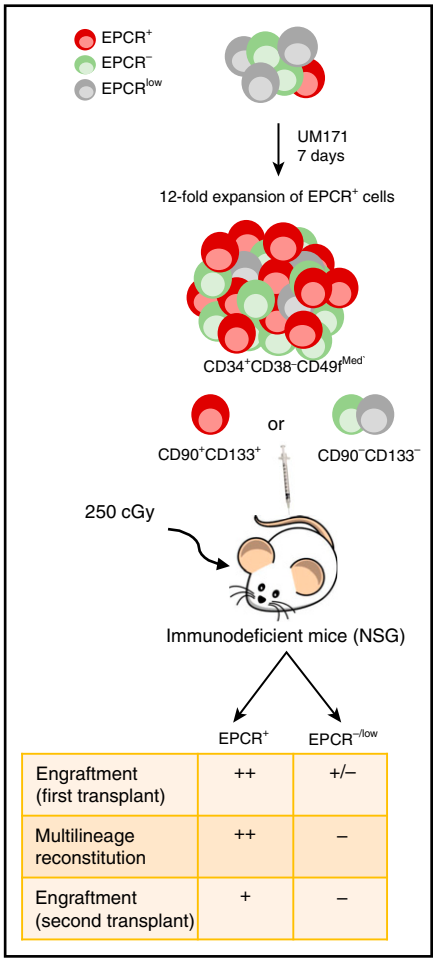


UM171 treatment of CD34-enriched CB cells induces the expansion of EPCR$^+$ cells. EPCR$^-$ and EPCR^{low} cells are present within CD34$^+$CD38$^-$CD49f^{Med} HSCs, but only EPCR$^+$ cells also express a more primitive phenotype defined by the expression of both CD90 and CD133. Injection of EPCR$^+$ sorted cells, but not EPCR$^{-/low}$ populations, into immunodeficient mice results in human engraftment with multilineage reconstitution.

addition, as EPCR may contribute to murine, but not human HSC self-renewal, it would be interesting to investigate the basis for this species difference. It should be noted, however, that because assessment of human HSC frequency and function in xenograft models may not accurately reflect human HSC biology as a result of possible difficulties in modeling interactions between human surface proteins and their cognate mouse homologs, these findings should be interpreted with caution. To address this potential issue, it will be important to correlate EPCR$^+$ cell frequencies in CD34$^+$ CB grafts and/or mobilized peripheral blood grafts with measures of HSPC function such as time to engraftment and chimerism levels following transplantation. Finally, as it is unclear which methods may ultimately be used to expand CB HSCs in the clinical setting in the future, it will be interesting to determine if EPCR marks HSCs using protocols that other investigators have published to expand human CB HSCs. Such investigations have the potential to further credential EPCR as a general marker of ex vivo expanded CB HSCs.

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

REFERENCES

1. Fares I, Chagraoui J, Lehnertz B, et al. EPCR expression marks UM171-expanded CD34$^+$ cord blood stem cells. *Blood*. 2017;129(25):3344-3351.
2. Fares I, Chagraoui J, Gareau Y, et al. Pyrimidoindole derivatives are agonists of human hematopoietic stem cell self-renewal. *Science*. 2014;345(6203):1509-1512.
3. Balazs AB, Fabian AJ, Esmon CT, Mulligan RC. Endothelial protein C receptor (CD201) explicitly identifies hematopoietic stem cells in murine bone marrow. *Blood*. 2006;107(6):2317-2321.
4. Iwasaki H, Arai F, Kubota Y, Dahl M, Suda T. Endothelial protein C receptor–expressing hematopoietic stem cells reside in the perisinusoidal niche in fetal liver. *Blood*. 2010;116(4):544-553.
5. Mehta RS, Rezvani K, Olson A, et al. Novel techniques for ex vivo expansion of cord blood: clinical trials. *Front Med (Lausanne)*. 2015;2:89.
6. Dorrell C, Gan OI, Pereira DS, Hawley RG, Dick JE. Expansion of human cord blood CD34$^+$CD38$^-$ cells in ex vivo culture during retroviral transduction without a corresponding increase in SCID repopulating cell (SRC) frequency: dissociation of SRC phenotype and function. *Blood*. 2000;95(1):102-110.
7. Notta F, Doulatov S, Laurenti E, Poeppl A, Jurisica I, Dick JE. Isolation of single human hematopoietic stem cells capable of long-term multilineage engraftment. *Science*. 2011;333(6039):218-221.
8. Majeti R, Park CY, Weissman IL. Identification of a hierarchy of multipotent hematopoietic progenitors in human cord blood. *Cell Stem Cell*. 2007;1(6):635-645.
9. Park CY, Majeti R, Weissman IL. In vivo evaluation of human hematopoiesis through xenotransplantation of purified hematopoietic stem cells from umbilical cord blood. *Nat Protoc*. 2008;3(12):1932-1940.
10. Gur-Cohen S, Itkin T, Chakrabarty S, et al. PAR1 signaling regulates the retention and recruitment of EPCR-expressing bone marrow hematopoietic stem cells. *Nat Med*. 2015;21(11):1307-1317.

