Molecular Measurable Residual Disease Detection in AML

Peter J.M. Valk

Department of Hematology, Erasmus University Medical Center, Rotterdam, The Netherlands

Risk-stratification of acute myeloid leukemia (AML) based on the presence or absence of recurrent somatic abnormalities has evolved substantially over the years and is now summarized in the current 2017 European Leukemia Net (ELN) recommendations.1 Our improved understanding of the molecular landscape of AML has resulted in better treatment decisions at the time of complete remission (CR) after induction treatment. High dose chemotherapy treatment results in CR rates of 80% and higher, however, the majority of these AML patients will eventually relapse. Thus, there is still a great need for adequate prediction of impending relapse to adapt treatment accordingly and improve outcome of AML patients at risk of relapse. Measurable residual disease (MRD) detection has already proven to have substantial value in predicting relapse when applied to AML in CR, but molecular MRD detection has been limited to only a subset of genetically defined AML subtypes.2,3,4,5 Next generation sequencing (NGS) enables the detection of the all, often patient-specific, mutations at diagnosis and in CR and is applicable to virtually every newly diagnosed patient with AML.6

Reliable detection of all mutant cell populations at diagnosis and during the course of disease is required before NGS-based MRD detection can be adopted in routine analyses. Sequencing artifacts are introduced during DNA isolation, library prep and the actual NGS-procedure, which makes sensitive detection of all possible mutations at low level (<0.1%) challenging.7 These sequencing errors can be reduced by, for example, the use of proof-reading polymerases, computationally or with various error-corrected NGS methodologies using molecular barcoding.7 These strategies have been shown to increase the specificity of low-frequency mutation detection in general, however, the most optimal approach for reliable detection of MRD in AML needs to be determined.

Recently, several studies addressed NGS-based MRD detection in relatively large AML cohorts from clinical trials, all demonstrating that “NGS-based MRD carries profound prognostic impact for patients with AML.”8,9,10,11,12,13,14,15 In these studies persisting mutations in CR were measured with gene panels,8,10,12 capture deep sequencing,8,10,12 or targeted sequencing.13,15 Only in the latter NGS-based MRD detection included error-correction using unique molecular identifiers, indicating that the other NGS-based MRD studies may not have been optimal. Another successful approach to correct for noise is the usage of site-specific error models.11 However, in this setting a series of reference samples to discriminate true MRD from noise is a prerequisite. Since NGS MRD seems to have consistent prognostic value technological improvements should be accomplished to further optimize relapse prediction in AML.

In the initial NGS-based MRD studies in larger AML cohorts,10,11,12,13,15 it became also clear that “gene mutations persisting in CR that are well-known to be associated with clonal hematopoiesis of indeterminate potential (CHIP),16,17 such as mutations in DNMT3A, TET2 and ASXL1 (DTA), do not impact on risk of relapse” (Fig. 1). After high dose chemotherapy, these AML patients are in a state of clonal hematopoiesis (CH), where mutations occurring late in leukemogenesis are eradicated but mutations also found in CHIP persist. Besides acquired mutations in DTA, other well-known pathogenic mutations such as those in TP53, PPM1D, JAK2, CBL, SRSF2, and SF3B1 have also been shown to be present in CHIP, however, at lower frequencies.16,17 It has to be demonstrated if and to what extent persisting mutations other than DTA represent either true residual leukemia or CH with and without increased risk of relapse, respectively. RAS pathway-related mutations such as those in FLT3, RAS, KRAS, PTPN11, and KIT are known to be late events in AML evolution (Fig. 1). These mutations are generally cleared by high dose chemotherapy. However, persistence of these mutations, representing frank leukemia, is clearly associated with a higher risk of relapse.18,9,10,11,12,13,14,15 Altogether, these results indicate that molecular MRD monitoring should possibly focus on these late events. AML patients with TP53 mutations fail to reach a CR or relapse quickly after induction treatment, irrespective of their molecular MRD status.11 Thus, certain subtypes of AML may whereas other may not benefit from NGS-based MRD testing. While the recent developments in NGS-based MRD detection represent major steps forward in predicting relapse, they remain imperfect. It is conceivable that the non-DTA mutations include a mixture of mutations representative of either true leukemia or CH. Therefore, it is expected that a better distinction of these two conditions will improve the prediction of AML patients with higher risk of relapse. The numbers of AML patients included in the initial studies did not allow an in detail analyses of less frequent mutations.11 Consequently, future studies will require larger cohorts of AML.
How does NGS-based MRD detection compare to the ‘golden standard’ multiparameter flow cytometry (MFC) MRD detection? Today, there are only limited studies with a decent comparison between NGS- and MFC MRD detection. Both these studies demonstrate that there is a concordance of 70% with regard to MRD detection using the 2 technologies, and that those patients with detectable MRD by both MFC and NGS have the highest risk of developing an AML relapse. Interestingly, however, those AML cases with discordant MRD detection results with NGS and MFC still carry prognostic value as well. We need to further improve the sensitivity of our NGS assays as well as our understanding of the biology of CH after high dose chemotherapy to better understand the discordant cases and determine whether we require both technologies or only one.

In most studies NGS-based MRD detection focused on AML patients at the time of CR after high dose induction treatment. However, AML patients with high risk of relapse can also be recognized by NGS-based MRD detection post-transplant. Ultimately, dynamic monitoring of the AML-specific mutations during the course of disease by NGS will be utilized to determine therapy responses in AML.

Currently, “the major limitations of the NGS-based MRD detection methodology relate to: limited sensitivity and specificity of the assay and the inability to correctly discriminate between residual leukemia and CH”. Improvements should be made in all these areas before NGS-based MRD detection can successfully be implemented in routine practice.

References

Figure 1. Clonal hierarchy of the different types of mutations in AML development and recurrence. Initiating mutations that result in clonal dominance, also present in healthy individuals with CH, are not, whereas, residual transforming mutations that arise later in leukemia development present in CR are associated with increased risk of relapse.


Application of error-corrected sequencing NGS-based MRD detection.

