exhaustion in either the CAR-19 products or the recipient with poor outcomes.[8,9] The studies in this issue of *Blood* instead examined tumor-intrinsic factors that may lead to clinical CD19 CAR-T cell failures through whole-genome sequencing (WGS) of the tumor or low-pass WGS of cell-free DNA from plasma.

The first study from Jain et al at Moffitt Cancer Center and the University of Miami used WGS to assess tumor samples from 49 patients with DLBCL treated with axicabtagene ciloleucel and found that find that pretreatment presence of complex structural variants, APOBEC mutational signatures, and genomic damage from reactive oxygen species predicted CAR-19 resistance. In addition, the recurrent 3p21.31 chromosomal deletion containing the *RHOA* tumor suppressor was strongly enriched in patients who failed CAR-T therapy. Of note, reduced CD19 expression, which has been identified as a mechanism of failure in CD19 CAR-T cell therapy for acute lymphoblastic leukemia with tisagenlecleucel, did not affect responses in this study. One limitation of the WGS strategy used in this study is that it requires viable lymphoma tumor tissue and matched germline DNA.

Cell-free DNA provides an alternative strategy for evaluating tumor genetic changes[10] and, in the second report, Cherng and colleagues at MD Anderson Cancer Center used a less-intensive assay requiring only a peripheral blood sample to evaluate CNAs. The authors undertook low-pass WGS of cell-free DNA from blood samples collected at the time of leukapheresis for product manufacture and analyzed samples from 122 patients (92% of whom received axicabtagene ciloleucel and 8% of whom received tisagenlecleucel). In multivariable analysis, Cherng et al found that a high focal CNAs score was the most significant pretreatment variable associated with inferior 3-month complete response rates and overall and progression-free survival. The study identified 34 unique focal CNAs, with deletion 10q23.3 leading to loss of *FAS* death receptor being the most prognostic for poor outcomes. By combining focal CNAs score with standard markers of increased tumor bulk (elevated lactate dehydrogenase and disease at >1 extranodal site), this study builds a risk model that may reliably stratify patients.

Both studies were retrospective and conducted at a single institution in 1 case and 2 institutions in the other, so require validation in other cohorts. In addition, both studies predominantly included patients who received axicabtagene ciloleucel. Whether these observations also apply to the other 2 commercial products thus remains to be seen. Nevertheless, these studies provide tumor-specific predictive factors that could be used to identify patients at high risk of relapse following CD19 CAR-T cells who may need additional therapies. Integrating these tumor-specific risk factors with other host and product factors that influence outcome may produce even stronger algorithms to predict success or failure.