DOI 10.1182/blood-2017-05-780007

● ● ● LYMPHOID NEOPLASIA

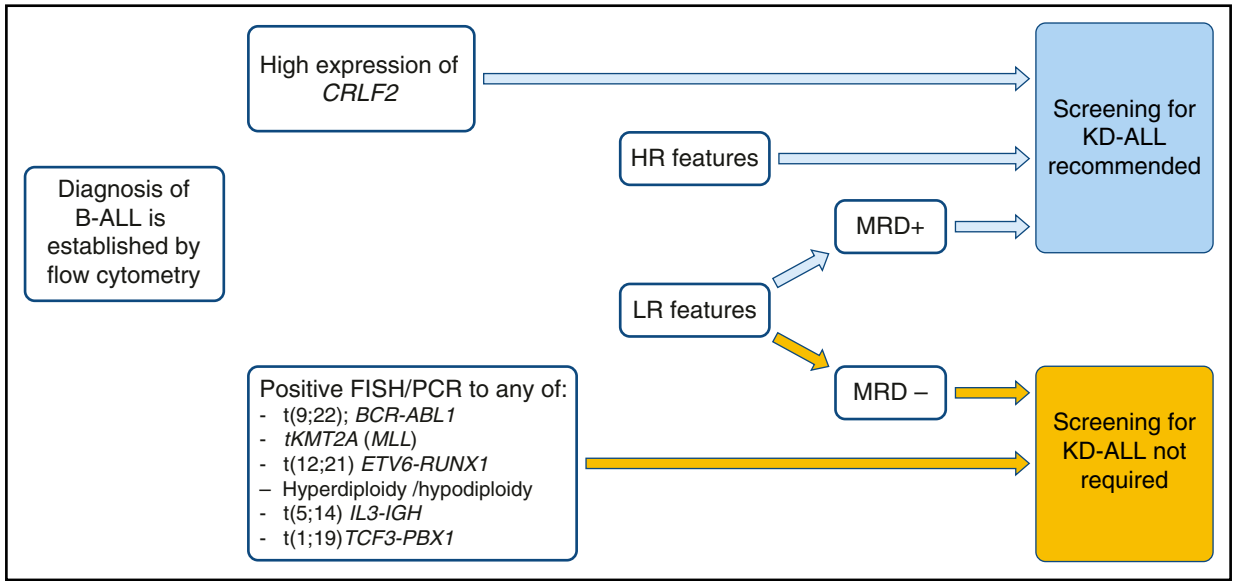
Comment on Reshmi et al, page 3352

Activated kinases in ALL: time to act

Yishai Ofran RAMBAM HEALTH CARE CAMPUS; ISRAEL INSTITUTE OF TECHNOLOGY

In this issue of *Blood*, Reshmi et al[1] report a study that defines a protocol for identifying kinase-driven high-risk (HR) features, known as "Ph-like" expression profile, in patients with acute lymphocytic leukemia (ALL). Revealing the underlying genetic aberration allows better prognostication and may point to potential therapeutic options for specific patients. Originally identified in pediatric patients, this Ph-like or kinase-driven ALL (KD-ALL) subtype has also been found to be common among adults.[2,3] The journey to the routine identification of these kinase-activating genetic alternations started 8 years ago and required extensive efforts and use of different laboratory methods to become feasible. The most important take-home message from this work is that the time has come for routine screening for kinase-activating alterations in ALL. Although this study is published before clinical outcome data of the patients enrolled in the Children's Oncology Group study have matured, the clinical significance of identification of KD-ALL is well established.[4] Reshmi et al confirm the complexity of the genetic alteration map of these potentially targetable aberrations. The authors also provide a working diagnostic paradigm starting with a simple gene expression screening test, which reliably identifies patients in whom genetic testing for kinase-activating alterations is futile. Of 202 patients whose suggested score for screening was below 0.5, only in 1 was a potentially targetable fusion detected (*HOOK3-FGFR1* genes).

Screening aims at identifying HR patients and patient-specific potentially druggable targets. The proposed laboratory protocol is complex. It requires the use of multiple sophisticated methods, is costly, and time consuming. Moreover, the expected



Schema for selecting B-ALL patients for KD-ALL screening. LR, low risk; MLL, mixed lineage leukemia.

turnaround time for results is several weeks. Thus, only a limited number of specialized laboratories are up to the challenge. Nevertheless, the time has come to devise a schema for effective kinase alteration screening, which should be taken into consideration in current practice (see figure).

