approach not only adds a new element to the therapeutic armamentarium, but offers the possibility of less-toxic treatments; in the case series, vemurafenib at the ultimate treatment dose was quite well tolerated. Although the response duration remains unproven, there is also hope that ECD and LCH, which appear to be cytogenetically less complex than metastatic melanoma, might develop resistance at a lower rate and that BRAF inhibitors, whether vemurafenib or others, could result in long-term responses.

The identification of another pair of diagnoses that have therapeutic responses based on the discovery of a recurrent gene mutation with the potential to activate aberrant signaling coupled with the development of an effective inhibitor further validates a genetically based treatment paradigm in which treatment options are less driven by histology than by DNA sequence. This approach is fast becoming the law of the land in diseases such as lung cancer, and the results of Haroche et al suggest that the benefit of targeted therapies could expand even to the rarest of the rare diseases.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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● ● ● MYELOID NEOPLASIA

Comment on Klco et al, page 1633

Demethylation demystification

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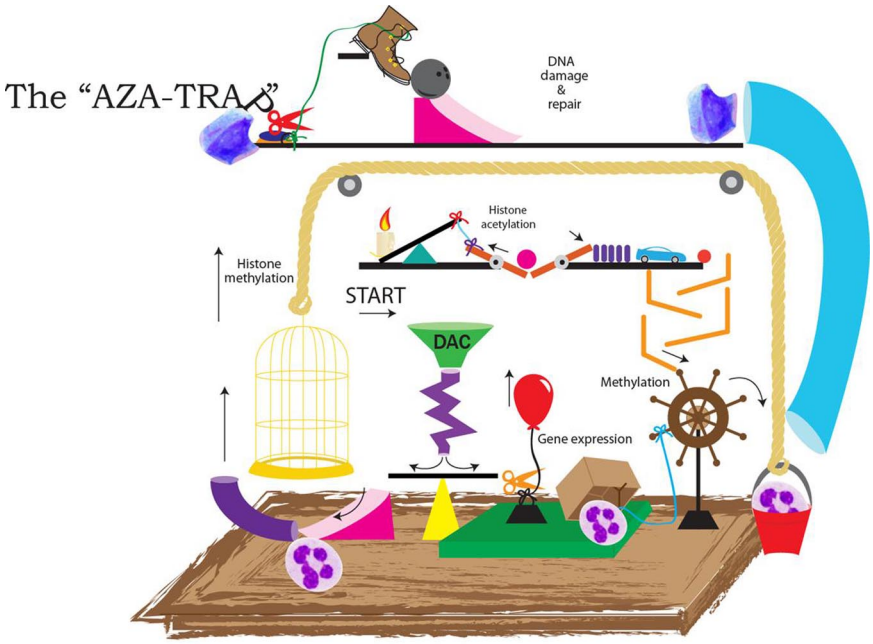
The ability of the DNA methyltransferase inhibitors (DNMTi) to induce terminal differentiation in fibroblasts was first noted by Taylor and Jones in 1979[1]; Silverman and Holland reported hematologic improvement in patients with myelodysplastic syndrome (MDS) in 1993.[2] That azacitidine improves survival in patients with high-risk MDS and acute myeloid leukemia with MDS features compared with a combined comparator group of supportive care, low-dose cytarabine, and intensive cytarabine plus anthracycline, while inducing trilineage normalization in approximately 15% of patients[3] makes the development of more potent, more specific drugs that behave like azacitidine imperative. The question is, how do the azanucleosides behave?

The incorporation of azacytosine nucleosides into DNA during S phase is followed by the formation of irreversible adducts with DNMT1,[4,5] depleting the cell of active enzyme. Subsequent replication cycles in the absence of active DNMT1 leads to progressive reversal of cytosine methylation. In vitro, methylation reversal at CpG islands is often associated with re-expression of the associated gene.[6] Thus, conventional wisdom assumes that cellular and clinical responses derive from a change in gene expression patterns resulting from promoter methylation reversal.

The problem is that such an association has been difficult to demonstrate. Several studies investigating changes in global methylation, methylation and/or expression of specific target genes, and genome-wide methylation and/or expression in patients receiving deoxyazacytidine (DAC) or 5-azacytidine (5AC), alone or in combination with other drugs, have failed to discriminate clinical responders from clinical nonresponders on the basis of baseline methylation/expression parameters or changes in these metrics (see figure).[7,8] Measurable changes in methylation appear necessary for clinical response (no doubt as a marker of drug bio-availability and adequate concentration) but have not been shown to be sufficient or mechanistically linked. These studies may be critiqued on the basis of the methodologies used, the times at which the tumor was sampled, and the heterogeneity of the patients treated.

