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REVIEW ARTICLE

Molecular methods for measurable residual disease in acute myeloid leukemia: where are we and where are we going?

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Abstract

Measurable residual disease (MRD) testing has become a standard practice for patients with acute myeloid leukemia (AML) following therapy. However, MRD testing in AML is not straightforward, and both molecular methods and multiparameter flow cytometric (MFC) methods demonstrate clinical utility. While MFC methods are potentially applicable to all AML patients, current molecular MRD methods must be individually tailored to the patient's AML disease genetics, a strategy that is currently applied for patients with acute promyelocytic leukemia, core binding factor AML, and AML with mutated *NPM1*. However, there is great interest in next-generation sequencing (NGS) methods for MRD assessment, an approach that could potentially be applied to all patients with AML. Current NGS methods have limited analytic sensitivity when compared with other molecular methods and MFC, but advances in NGS methods and informatic algorithms may improve NGS MRD testing in the near future. In this review, we discuss current recommendations for molecular MRD assessment in AML and discuss opportunities and challenges for MRD assessment by NGS methods.

Keywords Acute myeloid leukemia · Next-generation sequencing · Measurable residual disease

Introduction

The evaluation of post-therapy bone marrow specimens from patients with acute myeloid leukemia (AML) has historically relied on morphologic assessment. If fewer than 5% of marrow cells are blasts and if Auer rods (signifying an abnormal blast population) are not identified, then a patient is determined to be in a morphologic leukemia-free state, a prerequisite for complete remission (CR) [1, 2]. However, attainment of morphologic CR does not adequately predict outcomes in patients with AML, and it has become apparent that morphologic absence of leukemia is too crude a metric to guide optimal patient management [3, 4]. Manual differential counting is subjective, and counting relatively few cells (~ 500) leads to substantial measurement error, even under optimal theoretical circumstances, when dealing with low blast percentages [5]. Moreover, given the vast numbers of hematopoietic cells in the body, even 1% malignant blasts in a marrow sample would translate to billions of leukemic cells in the patient [6, 7]. Further, regenerating normal myeloblasts following chemotherapy cannot be reliably distinguished from neoplastic leukemic blasts by morphologic inspection. Therefore, techniques with greater clinical sensitivity than morphologic examination to monitor disease in AML patients are needed. Building upon successes in acute lymphoblastic leukemia (ALL) and chronic myeloid leukemia (CML) [8–10], the utilization of non-morphologic modalities to assess for measurable (minimal) residual disease (MRD) in AML has increased over the last several years.

There has been some hesitation to fully embrace MRD testing for patients with AML and to use these results to make treatment-related decisions due to a lack of randomized clinical trials [7]. However, clear associations between AML MRD status and the risk of frank relapse and inferior outcome [11, 12], as well as promising data from prospective non-randomized trials in which MRD positivity was used to guide subsequent therapy [13–16], have prompted the European LeukemiaNet to make several recommendations regarding MRD testing. These recommendations include (1) using MRD status as a factor in patient response criteria, (2) employing MRD status to drive post-remission therapy

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decisions and emphasizing the achievement of MRD-negative morphologic complete remission as a purpose of treatment, and (3) the utilization of MRD negativity as an end point in clinical trials as a surrogate marker for event-free survival [17].

Two primary methods exist for MRD testing: multiparameter flow cytometry (MFC) to assess for immunophenotypic abnormalities on blasts and molecular methods to assess for leukemia-associated genetic alterations. MRD status may be assessed either by MFC using a leukemia-associated immunophenotype approach that looks for a constellation of phenotypic markers previously seen at diagnosis in a specific patient or by a different-from-normal approach that looks for a discrete blast population with phenotypic characteristics unlike those of normal myeloblasts. Current consensus MFC MRD recommendations suggest using a combination of these strategies [12]. While MFC approaches can be attempted across AML subtypes (though with potentially less success in AML with certain phenotypes, such as those with monocytic differentiation [18]), molecular approaches must be tailored to the individual patient, and the wide availability of genetic panels designed for AML at diagnosis can lead to confusion among pathologists and clinicians regarding appropriate ordering of follow-up molecular MRD studies. Some molecular strategies for detecting MRD, particularly those targeting *NPM1* mutations and WHO entity-defining recurrent fusions (e.g., *PML/RARA*, *RUNX1/RUNX1T1*, and *CBFB/MYH11*), boast superior sensitivity when compared with MFC, but are limited in applicability due to the specificity of their genetic targets. Exciting new developments in MRD assessment by next-generation sequencing (NGS) technologies may offer a broader, more flexible evaluation for persistent disease, but are currently limited by subpar sensitivity and a lack of clarity regarding which molecular aberrations are tied to specific clinical outcomes in specific subsets of patients. As massively parallel sequencing becomes more commonplace and the significance of specific mutations becomes better understood, new advancements in MRD detection and clinical utilization are likely to follow. In this review, we touch on the history of using molecular methods in AML MRD detection, discuss current molecular approaches, examine the most recent recommendations for molecular MRD use and the application of this data in clinical decision-making, and what the future may hold for MRD as new technologies come into the fold.

