widespread lesions in a toddler with homozygous plasminogen deficiency came out in 1998.[5] Because of the shorter half-life of Lys-plasminogen, this toddler was treated with a continuous infusion initially, followed by daily infusions. In comparison, based on individual pharmacokinetic studies, Glu-plasminogen used by Shapiro et al allowed for infusions every 2 to 4 days in these 14 patients. All evaluable lesions in these patients improved or resolved within the 12-week study window, and most improved after only 4 weeks of treatment.

Adverse events were largely limited to headache, nasopharyngitis, and gastrointestinal distress (abdominal pain, nausea, and/or diarrhea). Reassuringly, bleeding complications appear minimal, with only 2 patients each reporting epistaxis, hematuria, or dysmenorrhea during their treatment with Glu-plasminogen.

An astute reader or a scholar of congenital plasminogen deficiency will notice that thromboembolic disease is not a sequela of congenital plasminogen deficiency and that the disease manifests solely with extravascular deposition of fibrin. Despite initial reports implicating heterozygous hypoplasminogenemia as a risk factor for thromboembolic disease, more recent epidemiologic studies have not found an increase in thrombosis compared with those with normal levels of plasminogen.[2] Furthermore, thrombosis has not been described in patients with homozygous or compound-heterozygous plasminogen deficiency.[2] This suggests that other pathways, such as those involving cathepsins, elastases, or matrix metalloproteinases, can cleave intravascular, but not extravascular, fibrin and highlights how much more we need to learn about fibrinolysis.

Undoubtedly, many hematologists (cardiologists, stroke-ologists, and vascular surgeons too) are already daydreaming about all the indications that a widely available, licensed Glu-plasminogen product may serve for a myriad of acquired conditions. However, let us remember that (1) this is still an ongoing clinical trial that has shown preliminary efficacy in improving extravascular fibrin deposition and (2) other hemostatic products marketed for congenital conditions that were used off-label for acquired disease have demonstrated less than stellar outcomes.[6,7] Instead, let us celebrate that a potential treatment is one step closer to helping patients with this devastating orphan disease.

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

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DOI 10.1182/blood-2018-01-828194

HEMATOPOIESIS AND STEM CELLS

Comment on Arndt et al, page 1311

Set(d1a)-ing novel links between HSC regulators

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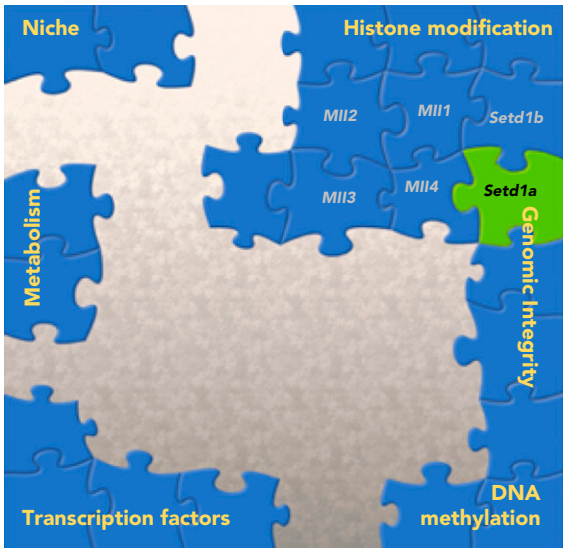
In this issue of *Blood*, Arndt et al demonstrate the unique role of the histone methyltransferase *Setd1a* in regulating hematopoietic stem cells (HSCs) and provide a connection between 2 drivers of aberrant stem cell function, epigenetic dysregulation and DNA damage.[1]

HSCs are responsible for maintenance of the hematopoietic compartment and for the restoration of the system during conditions of stress or loss of homeostasis. Regulation of stem cell fidelity is considered paramount, as these self-renewing cells ensure the capacity for life-long hematopoietic function. To safeguard HSCs, cells are imbued with cytoprotective features to mitigate alterations to their functional potential and are regulated by the orchestration of both intrinsic and extrinsic signals. As we continue to piece together the molecular guardians of HSC potential (see figure), it is becoming more evident that this complex network is interconnected and that key regulators may play multiple roles in this maintenance.

