after ablative host conditioning and provide further impetus to develop conditioning strategies that more potently suppress Treg reconstitution. Clinically, the use of fully myeloablative conditioning using TBI and hematopoietic stem cell rescue before ACT has been used to robustly deplete all host lymphocytes, including Tregs. Although such a strategy has been associated with improved response rates, the toxicity and complexity of this regimen can be a barrier to its widespread adoption. Recombinant immunotoxins targeting cells expressing CD25, such as denileukin difitox and LMB-2, have been used clinically to deplete Tregs in patients with cancer, but results have been marginal. In addition, as noted above, such an approach runs the risk of depleting tumor-reactive effector T cells. Thus, reagents that disarm the suppressive capacity of Tregs without having detrimental effects on effector T cells might be the best way to effectively translate the findings reported here. For example, combining ACT with blocking antibodies against cytotoxic T-lymphocyte antigen 4 (CTLA-4) might positively impact outcomes not only by inhibiting Tregs but also augmenting the effector functions of the adoptively transferred T cells. In support of such an approach, a post hoc analysis suggests a trend toward improved survival in patients previously treated with anti–CTLA-4 before receiving lymphodepletion and ACT. For this reason, a prospective trial testing the addition of anti–CTLA-4 with ACT would be warranted.

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

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Myeloid Neoplasia

Comment on Yan et al, page 2466

Cp-jeez! Aza-natomy!

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In this issue of Blood, Yan et al study the anatomy of azanucleoside methylation reversal.1

The azanucleoside analogues 5-azacytidine and decitabine improve hematopoiesis in approximately half of treated myelodysplastic syndrome patients2-4; in high-risk patients, 5-azacytidine improves overall survival compared with other typical clinical practice.2 Because both drugs are active inhibitors of DNA methyltransferase and can reactivate tumor suppressor genes silenced through cytosine methylation in CpG-rich promoter regions (so-called CpG islands), conventional wisdom assumes that these drugs exert their clinical effects through similar mechanisms. In fact, these drugs are commonly referred to as hypomethylating agents. Despite the biologically attractive dogma, attempts to identify specific genes or groups of genes whose methylation reversal can be associated with or predictive of clinical response have not resulted in clear causal relationships.4-6

Recently, the epigenome has been recognized as increasingly complex.7 While it has long been known that cancer genomes are hypomethylated outside of CpG islands, the significance of epigenetic changes in noncoding regions constitutes an area of intensive research due to the availability of newer sequencing technology. Yan and colleagues combined capture of methylated DNA with next generation sequencing (MethylCap-seq) to examine bone marrow samples obtained from patients during their first cycle of treatment with decitabine.1 Methylation on day 25 of treatment was compared with pretreatment bone marrow. Hypomethylation at 25 days after treatment was significant in genomic regions associated with CpG islands, CpG island shores, CpG islands, miRNA-associated CpG islands and promoters, RefSeq genes, and RefSeq gene-associated CpG islands (see figure). Clinical responders and nonresponders had similar changes in methylation in each region; however, nonresponders demonstrated a smaller extent of methylation reversal. Differentially methylated regions significantly clustered on the ends of all but 5 chromosomes.

This study should be seen as a demonstration of feasibility. Only 16 patients were included, and the only true discriminator between patients who subsequently developed clinical responses and those who did not was the extent of methylation reversal; decrease in global methylation can be measured using much simpler and inexpensive techniques! Nonetheless, as the significance of methylation changes in different genomic regions becomes clearer in both normal and malignant cells, exploration of differential methylation changes in specific regions in response to azanucleosides may lead to a better understanding of the mechanisms underlying the clinical activity of these drugs. Ultimately, such information may help in 2 directions: (1) clinically, understanding mechanism may lead to design of better drugs. And (2), at a more basic level, understanding the significance of perturbing methylation patterns of specific regions of the genome may better our appreciation of epigenomic organization and cellular regulation.

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PLATELETS & THROMBOPOIESIS

Comment on Ye et al, page 2484, and Al Hawas et al, page 2493

SNARing platelet granule secretion

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In this issue of Blood, Ye et al1 and Al Hawas et al2 clarify the roles of 2 key fusion proteins that regulate the agonist–stimulated release of bioactive factors from platelets, and thereby explain the defective hemostasis in patients with 2 rare genetic diseases.

On stimulation at sites of blood vessel damage, platelets release an array of soluble factors that facilitate platelet adhesion and other physiologic responses required for hemostasis and thrombus formation and remodeling. These factors are released from 3 types of storage compartments (α granules, dense granules, and lysosomes) after their fusion with the platelet plasma membrane or open canalicular system. Fusion requires
Cp-jeez! Aza-natomy!

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