated with reduced H3K79 methylation. Although this DOT1L inhibitor was well tolerated, it required continuous IV infusion due to rapid clearance.[8] Pinometostat was also well tolerated in a recent first-in-human study but again it required continuous IV infusion.[9] It had modest clinical effects, inducing complete remission in 2 of 51 MLL-fusion leukemia patients.[9]

The current study from Perner et al builds on a 2019 report from some of the same authors that identified 2 novel DOT1L inhibitors.[10] Although these new compounds each have markedly different structures and target binding modes, Perner et al show specific DOT1L methyltransferase inhibition comparable to pinometostat. However, in contrast to previous DOT1L inhibitors, these new compounds are effectively administered by oral or intraperitoneal routes and produce stable plasma drug levels that are well tolerated by immunocompromised mice.[1] Furthermore, in PDX mouse models of primary MLL-rearranged AML, the new compounds potently inhibit DOT1L-mediated H3K79 methylation, triggering leukemia differentiation and significantly reducing disease burden.[1]

By demonstrating that DOT1L inhibition can be achieved in AML in vivo with relative ease using these new compounds, Perner et al set the scene for further interrogation of DOT1L dependency in preclinical AML models. It is unclear whether the modest single-agent DOT1L inhibitor efficacy observed in the original AML patient trial[9] reflects suboptimal dosing and target inhibition, distinct sensitivity of different MLL-fusion proteins to DOT1L inhibition, and/or other factors. The new compounds characterized by Perner and colleagues should help resolve these questions. The field is now poised to define and refine the antileukemic potency and tolerability of DOT1L inhibitors in preclinical models and then hopefully in leukemia patients, either as monotherapies or in combination with other antileukemic agents.

Conflict-of-interest disclosure: R.A.D. has been a member of advisory boards for Celgene. ■

REFERENCES

1. Perner F, Gadrey JY, Xiong Y, et al. Novel inhibitors of the histone-methyltransferase DOT1L show potent antileukemic activity in patient-derived xenografts. *Blood*. 2020; 136(17):1983-1988.

2. Brien GL, Stegmaier K, Armstrong SA. Targeting chromatin complexes in fusion protein-driven malignancies. *Nat Rev Cancer*. 2019;19(5):255-269.

3. Meyer C, Burmeister T, Gröger D, et al. The MLL recombinome of acute leukemias in 2017. *Leukemia*. 2018;32(2):273-284.

4. Sarno F, Nebbioso A, Altucci L. DOT1L: a key target in normal chromatin remodelling and in mixed-lineage leukaemia treatment. *Epigenetics*. 2020;15(5):439-453.

5. Bernt KM, Zhu N, Sinha AU, et al. MLL-rearranged leukemia is dependent on aberrant H3K79 methylation by DOT1L. *Cancer Cell*. 2011;20(1):66-78.

6. Jo SY, Granowicz EM, Maillard I, Thomas D, Hess JL. Requirement for Dot1l in murine postnatal hematopoiesis and leukemogenesis by MLL translocation. *Blood*. 2011;117(18):4759-4768.

7. Nguyen AT, Taranova O, He J, Zhang Y. DOT1L, the H3K79 methyltransferase, is required for MLL-AF9-mediated leukemogenesis. *Blood*. 2011;117(25):6912-6922.

8. Daigle SR, Olhava EJ, Therkelsen CA, et al. Potent inhibition of DOT1L as treatment of MLL-fusion leukemia. *Blood*. 2013;122(6):1017-1025.

9. Stein EM, Garcia-Manero G, Rizzieri DA, et al. The DOT1L inhibitor pinometostat reduces H3K79 methylation and has modest clinical activity in adult acute leukemia. *Blood*. 2018; 131(24):2661-2669.

10. Stauffer F, Weiss A, Scheufler C, et al. New potent DOT1L inhibitors for in vivo evaluation in mouse. *ACS Med Chem Lett*. 2019;10(12):1655-1660.

DOI 10.1182/blood.2020007352