Epigenetic regulation of HSC function and fate decisions has been a burgeoning topic in recent years. This literature has established that histone modifications, DNA methylation, and ncRNA are cornerstones of the HSC regulatory network.[2,3] The role the MLL/SET family of histone methyltransferases plays in hematopoiesis has been of particular interest because of the association of leukemias with chromosomal rearrangements of MLL1. The MLL/SET family is composed of 6 members (Mll1/KMT2A, MLL2/KMT2B, MLL3/KMT2C, MLL4/KMT2D, SETD1A/KMT2F, and SETD1B/KMT2G), and they methylate lysine 4 of histone H3 (H3K4). Previous work has demonstrated using constitutive knockouts of the MLL/SET family that loss of these methyltransferases results in embryonic death.[4] Although all MLL/SET proteins are critical for development, each factor appears to have specific roles in regulating adult hematopoiesis and stem cell function,[1,4] including playing a role in white blood cell output, B-cell development, and erythropoiesis (see figure).[5,6] Waskow's group uses a series of conditional knockout mice to elucidate the function of *Setd1a* in the primitive hematopoietic compartment.[1] Global loss of *Setd1a* led to significant



H3K4 methyltransferases

	Adult hematopoiesis
Mll1	Self-renewal of HSCs Maintenance of HSC quiescence
Mll2	Functional macrophages
Mll3	Differentiation of primitive compartment
Mll4	Differentiation of primitive compartment ROS levels Mature B-cell regulation
Setd1a	Differentiation of primitive compartment DNA damage Maintenance of HSC quiescence Erythroid potential B-cell differentiation
Setd1b	----

Multiple factors contribute to the overall regulation of HSC potential. One family of histone modifiers, H3K4 methyltransferases, has been characterized, and Arndt et al provide novel data supporting a dual role of *Setd1a* in both epigenetic regulation and genomic integrity. ROS, reactive oxygen species.

reduction in peripheral blood reconstitution and early lethality, suggesting dysfunctional hematopoiesis causing death. To address the cell autonomous nature of this loss, competitive bone marrow transplants were performed, and again donor cells lacking *Setd1a* expression failed to contribute to peripheral blood. The authors further show a significant decrease in erythroid progenitors (common myeloid progenitors and megakaryocyte erythroid progenitors), confirming the role of *Setd1a* in erythropoiesis.[6] However, in contrast to the loss of more differentiated cells, the overall donor-derived Lineage$^-$ Sca-1$^+$ cKit$^+$ (LSK) compartment from the *Setd1a* knockouts was expanded, largely because of increased numbers of multipotent progenitors. To more definitely examine the effect on the stem cells, Arndt et al challenged the primitive compartment lacking *Setd1a* expression with proliferation, either by secondary transplants or 5-fluorouracil stress, and again recapitulated the significant loss of differentiation potential.

The authors demonstrate that many of the aberrant HSC functions were only manifest in environments of proliferative challenge.[1] These data may contribute to the discussion of the role HSCs play in steady-state hematopoiesis. Studies using transposon-tracking bar codes have supported the hypothesis that HSCs have minimal contribution to steady-state hematopoiesis and the drivers during adulthood are largely the progenitors cells.[7,8] Yet, the debate has been reopened as Sawai et al recently provided evidence supporting a larger contribution from HSCs in adult mice.[9] Loss of *Setd1a* in *Scl*-expressing cells (largely the primitive compartment) led to only mild perturbations in the blood over a 6-month follow-up,[1] perhaps lending support to the lesser role of HSCs during steady-state hematopoiesis.

To investigate the role of *Setd1a* in the cell cycle and cell division, the authors demonstrate a decreased number of quiescent cells in the primitive compartment lacking *Setd1a* expression. Surprisingly, elevated cycling activity was not correlated to an increase in ROS.[1] In fact, ROS levels were significantly decreased in the *Setd1a*-knockout cells. However, the authors show a significant increase in global DNA damage in these knockout cells and a significant decrease in expression of DNA damage repair pathway genes in LSK cells. This is interesting as the *Setd1a* knockout cells are less quiescent, which might have been correlated with increased repair and response pathways as many of the DNA damage repair and response genes are intimately tied to the cell cycle. Arndt et al correlate reduced expression of DNA repair genes to reduced levels of H3K4me3 at the promoter of several genes in DNA repair pathways in the *Setd1a* knockout suggesting a direct connection between DNA repair and this histone methyltransferase. The methyltransferase activity of *Setd1a* appears to be essential for the function, whereas other MLL/SET family members can also regulate hematopoiesis in an indirect fashion,[4] supporting diversity of their modes of action. Further, many of the phenotypes of this *Setd1a* knockout in the primitive compartment mirror the effects of the conditional knockout of Dpy30, a core subunit for the MLL family of H3K4 methyltransferases. Both knockouts show an expansion of the LSK compartment, loss of differentiation potential, and ROS decrease[10] supporting histone methyltransferase activity as playing a driving role in the regulation.