Patients identified by fluorescence in situ hybridization (FISH) or polymerase chain reaction tests to carry common translocations, such as *BCR/ABL* or others that define a specific entity according to the 2016 World Health Organization classification,[5] are excluded from further screening. The highest priority for gene expression screening testing is patients presenting with HR features, those with high *CRFL2* expression, and individuals with a minimal residual disease (MRD) level higher than 0.01% at the end of induction therapy. The results reported by Reshmi and colleagues support the incorporation of *CRFL2* expression assessment in routine flow cytometry evaluation for B-ALL patients. In the current cohort, 80% of patients presenting with high *CRFL2* expression carry a kinase-activating lesion. Both FISH and polymerase chain reaction may serve as substitutes to immunophenotyping in CRFL2 identification.

KD-ALL is more common among patients presenting with traditional HR factors. Reshmi and colleagues screened samples collected as part of clinical protocols from HR patients, patients with standard risk (SR) presenting with central nervous system or testicular involvement or those in whom MRD was detected after induction. In this subgroup of SR patients, KD-ALL was diagnosed in 17% of patients. The prevalence of KD-ALL among MRD-negative SR patients is not known.

Although the significance of this study is unequivocal, some areas of uncertainty remain. In 20% of patients presenting with high *CRFL2* expression, no culprit translocation was identified. Although the false-negative rate of a low gene expression screening score is documented by the authors, the rate of false positivity is still unknown. Furthermore, a kinase-affecting alteration was not detected in 42 (14.8%) of the 284 patients defined as positive by the suggested cutoff. It is unclear whether these patients suffer from KD-ALL with their underlying activating aberration yet to be defined or if this represents false positivity of the suggested screening method. Another outstanding question is the applicability of allogeneic stem cell transplantation (allo-SCT) in KD-ALL patients achieving MRD negativity. There are scant and conflicting data available on the actual risk of relapse in MRD negative KD-ALL patients, and the outcome data of such cases following allo-SCT are likewise limited. The risk may be different for patients with *ABL* or *CRFL2* translocation and could also be age-dependent. At least in adults, allo-SCT may be justified based on the poor general outcome reported.

Finally, it is tempting to match any found alteration with a drug that blocks the activated kinase. However, it should be emphasized that although KD-ALL patients are known to have a poor prognosis, the beneficial effect of *ABL*- or *JAK*-targeted therapies is currently based on anecdotal reports. The *CRLF2/JAK* aberration may be unstable in relapse[6] and therefore may not be effective in such cases. The clinical experience associated with the use of TKIs in *ABL*-activated cases seems promising.

Ongoing prospective pediatric studies could provide evidence regarding the targeted approach in KD-ALL. As long as a clear match between a kinase-activating genetic alteration and a specific drug is not established, one should also consider the use of some of the newly approved anti-ALL antibodies as therapeutic augmentation in these HR patients.

The study by Reshmi et al is a call for action. Screening patients for KD-ALL is to be encouraged, and prospective and retrospective studies are warranted to define the optimal clinical approach in these patients.

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

REFERENCES

1. Reshmi SC, Harvey RC, Roberts KG, et al. Targetable kinase gene fusions in high-risk B-ALL: a study from the Children's Oncology Group. *Blood*. 2017;129(25):3352-3361.

2. Jain N, Roberts KG, Jabbour E, et al. Ph-like acute lymphoblastic leukemia: a high-risk subtype in adults. *Blood*. 2017;129(5):572-581.

3. Roberts KG, Gu Z, Payne-Turner D, et al. High frequency and poor outcome of Philadelphia

chromosome-like acute lymphoblastic leukemia in adults. *J Clin Oncol*. 2017;35(4):394-401.

4. Ofran Y, Izraeli S. BCR-ABL (Ph)-like acute leukemia-pathogenesis, diagnosis and therapeutic options. *Blood Rev*. 2017;31(2):11-16.

5. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*. 2016; 127(20):2391-2405.

6. Vesely C, Frech C, Eckert C, et al. Genomic and transcriptional landscape of P2RY8-CRLF2-positive childhood acute lymphoblastic leukemia [published online ahead of print 6 January 2017]. *Leukemia*. doi:10.1038/leu.2016.365.

