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EDITORIAL

Revisiting the role of measurable residual disease in FLT3 mutated acute myelogenous leukemia

Håkon Reikvam[a,b] and Richard Dillon[c]

[a]K.G. Jebsen Center for Myeloid Blood Cancer, Department of Clinical Science, University of Bergen, Bergen, Norway; [b]Department of Medicine, Haukeland University Hospital, Bergen, Norway; [c]Department of Medical and Molecular Genetics, King's College, Guy's and St Thomas' NHS Foundation Trust, London, UK

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1. Introduction

Acute myelogenous leukemia (AML) is an aggressive hematological malignancy characterized by a block in the maturation and differentiation of immature myeloid blast cells [1,2]. AML is a heterogeneous disease, encompassing a myriad of molecular genetic alterations [1], among which the FMS-like tyrosine kinase 3 (*FLT3*) gene is recurrently mutated, occurring in approximately 25–30% of AML patients [3] and up to 40% in the group of cytogenetically normal AML [3]. The mutations can be categorized into two major types: *FLT3* internal tandem duplication (ITD) mutations and point mutations in the tyrosine kinase domain (TKD). The former is more common and has greater prognostic impact, being associated with increased relapse rates.

Responses to the initial chemotherapy regimen have traditionally been considered a strong prognostic marker, with the achievement of complete remission (CR) defined as morphological bone marrow evaluation showing <5% blasts without significant cytopenia. However, despite achieving CR, a significant number of patients will relapse. Over the last decade, measurable residual disease (MRD), which refers to the persistence of residual leukemic cells after treatment, has emerged as a powerful tool for physicians managing AML.

The European Leukemia Net (ELN) has introduced CR with MRD negativity (CR^{MRD-}) as a new response criterion [2,4]. MRD monitoring has become a crucial component of AML management, providing significant prognostic information and guiding additional treatment decisions [5]. Until recently, *FLT3* mutations were not considered suitable MRD markers; however, highly compelling data has recently emerged from a number of study groups, which are likely to lead to the adoption of *FLT3* MRD testing into routine clinical practice, alongside more established MRD assays. Here we discuss the status and future aspects of MRD monitoring in the subgroup of AML patients with *FLT3* mutations.

2. MRD monitoring in *FLT3* mutated AML-state of the art

Specific and sensitive techniques are essential for MRD monitoring, and several methods have been developed for MRD monitoring in AML. The main techniques are based on polymerase chain reaction (PCR), multiparametric flow cytometry, and most recently next-generation sequencing (NGS). Molecular testing using a validated quantitative PCR test is the recommended MRD methodology for patients with a validated, stable, and leukemia-specific genomic aberration including *PML:RARA*, *RUNX1:RUNX1T1*, and *CBFB:MYH11*. Additionally, a range of rarer but recurrent leukemia-specific fusion transcripts, for example, *NUP98:NSD1* and *KMT2A* rearrangements are under evaluation as MRD markers. Furthermore, in cases of AML with the nucleophosmin 1 (*NPM1)* mutation, usually occurring as four base pair insertion in exon 12 of the gene, MRD monitoring by quantitative PCR has been demonstrated to be a powerful tool for predicting relapse [6]. These established MRD markers are applicable to the majority of cases of *FLT3*-mutated AML, since *NPM1* mutations occur in approximately 55% of cases [3,7] and fusion transcripts in approximately 15% [8] (Figure 1). Hence, in many cases of *FLT3*-mutated AML, established PCR-based MRD assays may be used to refine prognostication and track disease [6,8]. Limitations of these assays include the phenomenon of persistent low-level MRD positivity at the end of treatment [9], which does not always predict relapse. In addition, the detection sensitivity of molecular MRD assays can vary depending both on the sample quality and baseline expression level, potentially leading to false-negative results. Furthermore, in the case of *NPM1* leukemias, a fraction of patients will develop relapsed disease from an *NPM1* negative clone, hence it will not be detected by *NPM1* MRD assays [10]. Despite these drawbacks, molecular MRD monitoring by RT-qPCR has been established as a significant tool for AML monitoring and prognostication.