Quantitative PCR–based methods

PCR-based methods were first proposed for the detection of MRD in ALL and CML in the late 1980s [19, 20] and continue to serve as a critical component of disease monitoring in these patients [8, 10]. Currently, approximately 60% of AML cases in younger adults (under age 60) carry a recurrent molecular lesion that can be monitored post-therapy by widely validated quantitative reverse-transcriptase polymerase chain reaction (RT-qPCR) assays [17]. As a result, current ELN MRD Working Party consensus guidelines recommend this method for MRD monitoring in patients with acute promyelocytic leukemia (APL), core binding factor (CBF) AML, and AML with *NPM1* mutations [12]. While bolstered by strong data that these lesions are highly stable through relapse, each of these molecular targets warrants some special considerations for optimal utilization. A number of other potential MRD targets can theoretically be assayed by qPCR in appropriate patient populations; these will also be briefly discussed.

Leukemia-associated fusions

RT-PCR methods to identify chimeric, leukemia-associated gene fusions are technically straightforward (Fig. 1a). Briefly, reverse transcription is performed on extracted RNA to generate complementary DNA (cDNA). While the genomic DNA intronic breakpoints may be highly variable in recurrently translocated genes, generally only a limited number of exons will combine to form leukemogenic chimeric transcripts at the RNA level. Therefore, relatively few primers are required to detect chimeric fusions using a cDNA template. Because forward and reverse primers are only near one another when the target fusion is present, non-neoplastic cells will not generate a product during the PCR reaction, leading to very low limits of detection with this approach (around 10^{-5}) [12].

Acute promyelocytic leukemia

Modern therapy regimens have transformed APL from "the most malignant form of acute leukemia" [21] to a member of the favorable risk category, with recent clinical trials reporting long-term overall survival from 88 to 99% and incidence of relapse ranging from < 1 to 5% [22–25]. However, the minority of APL patients who experience recurrence are at risk for adverse outcomes related to relapse-induced coagulopathy and treatment-induced differentiation syndrome [26], and early therapeutic interventions based on post-remission MRD positivity have been shown to facilitate better outcomes when compared with treating patients at frank clinical relapse [14, 15, 27]. Because ATRA-based therapies drive promyelocyte maturation instead of directly facilitating blast death, some patients require up to 10 weeks after therapy to achieve morphologic remission [27]. Not surprisingly, *PML/RARA* positivity by RT-qPCR can also persist for some time after completing induction therapy without representing treatment failure or clinically relevant residual disease. Therefore, *PML/RARA* quantification is recommended in APL patients at the