DOI 10.1182/blood-2017-05-780015

MYELOID NEOPLASIA

Comment on Malcovati et al, page 3371

Now I cuss less about ICUS

R. Coleman Lindsley DANA-FARBER CANCER INSTITUTE

In this issue of *Blood*, Malcovati et al show that somatic mutations can identify patients with unexplained cytopenias who have, or are at a high risk of developing, myeloid malignancies. This study provides clear evidence that supports integration of gene-panel sequencing into routine clinical evaluation of unexplained cytopenias.[1]

Definitive diagnosis of myelodysplastic syndrome (MDS) in a patient with cytopenias relies on demonstration of characteristic morphologic changes or MDS-defining cytogenetic abnormalities. However, many patients have a normal karyotype and lack the most distinctive pathologic features of MDS, such as ring sideroblasts or an excess of myeloid blasts. In such cases, it may be difficult to exclude or establish a definitive diagnosis, and a degree of diagnostic variability has been recognized. The term "idiopathic cytopenia of undetermined significance" (ICUS) describes a broad category of patients with persistent unexplained cytopenias that do not meet criteria for MDS. As a diagnosis, however, ICUS has been known to inspire a measure of frustration, or even the rare obscenity, among clinicians.

Recent studies have established an important paradigm: a sizeable fraction of patients with ICUS have MDS-associated somatic mutations, and these patients may share genetic and clinical characteristics with those who have bona fide MDS.[2,3] However, the natural history of patients with clonal versus nonclonal cytopenias has not been demonstrated, and evidence to guide clinical practice has been scant.

Malcovati et al evaluated mutations in a panel of 40 genes in a prospective cohort of 683 patients who presented for clinical evaluation of unexplained cytopenias and validated their findings in an independent cohort. On the basis of independent pathologic review, patients were determined to have myeloid neoplasm, ICUS, or "other" cytopenia. As was expected, most patients with myeloid neoplasms harbored canonical myeloid mutations, whereas these mutations were much less frequent in patients with ICUS or other cytopenias. Both the number of somatic mutations and the size of the mutant clone, as inferred from the variant allele fraction, had significant predictive values for myeloid neoplasm. However, there was marked heterogeneity in the clinical characteristics of specific mutations. For example, some mutations had high predictive value for myeloid neoplasm irrespective of co-occurring mutations, including those affecting RNA splicing (*SF3B1*, *SRSF2*, *SF3B1*), *JAK2*, and *RUNX1*. Others, such as mutations in *TET2*, *DNMT3A*, or *ASXL1*, had low predictive value unless paired with additional mutations. These results are highly consistent with data showing that somatic *TET2*, *DNMT3A*, and *ASXL1* clones are commonly found in the blood of aging individuals and may require cooperating genetic events to cause development of clinically apparent myeloid malignancies.[4,5]

Using these mutation patterns, Malcovati et al asked whether patients with ICUS could be segregated by molecular profile into groups with distinct outcomes or likelihood of clinical progression. They found that patients with clonal ICUS had a much higher rate of progression (14-fold higher) than did patients with nonclonal ICUS. Importantly, they further showed that patients with clonal ICUS defined by highly specific mutation profiles had similar clinical characteristics to those with low-risk MDS patients, including older age, male bias, overall survival, and risk of disease progression. Somatic mutation status has not yet been substantively integrated into the latest revision to the World Health Organization (WHO) classification scheme.[6] However, these findings should incite active discussion about whether the presence of highly specific mutation patterns in patients with ICUS provide presumptive evidence of bona fide MDS, even in the absence of definitive morphologic findings.

In contrast, does a negative molecular test have value in the evaluation of unexplained cytopenias? Many patients with unexplained cytopenias never develop a myeloid neoplasm. Prospective identification of this group of low-risk patients, who may need less invasive diagnostic testing and a more limited follow-up strategy, could reduce health care expenditures and provide more peace of mind for patient and physician. Malcovati et al show that the absence of somatic clonal mutations, particularly when paired with standard cytogenetic analysis, have a high negative predictive value. Similarly, patients with "mild" dysplastic changes and a negative mutation panel had exceptionally good outcomes, even though they formally fulfilled WHO morphologic MDS criteria. The predictive value of a negative test was enhanced by analyzing more genes, suggesting that there is utility in expanding the breadth of diagnostic gene panels.

Together, these findings have clear clinical implications, especially in the large population of patients with ICUS or MDS with mild dysplastic changes. However, several questions remain about how to fully integrate these findings into clinical practice. What is the role of bone marrow examination in the evaluation of unexplained cytopenias? Does a negative test obviate the need for a bone marrow study? Can a diagnosis of MDS be made without morphologic examination? How would prognostic models, such as the Revised International Prognostic Scoring System, be affected by refinement of MDS diagnosis to include MDS-defining mutation profiles? Future studies with ever-longer follow-up in longitudinal studies with comprehensive genetic annotation will be necessary to address these questions. At a minimum, staging of presumptive MDS by bone marrow