Immunophenotyping by multiparameter flow cytometry has been a cornerstone in establishing a diagnosis of AML, i.e. to determine myeloid or lymphoid lineage affiliation and distinguish AML from acute lymphoblastic leukemia (ALL). However, the technique has also been established for MRD detection, and two separate approaches have

CONTACT Håkon Reikvam Hakon.Reikvam@uib.no K.G. Jebsen Center for Myeloid Blood Cancer, Department of Clinical Science, University of Bergen, Bergen N-5021, Norway

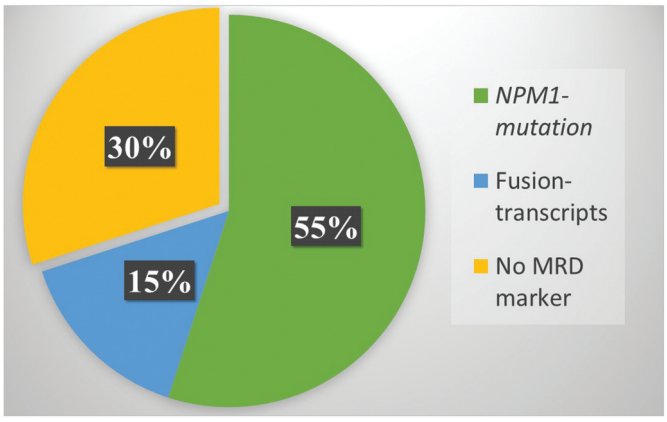

Figure 1. Molecular MRD targets in patients with *FLT3* mutated AML in the NCRI AML19 trial [8]. 55% of patients had co-occurring *NPM1* mutations, and approximately 15% patients had fusion transcripts (i.e. *CBFB:MYH11*, *RUNX1:RUNX1T1*, *NUP98:NSD1*, or other rare fusion genes), while for the remaining 30% of patients no validated molecular MRD target was present.

been developed: leukemia-associated immunophenotypes (LAIP) at diagnosis that are then tracked throughout the treatment course, and differences from normal (DFN) by identifying cell populations demonstrating deviation from normal antigen expression in myeloid cells during maturation [11]. Hence, MRD monitoring by multiparametric flow cytometry has been demonstrated to be effective and relevant in *FLT3*-mutated AML cases.

However, the technique is hindered by some obstacles and limitations. Firstly, it requires fresh cells, and preparing and sending to specialized laboratories present limitations [12]. Additionally, not all AML cases have an aberrant immunophenotype, and the phenotype may change during disease evolution, such as during relapse occurring from another clone. Furthermore, the technique requires substantial experience and expertise, and standardization of the methodology has proved challenging [12].

3. NGS-future and emerging approaches for MRD monitoring in *FLT3* mutated AML

NGS has gained considerable interest over the last decade and is now considered essential for accurate risk classification of AML patients at diagnosis [2]. Furthermore, high sensitivity NGS techniques could theoretically be used to evaluate MRD in virtually all AML patients, as recurring mutations are detected in almost all AML patients. However, some concerns regarding its clinical value for the prediction of relapse have been raised, since some mutations associated with clonal hematopoiesis often remain detectable after treatment. A study of 482 AML patients showed that the persistence of non-DTA (i.e. *DNMT3A*, *TET2*, and *ASXL1*) mutations during CR had a significant independent prognostic impact on relapse rates and overall survival [13]. Furthermore, comparison of NGS with multiparametric flow cytometry for the detection of MRD demonstrated that NGS had significant additive prognostic value [13].

Approximately 30% of *FLT3* mutated AML patients lack suitable markers for PCR detection (Figure 1), and multiparametric flow cytometry has, as mentioned, several limitations in this patient group. Accordingly, challenges and obstacles remain in the effort to establish effective, reliable, validated, and standardized MRD monitoring for *FLT3*-mutated AML cases. *FLT3*-ITD MRD detection by either PCR or NGS has been hampered by the great variety in the spectrum of *FLT3*-ITD, including the length, sequence, and site of the insertion, in addition to the variation in mutation allelic ratio and the apparent instability of *FLT3*-ITD which is usually a late event in leukemogenesis [3]. Hence, until recently, the approach of using *FLT3* itself to monitor treatment responses has not been recommended. However, advances in sequencing technology and bioinformatics now enable accurate detection of *FLT3*-ITD with very high sensitivity. Recent studies have demonstrated the feasibility and clinical utility of this approach (Table 1) [14–22], and growing interest in use of NGS as an MRD approach among *FLT3* mutated patients has emerged. In a study of 161 AML patients with *FLT3*-ITD mutation, NGS-based *FLT3*-ITD MRD was positive in 47 of 161 (29%) patients after two cycles of induction chemotherapy. The presence of *FLT3*-ITD MRD was associated with an increased risk of relapse and reduced overall survival. Interestingly, *FLT3*-ITD MRD provided additional prognostic information to established prognostic factors, including mutant *NPM1* detection or multiparameter flow cytometry [19]. Furthermore, recently a multicenter study demonstrated that AML patients in CR with detectable *FLT3*-ITD in the blood prior to allogeneic hematopoietic stem cell transplantation (allo-HSCT) had substantially increased relapse rates and worse survival [18]. Hence, NGS-based MRD has been demonstrated to be widely applicable to AML patients and highly predictive of relapse and survival, especially refining transplant and posttransplant management in AML patients [23].