Arndt et al have added an important piece to the "HSC regulation puzzle" that also highlights the importance of continuing to establish connections between regulators of HSCs (see figure). Waskow's group also demonstrates differential results depending if experiments were performed in vivo or in vitro.[1] Although not uncommon, it again suggests regulation by the niche needs to be factored into this overall regulation puzzle. Given the interesting decrease in ROS in these cycling cells, it will be also be relevant to examine the connection that *Setd1a* may play in HSC metabolism. Understanding the network of regulation controlling the HSC compartment is opening new means

to examine aging and diseases of the hematopoietic system, which are associated with loss of epigenetic regulation, accumulation of DNA damage, and environmental alterations. It will be interesting to expand these studies characterizing regulators, such as *Setd1a*, that simultaneously affect multiple facets of stem cell regulation, in normal hematopoiesis and in settings of dysfunction.

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

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DOI 10.1182/blood-2018-02-829077

LYMPHOID NEOPLASIA

Comment on Wood et al, page 1350

There is nothing minimal about residual disease

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Can the detection of measurable residual disease (MRD) become the standard approach for acute lymphoblastic leukemia (ALL)? In this issue of *Blood*, Wood et al[1] use a highly sensitive sequencing approach that is an improvement on conventional methods by both performance characteristics and potential for standardization.

The presence of MRD in the context of a morphological remission is associated with a higher relapse rate than MRD-negative cases in all leukemia subtypes of leukemia and across most (if not all) treatment modalities. Indeed, in chronic myeloid leukemia (CML), the measurement of BCR-ABL messenger RNA has become the standard of care for assessing disease burden and treatment response both in the community and in clinical trials.[2]

Although CML is the poster child of MRD direct treatment strategies, it can be argued that the data for MRD and response in ALL is even stronger. MRD has been associated with poorer event-free survival (EFS) and overall survival (OS) in both pediatric and adult ALL, though the reported experience in the pediatric arena dwarfs that in the adult setting.[3,4] Several pediatric studies in Europe and the United States have risk stratification treatment options based on postinduction and consolidation MRD status.[5,6] A recently completed National Cancer Institute–sponsored large meta-analysis of MRD in ALL found that the impact of MRD on overall and EFS was amazingly stable over types of populations (pediatric vs adult), subtypes of ALL (B, T, and Ph+), and assays used to detect MRD (flow cytometry vs polymerase chain reaction [PCR]; see below).[7] In all these situations, the hazard ratio of MRD negativity ranged from ~0.2 to 0.3.

If MRD is so important, then why is it not the standard of care? Simply said, it is not easy to do it well. MRD can be measured in several ways, including PCR of the immunoglobulin heavy chain (IgH) VDJ and/or T cell receptor (TCR) gene rearrangements or leukemia-specific fusion transcripts (eg, BCR-ABL), or by multiparametric flow cytometry (FC). These methods can have laboratory-to-laboratory variations in performance characteristics (eg, the sensitivity of detecting a leukemic cell in a background of normal cells) and can be very labor intensive (gene rearrangement PCR) or equipment intensive (FC). Consequently, standardization has lagged, making it difficult to use in clinical trials across academic centers, let alone in the community setting, and regulatory agencies have been (perhaps understandably) slow to adopt MRD in ALL as a surrogate in trials of new drug agents.

The study by Wood et al suggests a path forward to improve the sensitivity of MRD detection while offering potential standardization, utilizing a deep sequencing methodology of IgH and TCR rearrangements. Basically, a high-throughput sequencing (HTS) method has been devised with simultaneous use of a multiplex of upstream and downstream primers to sequence across all IgH and TCR rearrangements (see figure, panel A). This is in contrast to the conventional PCR method, which takes several steps to go from amplification of the patient specific rearrangement to the generation of patient specific primers and probes used in the subsequent MRD detection. Several small retrospective studies have suggested that the HTS method is more sensitive than state-of-the-art flow cytometry by an order of magnitude (or so).[8-10]

In the Wood et al study, HTS and FC for MRD detection at the end of induction chemotherapy were directly compared in 619 standard- or high-risk pediatric patients with newly diagnosed B-lymphoblastic leukemia (B-ALL). HTS detected a clonal rearrangement in 95% of samples, and 75% of these cases were found to have MRD detected after induction. Analyses revealed several important findings. (1) At the