

treatment. In agreement with recent studies by Alberti-Servera and collaborators,<sup>3</sup> Zhang et al show that, in some patients, there are obvious alterations in clonal composition from diagnosis to relapse, with some subclones progressively enriching throughout the course of chemotherapy. This evolutionary pattern, which the authors refer to as “clonal shift,” has been reported in T-ALL and many other cancers.<sup>3,4</sup> However, another pattern, coined “clonal drift,” was described by Zhang et al for T-ALL, in which there is no substantial shift in clonal composition but there are considerable changes in the transcriptional profile at relapse (see figure). Thus, 2 alternative evolutionary patterns occur in response to therapy that are characterized by either “dynamic” (clonal shift) or “stable” leukemic clones, with the variation occurring instead in the transcriptome (clonal drift). Fitness in this case likely reflects selection of traits that are epigenetically determined. In other words, “cell states” rather than “cell clones” are the subject of Darwinian selection—similarly to what Turati et al have recently reported for childhood B-cell acute lymphoblastic leukemia (B-ALL).<sup>5</sup>

What are the determinants of selection resulting in resistance in clonal drift? Commonly enriched “drifted” gene signatures show upregulation of genes such as *NFKB1A*, *SERPINB1*, *CD69*, and *MSI2* at relapse. The authors focus on *MSI2*, a logical choice considering that *MSI2* has been associated with poor outcome in ALL.<sup>6,7</sup> Now, 2 independent T-ALL cohorts provide evidence that high *MSI2* expression at diagnosis is associated with persistence of residual leukemia after induction chemotherapy. This hints at the possibility that *MSI2* may be a biomarker of resistance in T-ALL.

So, what leads to *MSI2* upregulation? Zhang and colleagues do not provide a definitive answer, although they do show that histone marks (H3K4me3 and H3K27ac) compatible with increased transcription initiation of *MSI2* are elevated upon relapse. The answer to the obvious next question is perhaps more relevant: how does *MSI2* promote resistance to therapy? Zhang et al show that *MSI2* binds to transcripts of the oncogene *MYC*, thereby stabilizing them and contributing to T-ALL cell viability and proliferation. In vivo evidence in a mouse model of activated NOTCH1-induced T-ALL provides correlative evidence that downregulating

*MYC* by pharmacologically inhibiting *MSI2* may be a valid frontline strategy to treat T-ALL. Important for relapse or refractory disease is the demonstration that *MSI2* overexpression induces in vitro resistance to daunorubicin, cytarabine, vincristine, and methotrexate, whereas knocking out *MSI2* sensitizes T-ALL cells to chemotherapy—an effect that is counterbalanced by *MYC* overexpression. The corollary is that *MYC* upregulation is critical for *MSI2*-mediated chemoresistance in T-ALL. In animal models, leukemia stem/initiating cells display high *MSI2* levels,<sup>8</sup> T-ALL leukemia initiating cells express high *MYC* levels, and pharmacological inhibition of *MYC* activity leads to T-ALL remission.<sup>9</sup> Conversely, B-ALL cells that escape chemotherapy are quiescent, with downregulation of *MYC* activation being a frequent feature of relapsed B-ALL.<sup>5</sup> Hence, the *MSI2*-*MYC* axis, which can drive resistance in T-ALL, is unlikely to play a role in B-ALL relapse.

Although many questions arise from the work by Zhang et al, such as whether *MSI2* expression in T-ALL cells may drive resistance also by impacting the normal immune cell compartment, one point of special interest is the demonstration that small molecule inhibition of *MSI2* sensitizes T-ALL cells to chemotherapy in vitro and substantially delays leukemia progression in vivo in patient-derived xenograft models when given in combination with daunorubicin or cytarabine. Although other combinations, including with glucocorticoids, still require analysis, these experiments highlight the potential of *MSI2* as a target for therapeutic intervention in relapse/refractory T-ALL.

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

## MYELOID NEOPLASIA

Comment on *Othman et al*, page 336

# MRD in AML: who, what, when, where, and how?

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In this issue of *Blood*, Othman et al use retrospective data to show the utility of measurable residual disease (MRD) monitoring of patients with *NPM1* acute myeloid leukemia (AML) in de novo treatment using venetoclax-based regimens.<sup>1</sup>