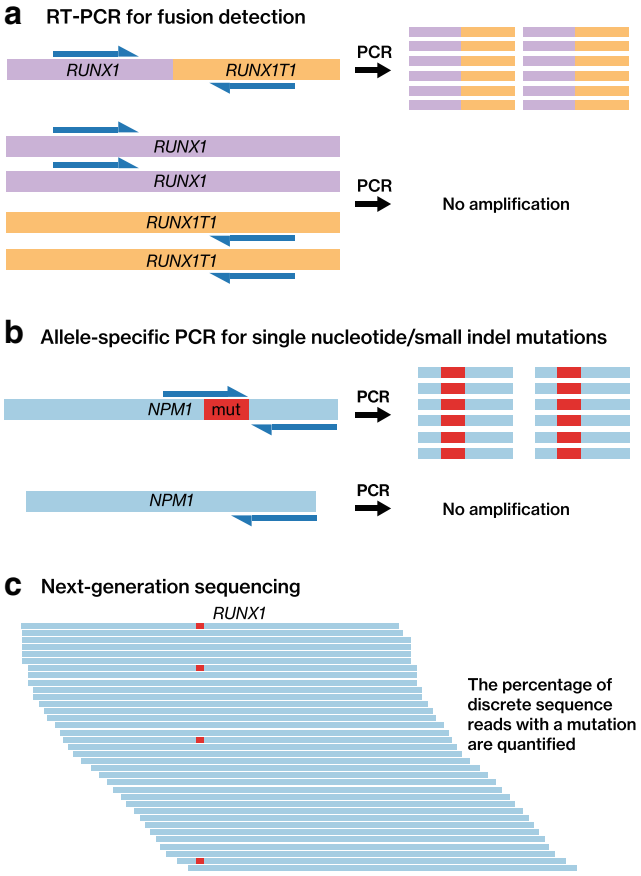

Fig. 1 Molecular approaches for measurable residual disease testing. **a** Leukemia-specific chimeric fusion transcripts are detected by reverse transcriptase polymerase chain reaction. Following reverse transcription of cDNA, primers (blue arrows) bind to the abnormal chimeric fusion transcript, leading to PCR amplification if it is present. In the absence of a fusion, the primers would bind many megabases apart or on different chromosomes, precluding amplification. This allows for very low limits of detection in specimens where a target fusion is present. **b** Mutation-specific primers can be designed for highly recurrent mutations and used in an allele-specific PCR reaction. In the example, a primer specific for an *NPM1* insertion mutation allows amplification of mutated *NPM1*. However, the mutant-specific primer does not efficiently bind wild-type *NPM1*, again leading to very specific amplification and allowing for low limit of detection. **c** Next-generation sequencing approaches theoretically allow for broad identification of mutations, as the number of individual sequencing reads carrying a mutation can be quantified. However, sequencing reads contributed by non-neoplastic cells are also present in the analysis, along with relatively high background sequencing error rates, limiting the ability to reach very low limits of detection with conventional approaches

completion of consolidation to predict for relapse risk, and post-induction assessment of MRD status is not indicated [26, 27]. Current European APL-specific consensus guidelines suggest that bone marrow aspirate material is the optimal specimen for APL MRD testing [27], though National Comprehensive Cancer Network (NCCN) guidelines suggest using peripheral blood as the MRD specimen [28].

Historically, qualitative RT-PCR methods were used for MRD monitoring in APL patients; however, more recent guidelines suggest using RT-qPCR techniques, with MRD positivity being established when increasing *PML/RARA* transcripts are identified in 2 consecutive samples [27]. In APL patients who have achieved MRD negativity following consolidation, current guidelines only recommend additional serial monitoring at 3-month intervals for high-risk patients (based on presenting WBC counts > 10×10^9/L) [27].

Core binding factor Leukemias

AML cases with t(8;21), inv(16), and t(16;16) (CBF AMLs) are also highly amenable to MRD monitoring by RT-qPCR-based methods, with the greatest prognostic value after consolidation therapy [29–31]. Patients with *CBFB/MYH11* fusions may particularly benefit from a molecular approach to MRD detection over MFC methods due to the myelomonocytic leukemia–associated immunophenotype of many of these cases, which may make blast identification by flow cytometric analysis difficult [32]. A prospective, non-randomized study was able to identify t(8;21) patients at high risk for relapse based on fusion transcript levels after second consolidation and demonstrated that these patients benefitted from allogeneic stem cell transplant when compared with additional chemotherapy, whereas low-risk patients had favorable outcomes with chemotherapy and autologous transplant [13].

Notably, using RT-qPCR as an MRD modality in CBF AML has two important limitations: (1) while very high and very low transcript levels strongly predict risk of relapse, intermediate levels inadequately stratify patients [32–36], and (2) a considerable number of patients in stable remission demonstrate persistent fusion transcript positivity [37–40]. These findings precluded the early widespread adoption of qualitative RT-qPCR approaches in CBF AML patients; however, subsequent studies established the value of RT-qPCR MRD monitoring with set fusion transcript level thresholds and with an emphasis on monitoring fusion transcript kinetics [29, 30, 33, 41, 42] in combination with multiparameter flow cytometry [32] for optimal prognostication. European LeukemiaNet guidelines recommend testing for CBF fusions in both blood and marrow specimens following induction therapy/prior to consolidation, at end of therapy/prior to allogeneic stem cell transplant, and at 3-month intervals for 2 years [12]. Of note, the interval between molecular relapse and morphologic recurrence is often very short in t(8;21) cases, prompting a suggestion that MRD status in the blood should be assessed at monthly intervals for at least the first year following end of treatment frequent intervals in these patients [42].

