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Leukemia (PH Wiernik, Section Editor)

Clinical Use of Measurable Residual Disease in Acute Myeloid Leukemia

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Opinion statement

Treatment of acute myeloid leukemia (AML) remains a high-risk venture for the patient suffering from the disease. There is a real risk of succumbing to the treatment rather than the disease, and even so, cure is much less than certain. Since the establishment of complete remission as a prerequisite for cure in the 1960s, a number of years passed before advanced techniques for detecting minute amounts of disease matured sufficiently for clinical implementation. The two main techniques for detection of measurable residual disease (MRD) remain qPCR and multicolor flow cytometry. When performed in expert laboratories, both these modalities offer treating physicians excellent opportunity to follow the amount of residual disease upon treatment and offer unparalleled prognostication. In some AML and age group subsets, evidence now exist to support the choice of both proceeding to allogeneic transplant and not doing so. In other AML subgroups, MRD has sufficient discriminative power to identify patients likely to benefit from allogeneic transplant and patients likely not to. After treatment or transplantation, follow-up by molecular techniques can, with high certainty, predict relapse months before bone marrow function deterioration. On the other hand, options upon so-called molecular relapse are less well tested but recent evidence supports the use of azacitidine both in transplanted patients and patients consolidated with chemotherapy. In conclusion, MRD testing during treatment is a superb prognosticator and a major tool when choosing whether a patient should be transplanted or not. The exact use of MRD testing after treatment is less well defined but evidence is mounting for the instigation of treatment upon rising MRD levels (pre-emptive treatment) before morphologically detectable relapse.

Introduction

Acute myeloid leukemia (AML) is a heterogeneous and highly aggressive malignancy and the most frequent acute leukemia among adults. Despite an ever-increasing understanding of the complex pathophysiology, treatment has not changed substantially over the past decades and outcome remains unsatisfactory with a median overall survival of less than 50% for patients treated with intensive chemotherapy. Most patients treated with cytarabine and anthracycline–based induction regimens achieve complete remission (CR) by cytomorphology as defined by < 5% myeloblasts in the bone marrow (BM), but relapse rates remain disappointingly high, which is caused by outgrowth of residual leukemia [1], which can be quantified by other methods than cytomorphology and is referred to as measurable residual disease (MRD).

Estimation of post-treatment risk of relapse includes patient-specific risk factors, such as age and comorbidity, and disease-specific risk factors including cytogenetics and molecular aberrations. In particular, the incorporation of molecular aberrations has refined disease-specific risk stratification schemas over the past few years [2••, 3•]. Here, pre-treatment prognosticators correlate with depth of remission and presence of residual leukemia following treatment. However, risk stratification remains challenging, not least in normal karyotype and elderly AML patients, due to limitations in individual applicability. Similarly, some good-risk patients perform poorly and vice versa.

Residual leukemia following any cycle of therapy may represent the sum of all resistance mechanisms (both patient-specific and disease-specific factors), and quantification of residual leukemia below the threshold of standard cytomorphology evaluation is a strong, independent prognostic marker of relapse risk, during and after therapy [4, 5]. Accordingly, it is well established that failure to achieve an MRD-negative CR or significant increase in MRD levels at any time point during or after treatment and irrespective of hematopoietic stem cell transplantation is strongly associated with dismal outcome [6, 7]. On the contrary, some intermediate risk patients, who become MRD negative after induction chemotherapy, will not benefit from allogeneic stem cell transplantation because of a low post-treatment risk of relapse [8].