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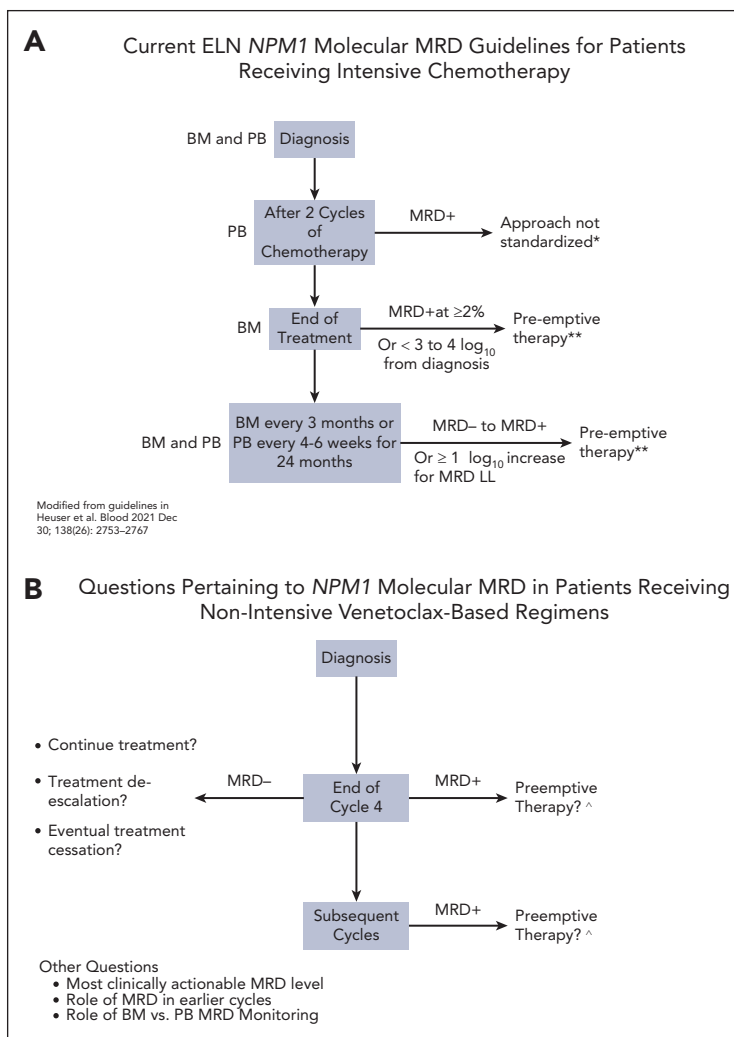
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MRD assessments have the potential to improve outcomes for patients with AML. However, there are still many open questions. For example, the 2021 European LeukemiaNet (ELN) updated MRD guidelines pertaining primarily to patients receiving intensive chemotherapy.<sup>2</sup> These guidelines recommend using quantitative or digital polymerase chain reaction (qPCR or dPCR) for MRD monitoring in molecularly defined subgroups, that is, *NPM1*-mutated and core binding factor AML (containing translocations involving *RUNX1-RUNX1T1* or *CBFB-MYH11*). MRD monitoring by multiparameter flow cytometry (MFC) is recommended for all other patients. Thus, it is unclear from these guidelines how to incorporate NGS-based monitoring, what depth of detection is needed for each of the modalities, what time points are optimal for MRD assessments, and the best source of assessment (bone marrow [BM] vs peripheral blood [PB]).

MRD monitoring for patients with *NPM1*-mutated AML undergoing intensive chemotherapy is based on work by Ivey et al.<sup>3</sup> Patients with *NPM1*-mutated AML who achieved complete remission (CR) with 2 cycles of intensive induction were studied. Persistence of *NPM1* PCR transcripts in PB after 2 cycles of intensive induction chemotherapy was associated with greater risk of relapse at 3 years. Relapse was also reliably predicted by a rising level of *NPM1* transcripts with sequential monitoring. The ELN guidelines recommend assessing MRD in *NPM1*-mutated AML with *NPM1* PCR in PB following 2 cycles of intensive chemotherapy, in the BM at the end of treatment, and in either BM every 3 months or PB every 4 to 6 weeks for 24 months after therapy completion (see figure panel A). These guidelines also define an entity of MRD at low level (MRD-LL) in *NPM1*-mutated AML as <2% but above the limit of assay detection. MRD-LL is associated with a low risk of relapse when measured at the completion of consolidation. These guidelines also recommend individualized treatment strategies to reduce the risk of relapse if specific MRD trends are noted. The MRD trend groups include MRD positivity defined as  $\geq 2\%$  in the BM at completion of consolidation, failure to achieve a 3 to 4 log reduction in either the BM or PB at completion of consolidation, or MRD relapse defined as conversion



Molecular MRD by qPCR: current guidelines for intensive chemotherapy and questions pertaining to less intensive venetoclax-based regimens. (A) Current European LeukemiaNet guidelines for monitoring molecular *NPM1* MRD testing for patients with *NPM1* mutations who are undergoing intensive induction chemotherapy regimens, though questions remain as outlined by the diagram. (B) There are no current guidelines for recommendations for MRD testing for patients with *NPM1* mutations receiving non-intensive venetoclax-based regimens. The diagram depicts current questions that exist surrounding monitoring *NPM1* molecular MRD testing in this treatment setting. \*Existing data have indicated that patients with MRD following induction chemotherapy may benefit from transplant, but approaches in this situation are not standardized. \*\*Preemptive therapy is recommended by ELN guidelines, but preemptive approaches are not standardized. Potential approaches include immediate transplant vs salvage therapy with either intensive chemotherapy or less intensive approaches for MRD eradication followed by potential transplant in appropriate candidates. Patients are encouraged to enroll in clinical trials. ^Preemptive therapy could include immediate transplant vs salvage therapy for MRD eradication followed by potential transplant in appropriate candidates.