Acute myeloid leukemia with mutated NPM1

NPM1 frameshift mutations have been identified in a large percentage of AML patients with normal karyotype [43] and

are associated with good prognosis in the absence of *FLT3* internal tandem duplications (ITD) [44–49]. Following the mutation's discovery, several studies evaluated *NPM1* mutations as an MRD marker [50–54]. Although many of these early reports were able to predict impending hematologic relapse in patients based on suboptimal reduction in mutant transcripts after therapy or by detecting an increase in transcript levels on serial sampling, most were not adequately powered to demonstrate statistical differences in overall survival based on MRD status. More recent studies have been able to clearly demonstrate the value of *NPM1*-based mutational MRD testing in predicting risk of overt relapse and inferior patient outcomes [55, 56]. Most studies on *NPM1* as an MRD marker have assessed the peripheral blood; either blood, marrow, or both have been suggested as an appropriate specimen types [12, 28]. As with CBF AML, *NPM1* MRD studies are suggested after induction/prior to consolidation therapy, at end of therapy/prior to transplantation, and at 3-month intervals for 2 years [12].

The rationale for using *NPM1* as an MRD target stems from several observations. Notably, *NPM1* mutations are considerably more stable in relapse than are *FLT3*-ITDs, which are often also present in these patients [47, 50]. *NPM1* mutant allele transcripts are highly expressed in leukemic cells, allowing modern RT-qPCR methods to reach sensitivities of 1 in 10^6 or 10^7 [17]. *NPM1* is also appealing because almost all mutations are in exon 12, with approximately 90% limited to 3 specific mutations (types A, B, and D) [43, 55], making an allele-specific RT-qPCR detection strategy that captures the majority of mutations relatively straightforward (Fig. 1b). However, different *NPM1* MRD assays offer differing degrees of coverage for specific *NPM1* mutation types, and so determining that a *NPM1* MRD assay will identify an individual patient's *NPM1* mutation is paramount before it is used for post-therapy monitoring. Identification of an *NPM1* mutation through a traditional fragment-based assay is not sufficient for this determination, as most common *NPM1* mutations (types A, B, C, D) all constitute 4 base pair insertions that appear identical upon fragment analysis. A reasonable approach would be to test a diagnostic specimen with the MRD assay of interest or by confirming the specific *NPM1* mutation type through sequencing analysis. Given the frequent monocytic phenotype of *NPM1*-mutated AML, molecular MRD testing also has advantages over more conventional MFC-based MRD approaches (Fig. 2). The ability to identify patients with inferior outcome in this category of generally good prognosis AML aids in determining which subset of patients will benefit from additional intervention, which has been shown to improve patient outcomes whether prompting transplant [57, 58] or additional chemotherapy [16]. While the great majority of relapses in patients with *NPM1*-mutated AML also harbor *NPM1* mutations, *NPM1*-wild-type relapses can occur, possibly stemming from a preleukemic clone of hematopoietic stem cells [59]. This finding suggests that MFC MRD approaches or broader molecular MRD approaches using NGS (discussed below) may have value in this group of patients, in addition to monitoring for *NPM1* mutations.

Additional MRD targets amenable to qPCR

Many other genetic anomalies are recurrently noted in AML [60], and these lesions may also serve as potential candidates for MRD monitoring. Translocations involving *KMT2A* (also known as *MLL*) on chromosome 11q are common in ALL and can serve as targets in ALL MRD detection [61]. Although less common in AML, MRD assessment of *KMT2A* fusion transcripts has been described in several small studies and seems to have some empiric prognostic value, particularly for predicting long-term remission when transcript levels remain low [62–65]. Several other reports have also demonstrated validity in using *KMT2A* partial tandem duplications (PTD) [66, 67], t(6;9) *DEK/NUP214* [68], t(1;22) *RBM15/MRTFA* [69, 70], and t(7;12) *MNX1-ETV6* [71] as MRD strategies. Logically, targeting t(9;22) in cases of *BCR/ABL1* positive AML (a provisional entity in the current WHO classification) [60] is also feasible as it is widely used in CML MRD assessment, but its clinical utility in this rare subtype of AML remains to be elucidated. While these targets are theoretically amenable to use as MRD markers, their scarcity and the complexity of clinical assay validation and maintenance have largely limited their availability as clinical tests.