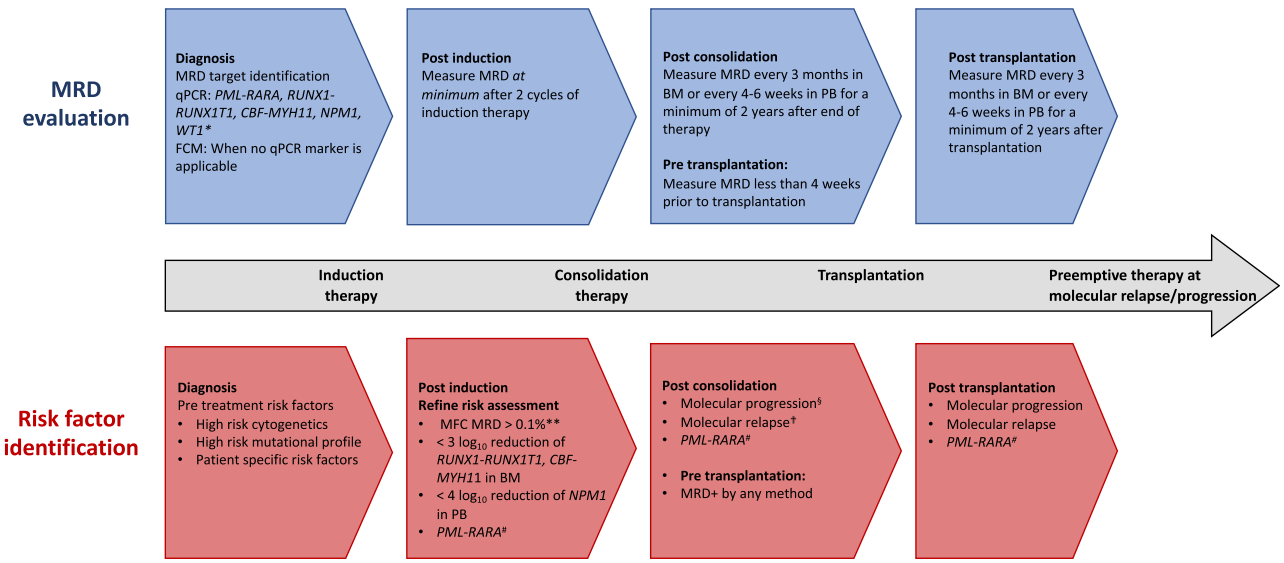

Fig. 1. MRD evaluation during treatment of AML patients according to ELN guidelines [9]. *WT1 should only be applied when no other marker is available. **MFC MRD tests with quantified MRD below 0.1% may still be consistent with residual disease. #PML-RARA: Conversion from undetectable to detectable is consistent with relapse . MRD negativity should be obtained after consolidation therapy. MRD monitoring can be ceased after achievement of MRD negativity in low-risk patients. §Molecular progression: increase of persistent molecular low copy numbers of ≥ 1 $\log_{10}$ between any 2 positive samples. †Molecular relapse: conversion of previously tested MRD negativity to an increase of ≥ 1 $\log_{10}$ between 2 positive samples.

The mounting acceptance and growing clinical use of MRD monitoring has led the European LeukemiaNet (ELN) MRD Working Party to recommend MRD testing in routine monitoring of AML patients, and the ELN AML Working Group has refined CR criteria by inclusion of MRD [2••, 9••]. While the clinical use of MRD assessment has been solidified in APL, in *NPM1*+ AML and core-binding factor leukemia (t(8;21)+ and inv(16)+), some challenges persist. Standardization and harmonization of MRD methods and prospective evaluation of MRD intervention in randomized clinical studies are highly needed [10•].

In this review, we will aim at summarizing the current clinical status of MRD surveillance in AML after induction and consolidation, the MRD detection before allogeneic stem cell transplantation, and the use of MRD measurements as a follow-up tool after chemotherapy or transplantation (Fig. 1).

MRD quantification methods

Despite the fact that achievement of CR by cytomorphology is a prerequisite for cure, it has long been recognized that such response evaluation is limited by poor sensitivity, quality, and considerable interobserver variability. By multiparameter flow cytometry (MFC) and real-time quantitative polymerase chain reaction (qPCR), residual disease can be identified down to levels of $1:10^4$ to $1:10^6$ contrasting cytomorphology levels of ~ 1:20. Presence of any residual leukemia following treatment as measured by either MFC or qPCR entail a worse prognosis [7, 9••, 10•, 11]. By FCM, more than 90% of AML patients can be evaluated for MRD status and qPCR covers about 25–30% of patients. Lack of standardized MRD techniques, genetic heterogeneity, and lack of universal antigenic leukemia markers deteriorate current MRD assessment by qPCR and MFC. New techniques (NGS and digital PCR) are fast gaining ground and will, undoubtedly, influence future MRD assessment in clinical use. For a more thorough review on MRD assessment techniques, the reader is kindly referred to the recent consensus paper by the ELN MRD Working Party [9••].

Multicolor flow cytometry

It is well documented that detection of residual leukemia by MFC is an independent prognostic factor for relapse, relapse-free survival (RFS), and overall survival (OS) [12, 13]. To define the best MFC MRD immunophenotype, a combination of LAIP-based and different-from-normal-based approaches using at minimum 8 colors is recommended to obtain satisfactory specificity of aberrancies. Single-tube quantification of leukemic stem cell burden also seems as a promising strategy [14]. The advantages of MFC pertain to wide applicability, short turn-over time and high specificity, whereas the method is limited by lack of interlaboratory harmonization and standardization, interpreter variability, and variable sensitivity. In particular, detectable MRD below 0.1% needs further study for clinical translation.

qPCR

Real-time quantitative polymerase chain reaction is the preferred method of MRD assessment in AML since it is more reliable and sensitive and, to a larger extent, has been standardized. The drawback is its limited applicability covering 25–30% of patients. The leukemia-associated chimeric fusion genes *PML-*

RARA, *RUNX1-RUNX1T1*, *CBFB-MYH11*, and mutant *NPM1* are by far the best-validated MRD markers and transcript levels importantly inform therapy. Overexpression of *WT1* is informative in selected cases but should be used only when no other alternative is available because of lower sensitivity and specificity.

