There is now a biological traffic light for predicting successful TFR.[10] Patients with *BCR::ABL1*$^+$ granulocytes have a red light, with residual disease still present. In these patients, a proactive switch to improve the depth of molecular response or a prolonged treatment with the same TKI should be required before considering discontinuing the therapy. Considering the poor results obtained in patients attempting a second round of discontinuation after the first TFR failure, a "red light" should help mitigate clinical failure. What remains to be done in the near future? In laboratories that can perform these new monitoring methods, an initial evaluation should better identify patients for whom therapy may be suspended safely, identifying the best timing for TFR, while also providing monitoring that can identify an early recurrence. In the near future, these biological data could be combined with new prognostic factors (eg, immunological, next generation sequencing) to better shape a withdrawal strategy.

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RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Lv et al, page 2198

Core transcription balancing erythropoiesis

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In this issue of *Blood*, Lv et al[1] demonstrate that a component of the core transcriptional elongation machinery called HEXIM1 can induce a fetal-like gene signature in erythroid precursor cells.

Coordinated checks and balances maintain steady rates of erythropoiesis. Foundational studies have resolved some of the molecular logic directing speed and fidelity of erythroid progenitor differentiation into mature red blood cells.[2] A soundly appreciated mechanism promoting erythroid gene transcription utilizes the GATA-binding protein-1 (GATA1) along with numerous GATA factor-associated proteins.[3,4] GATA1 promotes the expression of genes that encode for hemoglobin protein subunits and cell cycle regulatory genes, and it also represses the genes not required for or detrimental to erythropoiesis.[3,5] However, only ~1% of potential binding motifs are occupied by GATA1 protein in erythroid cells.[6] Since GATA1 activities are so critical for erythropoiesis, it is important to understand how common transcriptional machinery works with GATA1 to control selective gene expression and the rules for GATA1-mediated gene activation or repression.

Prior studies have hinted that positive transcription elongation factor-β (pTEFβ) may be an important contributor to directing specificity and transcriptional activity of GATA1-containing complexes.[7-9] One component of the pTEFβ complex known to negatively regulates its activity—hexamethylene bisacetamide inducible (HEXIM) protein—is highly expressed in erythroid cell types.[8] HEXIM1 overexpression alters normal pTEFβ activity, which in turn instigates accelerated erythroid progenitor proliferation and differentiation.[8] The opposite phenotype is induced when HEXIM1 is downregulated.[8]

Among the insights revealed by Lv et al, the authors demonstrated that HEXIM1 overexpression shifts the transcriptional program of erythroid precursors toward a fetal-like gene signature.[1] At the exemplary "beta-globin" locus, encoding multiple hemoglobin protein subunits, cells overexpressing HEXIM1 had moved the GATA1 protein away from the adult (HBB) gene and toward the HBG gene. This pattern associated with changes to globin subunit mRNA transcript levels, suggesting they are related. To test whether the molecular and cellular phenotypes were dependent on pTEFβ activity, the authors used both a mutation that blocks HEXIM-mediated pTEFβ release of RNA polymerase II and a CDK9 inhibitor. Overexpressing this mutant did not increase erythroid precursor cell proliferation and colony-forming ability, as was observed with HEXIM1 expression. Using sophisticated tools to interrogate nuclear organization, HEXIM1 induced changes in HEXIM co-occupancy with RNA polymerase at cell cycle regulatory genes, which were absent in cells expressing the mutant

HEXIM1 protein. Consequently, there were distinct changes to nuclear organization induced by the wild-type HEXIM1 compared with the mutant version. These findings suggest that HEXIM1 might function in both a pTEFβ-dependent and -independent fashion.

HEXIM1 overexpression both increased and decreased erythroid gene transcription. The authors next explored whether there were molecular correlations predicting gene activation compared with repression. Within these data, HEXIM1 overexpression appeared to induce GATA1 protein mislocalization at specific chromatin sites. At most sites, GATA1 occupancy associated with increased transcription. Intriguingly, HEXIM1-induced changes to chromatin accessibility did not associate with sites occupied by KLF1 or TAL1, which are often complexed with GATA1. Although it remains unclear whether the GATA1 occupancy changes are directly induced by HEXIM1 or if GATA1 is mislocalized due to altered transcriptional programs, these findings add important context clues to the mechanisms controlling the switch from fetal to adult erythropoiesis.

Paused RNA polymerase II release is a crucial balance point for erythroid gene transcription,[10] and this study implicates HEXIM1 in that process. Several important questions are raised from this work regarding the necessary checkpoints for homeostasis during developmental and adult erythropoiesis. What is the role of enhancer bound GATA factor complexes in regulating pTEFβ activity at gene promoters? How does pTEFβ activity initiate transcriptional programs associated with fetal erythropoiesis, and what is the mechanistic relationship between fetal programs and regenerative or "stress" erythropoiesis induced by acute blood loss? Does pTEFβ participate in GATA switching mechanisms, where GATA2 is replaced by GATA1 on select chromatin sites to promote terminal erythropoiesis? Does HEXIM1 have redundant or unique functions compared with HEXIM2, which is also highly expressed in erythroid cells? This study provides tractable inroads to critically address these questions. Coordinated transcriptional programs ensure a relatively constant number of developmentally appropriate red blood cells to meet its oxygen-carrying needs. Disease-associated perturbations in these mechanisms can lead to anemia or polycythemia.

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