Other theoretic targets, such as *FLT3*-ITD and *CEBPA* mutations, are not generally considered to be reliable MRD candidates. *FLT3*-ITDs commonly arise in a subclone of blasts, and while they are often present at relapse, *FLT3*-ITD-negative relapses sometimes occur in patients with AML that harbored *FLT3*-ITD at diagnosis [72, 73]. Additionally, conventional fragment analysis methods used to determine *FLT3* status lack the analytic sensitivity generally required for MRD analysis. However, the identification of *FLT3*-ITDs following therapy may have prognostic value [58, 72], particularly in the era of therapeutic *FLT3* inhibition, and informatics methods to more reliably identify *FLT3*-ITDs through NGS approaches have been developed [74]. *CEBPA* is a notoriously difficult gene to assess, as it is highly GC-rich and difficult to sequence by NGS techniques [75]. *CEBPA* mutations are relatively heterogeneous without hotspots, making development of targeted MRD assays difficult.

Next-generation sequencing

Current consensus guidelines do not include NGS as a tool for assessing MRD. However, NGS approaches are likely to become increasingly important in monitoring AML patients

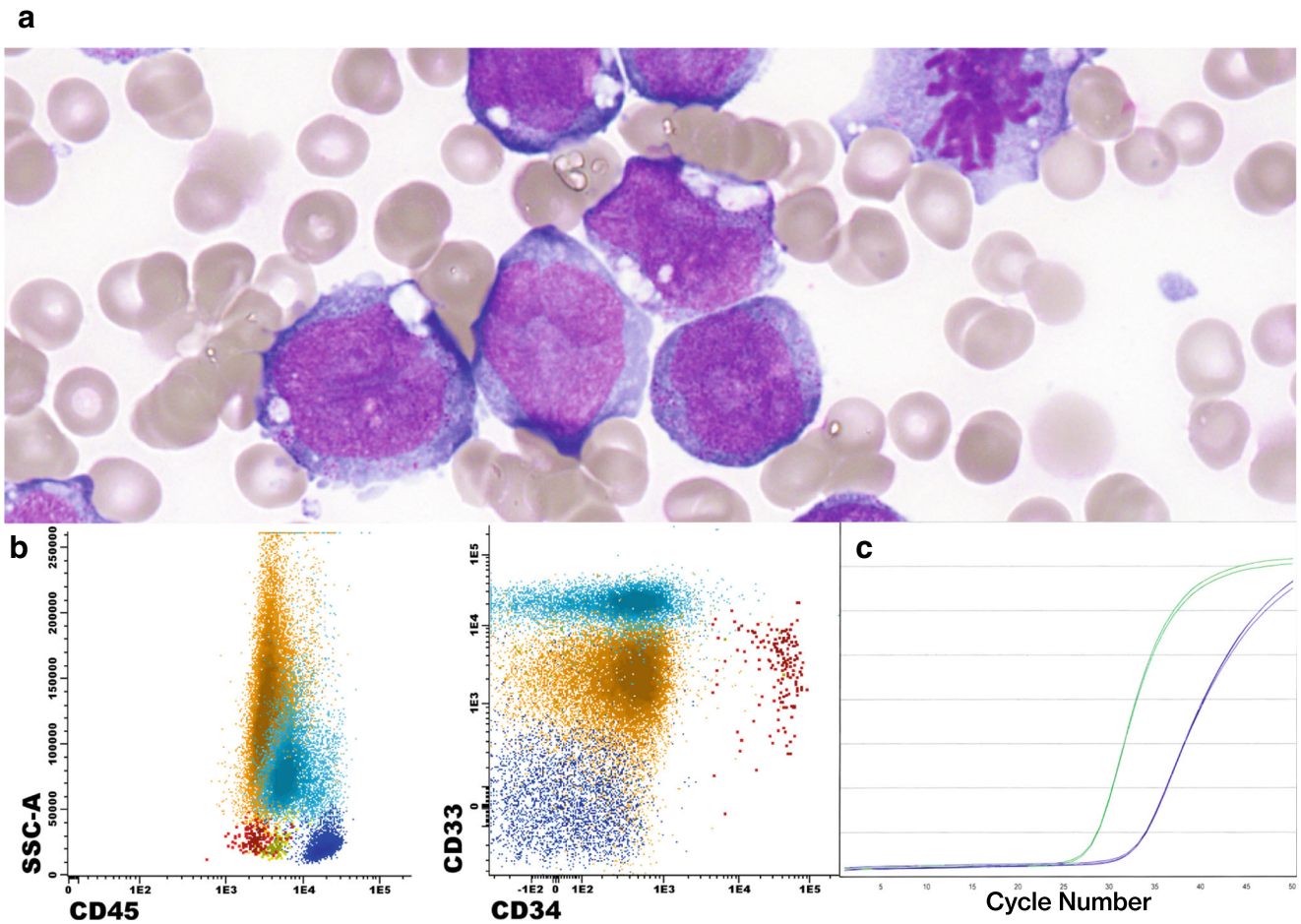

Fig. 2 Phenotypic measurable residual disease approaches may be limited in *NPM1*-mutated acute myeloid leukemia. **a** An example of an *NPM1*-mutated acute myeloid leukemia is shown, with numerous monoblasts and promonocytes (Wright-Giemsa, × 1000). However, flow cytometric analysis (**b**) demonstrates an expansion of CD34-negative cells with monocytic antigen expression (light blue) that are not reliably distinguished from monocytes by flow cytometry. This phenotype would be difficult to impossible to detect in the MRD setting by multiparameter flow cytometry, but quantitative *NPM1* mutation testing would demonstrate the presence of persistent disease. **c** Representative image of quantitative *NPM1* testing showing a positive result with a quantitative allele-specific RT-PCR assay. The positive *NPM1* result is represented by the blue curve; an internal housekeeping gene control, *ABL1*, is represented by the green curve

following therapy. NGS methods are able to provide real-time sequencing data on thousands upon thousands of DNA strands as they are extended base-by-base in a massively parallel fashion. The result is nucleotide-level sequencing data on numerous, overlapping DNA strands that, when oriented by computational informatics software, can be overlaid to determine the sequences of long stretches of DNA with far greater speed and efficiency than prior PCR methods or Sanger sequencing (Fig. 1c). An important aspect of NGS input is target enrichment, in which numerous copies of the same region of DNA are captured and amplified prior to sequencing. Parallel sequencing of many copies of the same DNA region allows for statistical assurance of the accuracy of the sequence and, in samples with sequence heterogeneity (e.g., samples containing a mixture of non-neoplastic cells and clonally related neoplastic cells), parallel sequencing facilitates the identification of mutations and the quantification of mutational burden expressed as a variant allele frequency (VAF).