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MYELOID NEOPLASIA

Comment on Li et al, page 516

AML: making residual disease more measurable

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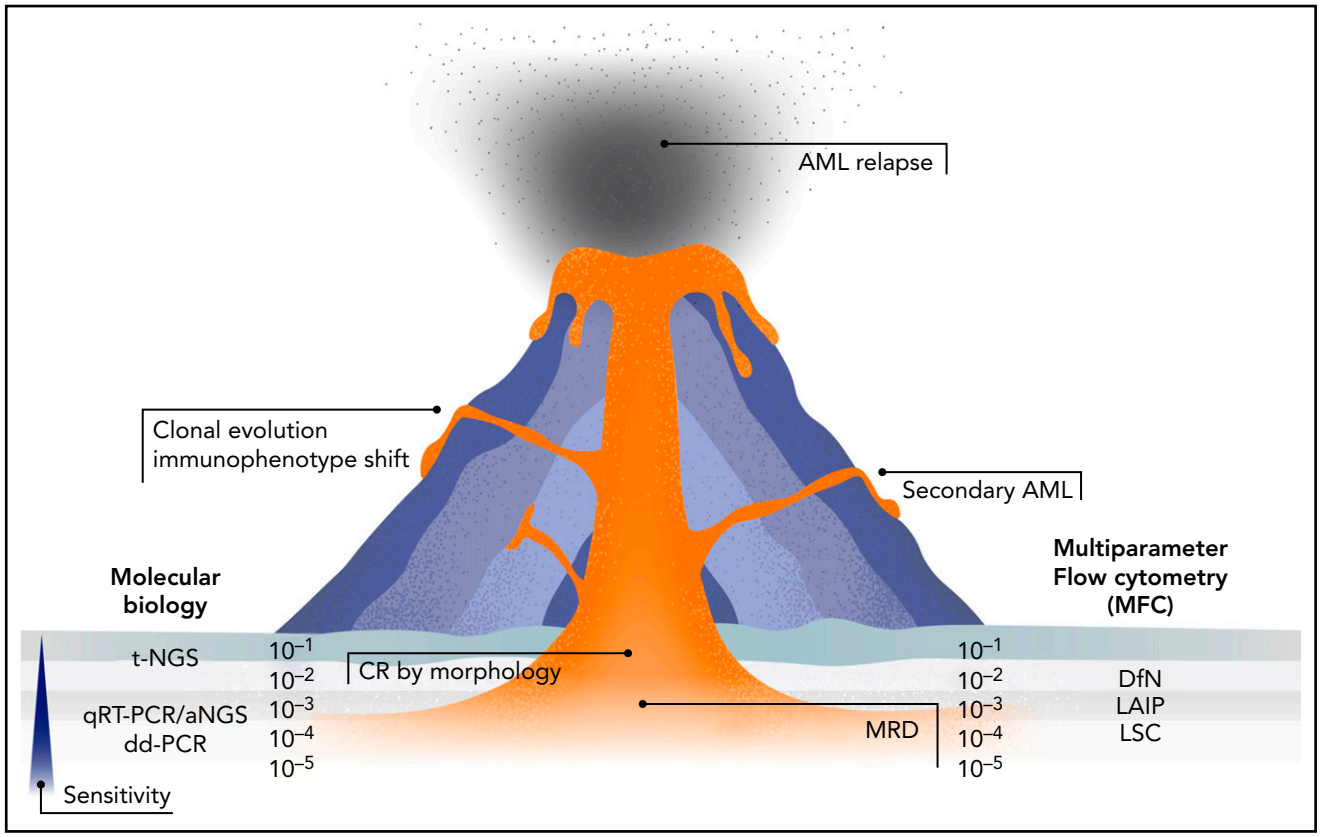
In this issue of *Blood*, Li et al[1] show that detection of leukemic stem cells (LSCs) may predict relapse and overall survival (OS) after allogeneic hematopoietic stem cell transplantation (HSCT) in patients with acute myeloid leukemia (AML) better than classical measurable residual disease (MRD) by multiparameter flow cytometry (MFC).

The power of MRD to predict outcome in AML has long been established and led to the inclusion of MRD positivity after induction or consolidation chemotherapy as one of the factors that define high-risk AML (see Figure). The importance of MRD is emphasized by the inclusion of MRD-negative complete remission (CR) as a treatment goal in AML in the 2017 and 2021 European LeukemiaNet (ELN) MRD guidelines.[2]



Sensitivity of molecular and multi-parametric flow cytometry methods for the detection of MRD in patients with AML. MRD positivity is associated with disease relapse. However, clonal evolution, immunophenotype shifts, or the onset of secondary independent leukemias may occur during follow-up and may escape MRD detection. aNGS, amplicon next-generation sequencing; ddPCR, digital droplet polymerase chain reaction; qRT-PCR, quantitative reverse transcriptase PCR; t-NGS, targeted NGS.

However, about 25% of patients with AML eventually relapse despite MRD negativity after the first 2 cycles of chemotherapy. Technical issues of sensitivity and specificity may limit MRD evaluation. From a biological standpoint, relapse may originate from a minor population of chemotherapy-resistant leukemic cells that escape MRD detection, but eventually lead to leukemic cell regrowth. These chemotherapy-resistant cells have been shown to be particularly abundant in the LSC fraction.[3]

To identify the residual LSCs, Li et al adopted a validated 8-color MFC approach consisting of a cocktail of 6 antibodies (CD56, CD22, CD11b, CD7, Tim-3, and CLL-1) in addition to CD34 and CD38 stem cell markers.[4] A total of 360 patients prospectively randomized into a training and a validation cohort were studied by using an informative positivity cutoff value of 0.004. By using this method, the authors were able to significantly improve relapse prediction compared with the standard leukemia-associated immunophenotype (LAIP)/different-from-normal (DfN) approach (C-index: 0.76 vs 0.69; Youden index: 0.58 vs 0.37).