Possible consequences of treatment efficacy evaluation

In patients receiving intensive chemotherapeutic treatment, information on prognosis could, in theory, be used to both intensify and de-escalate treatment (Fig. 2).

In younger fit patients, excellent MRD results could, in theory, provide the basis for de-escalation whereas inadequate MRD response could result in treatment escalation even in patients who have achieved morphological complete remission.

In either younger patients with significant comorbidities or older, fit patients, a satisfactory MRD result could entice the treating physician to follow up

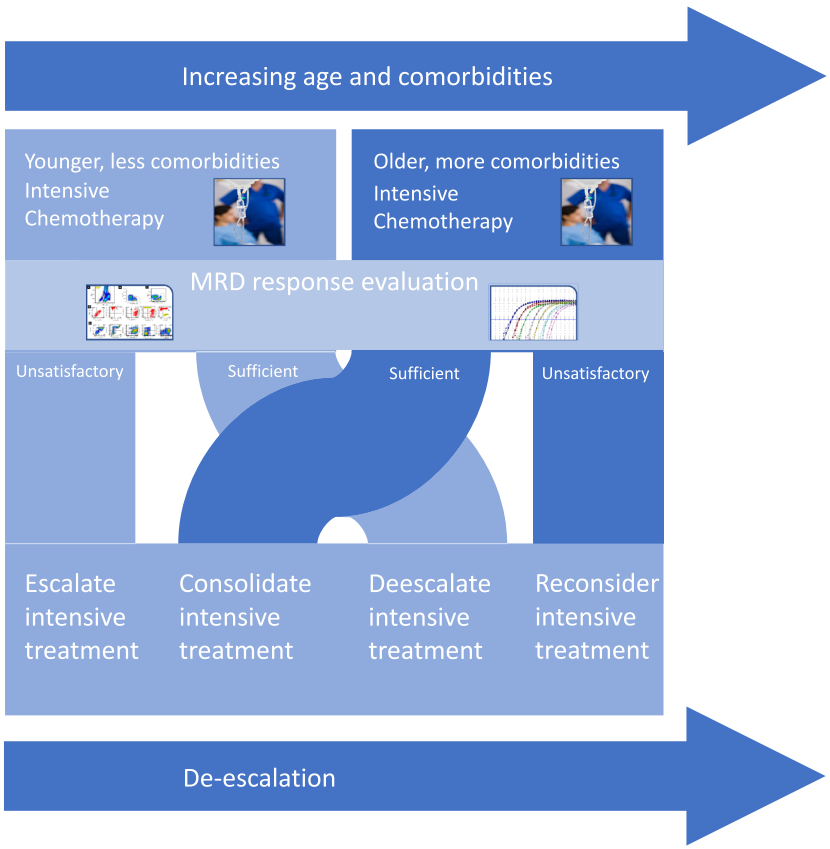

Fig. 2. Treatment strategy in different patient groups depending on age, comorbidities, and MRD assessment**.** Depending on whether the MRD response is unsatisfactory or sufficient, younger, more fit, patients can be either dose escalated or de-escalated, respectively. In older patients, less fit, the reaction to MRD response is opposite; an unsatisfactory response could lead to de-escalation in the form of reconsideration of whether the patient can be treated with curative intent. A satisfactory MRD response could lead to consolidation with further intensive treatment.

on the success with further intensive treatment, possibly a transplant, whereas a disappointing MRD result would force both patient and physician to re-evaluate if an intensive, potentially curative treatment really would be the best option for the patient.

In the following, we will evaluate the options for treatment escalation and de-escalation in various patient and AML subsets and consider the evidence, or lack of evidence, for different strategies.