The ability to assess the mutational status and VAF of a multitude of genes makes NGS a potentially valuable tool for MRD assessment in biologically diverse entities such as AML. NGS platforms could offer utility as an MRD platform for a substantially larger percentage of AML patients when compared with conventional qPCR methods. Data supporting the use of NGS panels as an MRD modality are sparse but encouraging thus far. Recent large-scale evaluations have demonstrated that 89–93% of AML patients have at least 1 mutation that can serve as a potential marker of residual disease and that persistence of NGS-detected mutations post-therapy is a poor prognostic indicator [76, 77]. Zhou et al. also demonstrated that patients meeting at least 1 criterion (pre-therapy mutations in *CEBPA* (monoallelic), *CSF3R*, or *NRAS*, or post-therapy MRD positivity on a 34-gene panel) had a higher risk of relapse and decreased overall survival and benefitted from transplant [78]. The results of an ongoing clinical trial (NCT02756962), designed to assess the value

of treating AML patients based on NGS-derived MRD status, are expected to highlight the clinical utility of this approach.

However, the main limitation of NGS is its relatively poor limit of detection, with most current clinical platforms only reliably identifying variant alleles at frequencies of 1–2% [79], and many clinical laboratories institute even higher, more conservative thresholds for mutation calls, at around 5%. The background sequencing error rate is highly dependent upon the sequence context, and individual, variant-specific error profiles would need to be generated or error-correction approaches applied to drive the limit of detection lower [76, 80]. The limit of detection by NGS is also dependent on the nature of the mutation in question, as small insertion/deletion mutations could theoretically be identified at lower VAFs than are single nucleotide variants, due to the underlying error profiles of sequencing. As current guidelines for MRD negativity require an assay with a limit of detection of 0.1% or lower [12], conventional NGS approaches generally cannot meet this target. However, these limitations have not prevented some recent studies from demonstrating that MRD assessment by NGS has prognostic utility [76, 77], with the caveat that negativity using conventional NGS platforms should not be accepted as definitive evidence of MRD-negative status [81].