from MRD negativity to MRD positivity or increase of MRD  $\geq 1 \log_{10}$  between any 2 positive samples for patients with MRD-LL. Allogeneic stem cell transplantation may improve patient outcomes with *NPM1*-mutated AML who have suboptimal molecular responses after induction therapy.<sup>4</sup> Preemptive therapy may benefit patients in these settings as persistent *NPM1* MRD pretransplant has been associated with worse posttransplant outcomes.<sup>5,6</sup> The ELN MRD guidelines recommend treatment on clinical trial whenever possible to establish an

evidence-based approach for patients with MRD persistence or relapse.

There are currently no established guidelines related to MRD monitoring for patients receiving less intensive induction regimens, in which venetoclax-based regimens are the new standard of care.<sup>7,8</sup> The VIALE-A study led to approval of venetoclax combined with azacitidine for patients with newly diagnosed AML who are ineligible for intensive chemotherapy. A follow-up study reported on MFC for MRD assessments

on VIALE-A and found that 41% of patients achieved MRD negativity (defined as  $<10^{-3}$ ) during treatment. Of these patients, MRD negativity was achieved in 52% by the end of cycle 4 with the rest occurring later in treatment. Patients who achieved MRD negativity had superior overall survival (OS) compared with patients who did not.<sup>9</sup> Early retrospective data also suggest that there may be patient populations able to stop venetoclax-based therapy without relapse with MRD negativity by MFC being a major predictive factor.<sup>10</sup>

The study by Othman et al is a retrospective review of patients with newly diagnosed AML with *NPM1* mutations who achieved CR using regimens containing venetoclax with low-dose cytarabine or hypomethylating agents. This study assessed the impact of *NPM1* MRD by RT-qPCR and found the deepest MRD responses ( $\geq 4 \log_{10}$  reduction from baseline) were predictive of better 2-year OS, with 44 patients (58%) achieving BM MRD negativity and a further 14 (18%) achieving a reduction of  $\geq 4 \log_{10}$  from baseline as their best response. Achievement of BM MRD negativity at the end of 4 cycles of treatment was the factor associated with greatest improvement in OS on multivariable analysis. A subset of the patients with MRD negativity also stopped further treatment with a 2-year treatment-free remission rate of 88%. The authors also showed that event-free survival was worse for patients who were MRD<sup>-</sup> in the PB but MRD<sup>+</sup> in the BM compared with patients with MRD negativity in both sources.

This study provides valuable insight into the kinetics and prognostic significance of molecular MRD by *NPM1* qPCR for patients with *NPM1*-mutated AML treated with less intensive venetoclax-containing regimens. It raises several important questions for additional investigation (see figure panel B). Some patients who achieved MRD negativity were able to stop therapy without experiencing relapse, raising the question of whether certain patients may be cured with venetoclax-based regimens. Future prospective studies of treatment deescalation/cessation for patients achieving MRD<sup>-</sup> remissions by certain time points would be informative, including how to monitor these patients after treatment

cessation. Conversely, could patients who do not achieve MRD negativity by early time points benefit from changing therapies? Another uncertainty is the optimal MRD threshold for clinically actionable decisions. In this study, patients with detectable MRD but  $\geq 4 \log_{10}$  reduction had survival outcomes that were worse than MRD<sup>-</sup> patients but better than those who achieved  $<4 \log_{10}$  reduction. Thus, it is not clear how patients with detectable, but lower, amounts of MRD should be approached. Patients with MRD-LL ( $<2\%$  positive) after intensive consolidation are at low risk of relapse, but it is unclear if this is applicable for patients treated with lower-intensity regimens.

This study represents a step forward in continued efforts to understand the significance of MRD and incorporation into clinical decision-making for patients treated with venetoclax-based regimens. It paves the way for further studies exploring how to better define who, what, when, where, and how to use MRD status optimally.

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## PLATELETS AND THROMBOPOIESIS

Comment on *Ellis et al*, page 342

# Platelet size matters

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**In this issue of *Blood*, Ellis et al<sup>1</sup> report stunning observations on the role of glycoprotein Iba (GPIba) and filamin A (FlnA) in thrombopoiesis using cutting-edge microscopic techniques. Their results may impact the general view of thrombopoiesis, bringing the role of platelet size into focus.**