NGS-based MRD monitoring is currently limited by the fact that these techniques require experienced laboratory and bioinformatics personnel, in addition to the time-consuming procedures and the economic costs associated with the techniques (Table 2). Further laboratory and clinical studies are needed to determine whether routine DNA-sequencing testing for residual *FLT3* variants can improve outcomes for this patient group.

4. Expert opinion

Despite considerable improvements, the last decade, several aspects of MRD monitoring in *FLT3*-mutated still AML remain uncertain. This uncertainty revolves around different assays, the ideal time to measure, use of blood or bone marrow, and establishing the optimal thresholds for classifying a patient as MRD positive or MRD negative. *NPM1* PCR appears to be a powerful prognostic test for patients with concomitant *NPM1* and *FLT3* mutations although for those without *NPM1* mutations, there are still unanswered questions. Multiparametric flow cytometry has its limitations, and NGS is still expensive and labor-intensive. The establishment of a reliable and reproducible PCR-NGS assay that can detect *FLT3* DNA sequences currently seems to be an emerging technique of great value. Additional preclinical and clinical studies to validate and standardize the method and reliably demonstrate its clinical utility are now required

Table 1. Studies assessing molecular MRD monitoring in *FLT3* mutated AML. the table summarizes the most important clinical studies assessing MRD in *FLT3* mutated AML and includes performed methods, sample source, the time point of monitoring, and the main conclusion of the study.

Methods	Source	n	Timepoint	Main findings	References
NGS DNA sequencing for *FLT3*-ITD and TKD	PB	339 + 412*	Pre allo-HSCT	Persistence of *FLT3*-ITD was associated with increased relapse rates and worse survival	[18]
PCR-NGS for *FLT3*-ITD	BM or PB	104	Pre allo-HSCT	*FLT3*-ITD MRD positivity was associated with increased risk of relapse and poorer overall survival	[17]
NGS-based *FLT3*-ITD	BM or PB	161	After two cycles of induction chemotherapy	NGS-based detection of *FLT3*-ITD MRD identified patients with profound risk of relapse and mortality post-transplant	[19]
NGS-based *FLT3*-ITD	BM or PB	321	After one or two cycles of induction chemotherapy	MRD negativity associated with better prognosis, but the FLT3 inhibitor quizartinib increased overall survival in MRD positive patients	[20]
NGS-based *FLT3*-ITD	BM or PB	142	After two cycles of induction chemotherapy	MRD negativity was the strongest independent favorable prognostic factor for relapse and overall survival	[21]
NGS-based *FLT3*-ITD	BM	356	Pre and post allo-HSCT	MRD negativity was associated with increased relapse free survival, but the FLT3 inhibitor gilteritinib increased relapse free survival in MRD positive patients	[22]

*Patients in discovery and validation cohort.
Abbreviation: FG, fusion genes; RT-qPCR, real time- quantitative polymerase chain reaction, NGS, next generation sequencing; BM, bone marrow; PB; peripheral blood; MRD; measurable residual disease, allo-HSCT, allogeneic hematopoietic stem cell transplantation.

Table 2. Advantages and disadvantages using NGS as an MRD marker for *FLT3* mutated AML.

Advantages	Disadvantages
• Applicable to all *FLT3* mutated AML patients. • Highly sensitive. • ctDNA allows for use of blood. • Potential for standardization of multiplexed platforms. • Information of clonal evolution if other mutations tracked. • May allow early clinical intervention, i.e. proceeding to allo-HSCT or introducing FLT3 inhibitors.	• Still lack of standardization and validation. • *FLT3*-ITD negative relapse can occur. • In need of bioinformatic advanced competence. • Time consuming. • Expensive.

as a matter of priority. Furthermore, the possibility of early diagnosis of molecular relapse offers a window of time for intervention to prevent relapse, although appropriate interventions, which could include allo-HSCT and/or FLT3 inhibition, remain incompletely defined.

In any case, we can be optimistic about progress in both diagnostics and treatment for this subgroup of AML patients and hope that in the near future, we can tailor and target treatment even more for this patient group.

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Author contributions

H Reikvam initiated the work, wrote, and revised the manuscript. R Dillon supervised the work, wrote, and revised the manuscript.

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