The significance of specific mutations as MRD markers in AML is an area of intense study that remains to be fully understood. This difficulty is at least in part due to heterogeneity in the mutational landscape among patients, making detailed statistical analyses difficult. Some investigators have partially circumvented this limitation by assessing the cumulative prognostic value of genes in shared pathways [77, 82] or by performing subanalyses after removing single or groups of genes from the statistical analysis [76, 77]. These strategies have shed some light on genes that may serve as reliable MRD candidates. Using this approach, and based on observations that these mutations are often cleared in patients that experience sustained remissions, *FLT3* (both *FLT3*-ITD and *FLT3* tyrosine kinase domain mutations), *NRAS*, *KRAS*, *PTPN11*, and *KIT* appear to be promising MRD candidates. Several small studies have also highlighted value in using *IDH1*/*IDH2* mutations as MRD targets, either via NGS [83, 84] or digital droplet PCR [85].

The prognostic implications of other mutations, particularly founder-type mutations that are strongly associated with clonal hematopoiesis of indeterminate potential (CHIP), are more difficult to discern, a problem that was recently extensively reviewed by Hasserjian and colleagues [81]. Clones with mutations in *DNMT3A*, *TET2*, and *ASXL1* (collectively referred to as "DTA mutations") often persist or expand after AML-directed chemotherapy, as they are relatively chemoresistant [86]. The proposed relevance of DTA mutations in MRD assessment differs in various studies. Hirsch et al. noted that the persistence of CHIP lesions did not impact prognosis when assessed individually, but patients with ≥2 persistent mutations (presumably including CHIP lesions) did have poorer outcomes [82]. In contrast, several recent studies identified no prognostic value in persistent DTA mutations [76, 77]. Mutations in several non-DTA genes (e.g., *BCOR* and *SRSF2*) are also thought to be related to underlying CHIP, although these genes are less commonly encountered in AML and have not been assessed as rigorously [76, 81, 87].

Mutations in other genes, including *TP53* and *RUNX1*, may have MRD utility in select patients but should be interpreted with caution. *TP53* mutations are often a component of CHIP and thus lack clear-cut utility as an MRD marker [81]. However, excluding *TP53* mutations from MRD statistical assessments weakened the prognostic value of mutation clearance in one analysis [77], and relapses appeared to occur in the majority of a small number of patients with persistent TP53 mutations post-therapy [82, 87]. Although *RUNX1* mutations are a common finding in AML and define a new provisional diagnostic category in the current WHO classification [60], their utility as MRD targets may be limited due to their frequent presence in CHIP [81]. However, at least one study has demonstrated some value in detecting *RUNX1* mutations in the setting of MRD [88]. Systematic implementation of NGS as an MRD strategy may be helpful in clarifying some of these areas of uncertainty, but it is clear that not all mutations have equivalent clinical implications for the patient, markedly complicating interpretation of MRD testing performed by NGS.

Future directions

Recent reports demonstrating improved analytic sensitivity of NGS-based methods by optimizing various aspects of workflow and informatics [89] or by utilizing massively multiplex digital PCR with primers that preferentially amplify variant alleles over wild type sequences [90] indicate that clinical NGS testing may someday boast sensitivities comparable to PCR-based approaches. NGS is particularly well-suited for monitoring AML cases that undergo clonal or immunophenotypic evolution after therapy or assessing for the emergence of unrelated, possibly therapy-induced, AML clones [91], a phenomenon that can undermine MFC- and qPCR-based MRD strategies [53, 92]. The finding of an unrelated AML clone may have implications for clinical trial eligibility, and with the emergence of more tailored AML therapies, repeated genetic characterization of AML at follow-up and relapse will continue to increase in importance [81]. Novel NGS strategies are also amenable to the identification of recurrent fusions and other large gene aberrations, such as tandem duplications, possibly allowing for NGS assessment of MRD to encompass biomarkers that have been traditionally evaluated by RT-qPCR [93, 94]. With continued

technical advances, NGS platforms may allow molecular MRD testing to become closer to a one-size-fits-all approach, analogous to the current situation with MFC, rather than the more limited, individually tailored approach that is currently necessary and that is more likely to engender test ordering error and misinterpretation.