In this issue of *Blood*, Klco and colleagues treat primary acute myeloid leukemia bone marrow samples with DAC in a stromal coculture system for 3 days before analyzing changes in the methylome and expression profiles.[9] Methylation array data showed high methylation levels in some promoters, but also in gene bodies and 3′ untranslated regions. Furthermore, decitabine induced hypomethylation–favored areas with higher baseline methylation and extent of methylation reversal appeared to correlate with degree of initial methylation. Thus, methylation changes were frequently more extensive in gene bodies than in CpG islands, similar to a recent study by Yan et al.[10] Post-mock and Post-DAC treatment samples of each leukemia cluster with themselves in unsupervised analysis, rather than with other treated samples, and correlation between changes in methylation and gene expression was "subtle," and did not apply to CDH1 or CDKN2B, 2 frequently methylated tumor suppressor genes in myeloid neoplasms.

This well-done study by Klco et al parallels the clinical experience, demonstrating a lack of demonstrable direct connectivity between methylation reversal events in response to azanucleosides and canonical early changes in gene expression. It is therefore not surprising that connecting such changes to clinical responses that manifest several months later has



In this Rube Goldberg contraption, leukemic cells are entered at the "Start" arrow, treated with an azanuceloside analog, and emerge as normal neutrophils. Along the way, a variety of epigenetic targets and DNA damage and repair pathways are encountered. Klco et al illustrate that despite state-of-the-art genomic technology, the mechanisms accounting for the clinical activity of DNMT inhibitors in myeloid leukemias remain complex and unclear.

been impossible. Building a better aza mousetrap is a laudable goal; if only we could figure out how the aza trap works.

Conflict-of-interest disclosure: S.D.G. receives research funding from the Celgene Corporation. The remaining author declares no competing financial interests. ■

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● ● ● PHAGOCYTES, GRANULOCYTES, & MYELOPOIESIS

Comment on Afonso et al, page 1644

ECM: chemoattraction but not adhesion

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The mechanisms that regulate 3-dimensional (3D) neutrophil chemotaxis are poorly understood. In this issue of *Blood*, Afonso et al demonstrate that the collagen receptor Discoidin domain receptor 2 (DDR2) promotes neutrophil chemotaxis in 3D by triggering matrix metalloproteinase (MMP) activity and the generation of chemotactic collagen peptides.[1]

Neutrophil-directed migration through interstitial tissues is critical for host defense. However, there is a limited understanding of how the extracellular matrix (ECM) regulates neutrophil motility within interstitial 3D tissues. Integrins are well established as essential regulators of cell adhesion during migration, in particular for mesenchymal cells. In contrast, substantial recent evidence suggests that although integrins are critical for 2D leukocyte adhesion and motility, they can be dispensable for interstitial leukocyte motility. Indeed, neutrophils lacking all integrins can still migrate directionally in 3D matrices.[2] However, it is important to note that it is not clear how integrins modulate the efficiency of neutrophil chemotaxis within interstitial tissues.

Afonso et al now show that the nonintegrin collagen receptor DDR2 mediates persistence of 3D interstitial neutrophil motility independently of its adhesive function (see figure).[1] The ECM commonly functions as a physical barrier to cell motility, which includes the basement membrane where MMPs are needed to degrade the matrix to facilitate cell movement. However, in general, neutrophil motility in interstitial tissues is thought to be independent of matrix degradation due to the ability of neutrophils to squeeze through small spaces. Accordingly, neutrophils are generally thought to lack matrix-degrading adhesive structures known as podosomes that are seen in other leukocytes like dendritic cells or macrophages or invadopodia of cancer cells. Intriguingly, ECM can also be a source of chemotactic peptides through the action of MMPs, as is the case for collagen exposed to the MMPs MMP-8 or MMP-9.

It is well known that neutrophils are an abundant source of MMP-9 and that MMP-9 levels are increased in inflamed tissues. In fact, MMP-9 has been reported to generate the collagen fragments N-acetyl Pro-Gly-Pro (Ac-PGP) that can mediate neutrophil chemotaxis.[3] Moreover, it has been shown that these collagen fragments can perpetuate a cycle of chronic inflammation through the chemokine receptors CXCR1 and CXCR2 leading to the production of more MMPs and neutrophil recruitment.[4] Afonso et al now show that DDR2 binding to collagen in circulating neutrophils mediates the production of MMP-8, which in turn can also lead to the generation of collagen fragments that mediate efficient chemokine-induced motility. It is not