Comment on Pagani et al, page 2192

A traffic light for TFR by lineage-specific MRD

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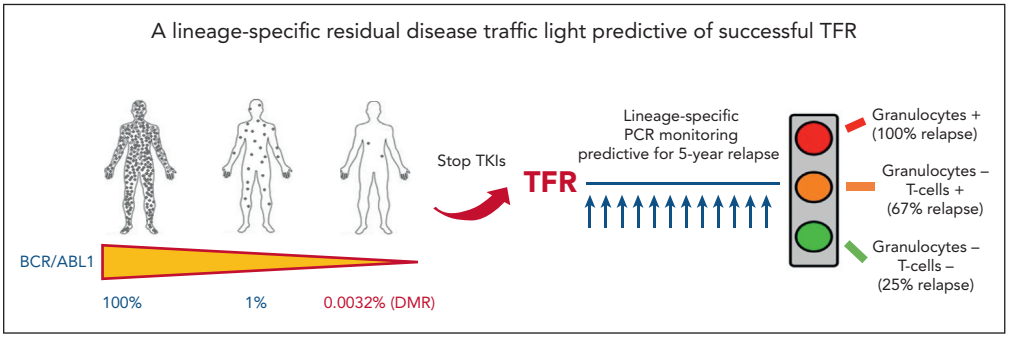
In this issue of *Blood*, Pagani et al[1] demonstrate that the residual *BCR::ABL1* DNA level detected in granulocytes and T cells can be used as a predictive measure for the successful discontinuation of tyrosine kinase inhibitors (TKIs) in patients with chronic myeloid leukemia (CML). Based on these results, a sensitive prognostic model capable of identifying the probability of recurrence at 60 months was designed.[1] Do we finally have in our hands a tool that can indicate the potential of success after discontinuation of TKIs?

Over the past 15 years, the outcome of patients with CML has improved dramatically following the introduction of TKIs, with overall survival now approaching that of healthy people.[2] The use of second-generation TKIs as first-line treatment has resulted in faster achievement of molecular response and increased the rate of deep molecular response (DMR, or MR4 and MR4.5 corresponding to 0.01% and 0.0032% *BCR::ABL1* ratio in International Scale [IS], respectively).[3] The concept of treatment-free remission (TFR) has been introduced as a possible goal for patients in the chronic phase with a long-lasting DMR. The first French trial, the STIM study, showed that patients with long-term MR4.5 can safely stop treatment, with approximately 40% of them remaining in remission (ie, no molecular detection of disease) without therapy.[4] Several studies and real-world evidence have shown that the percentage of patients who maintained the remission after discontinuation increases to 50% through 60% if the loss of major molecular response (0.1% in IS) was considered as the threshold to resume the treatment.[5] Prognostic factors for a successful discontinuation have been extensively studied, but only the duration of treatment for more than 5 years and the duration of a DMR for more than 3 years have been internationally confirmed as the optimal prerequisites for effective long-lasting discontinuation.[3,5] In recent years, the search for new methodologies capable of increasing the sensitivity of real-time quantitative polymerase chain reaction monitoring has been explored. The use of digital droplet polymerase chain reaction (ddPCR) seems to provide a valid tool for the subgroup of patients attempting TFR, with a better sensitivity and specificity.[6] DMR confirmed by ddPCR appears to increase the likelihood of success after discontinuation.[7] Shorter *BCR::ABL1* halving time after the start of first-line treatment was also found to correlate with the likelihood of success, as was reported in an earlier study by the same group.[8] Currently, there is still an ongoing discussion about the appropriate cutoff to use for ddPCR to define the increased risk of recurrence after discontinuation. Indeed, in this study, Pagani et al sought to dissect the value of residual disease by sorting the different leukocyte fractions followed by a DNA-based patient-specific PCR in the different leukocyte lineages. In a prospective comparison of patients who relapsed vs those who maintained remission, *BCR::ABL1* DNA was significantly detected in granulocytes and T cells but not in monocytes, B cells, or natural killer cells. Three groups of patients were defined based on the detection of *BCR::ABL1* in granulocytes and/or T cells, with different probability of recurrence at 60 months, in a model providing an accuracy of 77% (see figure). In multivariate analysis, detection of residual disease in granulocytes remained the only prognostic factor that could identify patients at risk of relapse in 2 different models that included T lymphocytes and halving time.

The results of this study expanded on previous reports by the same group on measurable residual disease (MRD) in patients attempting TFR,[9] specifically that finding of *BCR::ABL1*$^{+}$ lymphocytes at presentation and detectable disease in this subset of cells in TFR denoted the persistence of a multipotent progenitor. Sorting different subsets of leukocytes seems to increase the predictive accuracy in defining the risk of relapse. The increased sensitivity of the method requires that laboratories are familiar with the use of DNA-based patient-specific PCR. As with ddPCR, this is not yet currently used by all centers and will require centralized laboratory analysis of samples for patients attempting TFR in specific laboratories.



A lineage-specific residual disease traffic light predictive of successful TFR.

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