The $CD34^{+}CD38^{-}$ LSC cocktail technique, that was recently validated in a multi-laboratory study with a common sample preparation procedure and a detailed gating protocol, yielded reproducible results with minimal inter-laboratory variations.[5] In the HOVON102/SAKK trial, the comparative analysis of standard MRD with $CD34^{+}CD38^{-}$ LSCs in 242 patients demonstrated that MRD positivity and persistence of LSCs in CR were associated with a very poor outcome (3-year OS, 0%). Conversely, MRD-negative/LSC-positive, MRD-positive/LSC-negative, and MRD-negative/LSC-negative patients had a cumulative 3-year OS of 53%, 68%, and 66%, respectively.[6] In a post hoc analysis of the MRD-guided GIMEMA AML1310 trial, despite the low number of patients tested with both techniques, we were similarly able to confirm that LSC persistence after consolidation was associated with decreased 3-year OS in both MRD-negative (67% vs 86%; $P = .44$) and MRD-positive (<20% vs 75%; $P = .041$) patients.[7]

Conversely, about 15% of MRD-positive patients do not relapse. In these cases, MRD by LAIP/DfN may be non-informative, as in blasts with mature monocytic or stem cell phenotypes, the latter of which are potentially identifiable by the method described by the authors. In some cases, the residual positive cells may not be able to generate clonal progenies. In the context of HSCT, this may also be a result of the immunologically mediated graft-versus-leukemia effect. In these situations, a combined LSC-molecular approach may be the ideal choice. This may apply as a general principle, and indeed the analyses of MRD using a combination of the LSC cocktail and quantitation by quantitative reverse transcriptase polymerase chain reaction of recurrent translocations, NPM1 mutations, and MLL rearrangements, confirmed the superiority of the integrated approach for predicting relapse within specific molecular subgroups.[1]

In general, the LSC-MRD approach takes advantage of the stability of the LSC phenotype, despite clonal evolution and immunophenotype switch in blasts frequently found at AML relapse. However, aberrant LSC phenotypes may differ in specific genetic subgroups. Our group recently demonstrated that in FLT3-mutated AML, the CD34/CD123/CD25/CD99 antibody combination reliably identifies LSC populations expressing FLT3-internal tandem duplication (ITD) mutations at high levels.[8] These LSCs are identifiable at diagnosis in patients in whom the FLT3-ITD mutation is subclonal in blasts (as shown by capillary electrophoresis) and becomes detectable at the time of relapse. These data indicate that a complete molecular evaluation should be combined with MFC at the time of leukemia progression or relapse so that newly developed disease clones will not be missed.

Compared with the more traditional MFC approach, MRD positivity at the LSC level in the study by Li et al also predicted the results of LAIP/DfN and was associated with a longer time to hematologic relapse. This may provide a valuable window of opportunity for optimizing treatment at the stage of LSC-positive CR, with an eventual benefit in the patients' clinical outcome.

The MRD study by Li et al, as with other studies, has been performed in the conventional chemotherapy setting, including intensive induction/consolidation and HSCT. It will be interesting to validate the results obtained by the LSC cocktail approach in the context of less intensive treatment approaches such as in protocols that use venetoclax combined with hypomethylating agents. The synergistic activity of the drugs led to the achievement of MRD negativity by LAIP and DfN techniques in 41% of patients, including those with poor-risk karyotypes and those who achieved composite CR.[9] LSC-based MRD monitoring may be well suited for venetoclax combinations that have been shown to target LSC metabolic vulnerabilities, including amino acid metabolism and oxidative phosphorylation.[10]

In conclusion, quantitative evaluation of LSCs represents a crucial step for guiding AML treatment, including HSCT consolidation. Indeed, LSC estimates may complement the study of standard MRD, improving our capability to unravel the chemotherapy sensitivity of the leukemic progenitors, and it may be used as a biomarker of the efficacy of drugs targeting specific LSC antigens or pathways.

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