Conclusions and take-home points

MRD detection in AML is a challenging and dynamic endeavor, due to both advancements in our understanding of AML biology and new technological approaches. MFC modalities have typically been favored over PCR-based approaches due to their applicability in a greater percentage of patients. However, MFC assays are difficult to standardize and may be problematic for cases with certain phenotypes (e.g., myelomonocytic cases) and cases with immunophenotypic shift secondary to clonal evolution. Current guidelines emphasize that molecular MRD studies are indicated for patients whose AMLs harbor *PML/RARA, RUNX1/RUNX1T1, CBFB/MYH11,* or *NPM1* mutations, while MFC should serve as the primary MRD method for other AML subtypes [12]. For AML patients with less commonly encountered stable fusions, qPCR could also serve an empiric role in disease monitoring, but lack of widespread assay availability is a major limiting factor.

Newer NGS approaches are promising but are partially compromised by suboptimal analytic sensitivity and an incomplete understanding of the importance of specific mutations in the post-therapy setting. However, recent improvements in assay workflow and informatic processing may close the gap in sensitivity between NGS and qPCR. Even with a relatively poor limit of detection, several studies highlight the prognostic value of NGS as an MRD strategy, and an ongoing clinical trial will likely further strengthen our understanding of how MRD information can best be used to improve patient outcomes. MFC and MRD testing by NGS are orthogonal approaches that may best be used in parallel, as the combined data may best identify patients at risk for relapse [76]. A current practical limiting factor for the uptake of NGS MRD methods in AML relates to issues surrounding test reimbursement, as these studies may not currently be covered by payors despite the emerging data regarding clinical relevance [95].

MRD studies in AML patients are now suggested as part of the standard follow-up of the disease (Table 1). Molecular MRD studies are generally indicated following induction/prior to consolidation, at the end of treatment/prior to bone marrow transplant, and at 3-month intervals following end of therapy for patients with CBF AML, and AML with mutated *NPM1*, while APL patients should be monitored after consolidation and at 3-month intervals in high-risk patients (those with initial WBC counts > 10×10^9/L) [12, 27]. Other patients should be followed by MFC MRD studies; however, incorporation of NGS studies may also be reasonable, with the caveat that NGS negativity is not currently equivalent to MRD negativity, due to the suboptimal limit of detection of NGS.

Table 1 Summary of molecular MRD testing in AML [12, 26]

MRD studies should be considered following induction/prior to consolidation therapy, at the end of therapy/pre-transplant, and at 3-month intervals following therapy for most patients for 2 years*
DNA and RNA should be extracted from bone marrow and peripheral blood for molecular MRD testing at the timepoints indicated above.
Molecular RT-qPCR methods should be used for patients with *RUNX1/RUNX1T1, CBFB/MYH11, PML/RARA,* and *NPM1* mutations.
Patients without the above genetic lesions should be monitored by multiparameter flow cytometry performed on marrow.
Positive NGS results could have clinical importance in the follow-up setting, but not all mutations have equivalent prognostic significance. Negative NGS results cannot currently indicate absence of MRD.

Abbreviations: *MRD* measurable residual disease, *NGS* next-generation sequencing, *RT-qPCR* quantitative reverse-transcriptase polymerase chain reaction

*Patients with acute promyelocytic leukemia should be assessed post-consolidation, and low-risk acute promyelocytic leukemia patients may not need ongoing post-therapy monitoring if RT-qPCR negative

As follow-up testing for AML patients is complex and tailored to the patient's initial disease genetics, institutions should consider developing standardized workflows and procedures to facilitate testing and to avoid inappropriate test usage. Quantitative molecular MRD studies require RNA as a template, and so laboratories should establish procedures to extract both DNA and RNA from bone marrow specimens from patients with AML. Hematopathologists should consider reaching consensus with local molecular pathology and clinical colleagues to determine the appropriate use of available myeloid NGS testing in the follow-up setting, and pathologists should remain abreast of changing guidelines and technologies in this rapidly evolving field.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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