To transplant or not to transplant

In AML, the classical escalation strategy would be to proceed to allogeneic transplant. However, in parallel with increasing rates of AML patients receiving allogeneic transplantation, a satisfactory MRD result could equally well allow for de-escalation such as choosing not to transplant [8]. Balsat et al. were able to show that patients aged 18–60, *NPM1*+ and *FLT3*-ITD+, did benefit from an allogeneic transplantation in first remission if a *NPM1* MRD reduction of less than 4 $\log_{10}$ in peripheral blood (PB) was observed. If a higher than 4 $\log_{10}$ reduction was observed, no benefit from first CR transplant could be detected for these patients [15••]. Similarly, Zhu et al. [16] showed that, in *RUNX1-RUNX1T1*-translocated AML patients, aged 14–60 years, an insufficient MRD response (less than 3 $\log_{10}$ reduction) benefited from transplant in first CR, whereas patients with a good MRD response (more than 3 $\log_{10}$ reduction) did worse when transplanted in first CR. Note that, while the studies produce concomitant results, the threshold for a satisfactory response is different (4 $\log_{10}$ reduction for *NPM1*+ *FLT3*-ITD+ AML, 3 $\log_{10}$ for *RUNX1-RUNX1T1*-translocated AML). How can these results then be transferred to other age groups and AML subtypes? Evidently, not without loss of evidence. As demonstrated, the relapse kinetics for different AML subtypes is shown to be varying, hampering generalizability. Generalization across age groups will be equally difficult. The two studies cover only younger AML patients. While the genotypes included in the two papers do appear in older patients, they more rarely do so, and when they do, with less positive prognostic impact [17–19].

Apart from the abovementioned studies, Freeman et al. [20••] recently found a trend towards transplantation being better in intermediate risk, NPM1- patients, FCM MRD+, but a trend against transplantation when the same patient where FCM MRD-. These studies represent the only formal comparisons of transplant success based on MRD that has been published. In general, in eligible patients with a relapse risk above 35–40%, allogeneic transplant can be considered, even if this number should probably be modified based on transplant risk factors [8]. As such, to have clinical impact, MRD measurements should identify patients with a relapse risk lower than this number. In younger (below age of 60) *NPM1*+ AML patients, it was demonstrated by Krönke et al. [21] that *NPM1* negativity in BM after 2 courses of chemotherapy yielded a relapse rate as low as 6%. This was achieved by only 19% of patients but evidently, these have an excellent prognosis. Ivey et al. [22••] similarly tested younger *NPM1*+ AML but focused on *NPM1* levels in PB. Here, 164/194 patients (85%) tested negative after second course of chemotherapy with a relapse rate of 35%. Interestingly, this was irrespective of *FLT3*-ITD mutational status. Relapse rates were 35% in both *FLT3*-ITD+ and *FLT3*-

ITD- patients. *DNMT3A* status did influence the relapse risk; however, *DNMT3A*+, MRD- patients relapsed in 52% of cases compared to 29% of *DNMT3A*+ MRD- cases.

A transplant strategy in younger patients

Thus, a transplant strategy for young patients with *NPM1* mutation materializes. Based on the Balsat et al. study [15••], transplant should be avoided in *NPM1*+ and *FLT3*-ITD+ patients who experience a 4 $\log_{10}$ *NPM1* reduction after the first course of chemotherapy. In an extrapolation from this study, patients with a similar excellent outcome after the first course of chemotherapy but *NPM1*+*FLT3*-ITD- probably should not be transplanted. At a lower level of evidence, employing the 35–40% relapse rate as a guide to whom to transplant, patients who become *NPM1*- in BM after first course of chemotherapy probably should not be transplanted either. In patients who are *NPM1*+ in BM, patients who are *NPM1*- in PB transplant should be considered mainly in cases harboring the *DNMT3A* mutation.

In younger (below the age of 60) patients with *RUNX1-RUNX1T1* or *CBFB-MYH11*, transplant is generally advised against [2••]. Data from Yin et al. [23] suggests that bad MRD responders could be identified applying thresholds of an absolute transcript count of 100 for *RUNX1-RUNX1T1* and 10 for *CBFB-MYH11*. This is in conjunction with the finding of Zhu et al. [16], and thus, poor MRD responders in these AML subgroups probably could be considered candidates for transplantation.

In a study worth mentioning, Terwijn et al. [24] found a relapse rate of 40% if patients were MRD- by MFC after course 2 in a combined cohort of good, intermediate and adverse risk patients. The inclusion of good risk patients as well as *NPM1*+*FLT3*-ITD+ patients in the cohort could suggest that, if these patients were removed, flow cytometric MRD assessment could be too insensitive to detect the patients with a relapse risk lower than 35–40%. However, Freeman et al. [20••] studied the intermediate risk *NPM1*- patients, and, as mentioned above found, a trend towards MFC discriminating patients benefiting and not benefiting from transplantation even if the relapse rate in this study in the MRD- patients was 50%. Thus, at least in intermediate risk, *NPM1*- patients, MFC assessment of MRD is useful in determining whether patients should be transplanted.

It remains to be seen if a small subset of adverse risk patientsthat can avoid transplant can be identified by MRD. These patients will be bound to be rare and probably only detectable in studies performed by large cooperative research groups. For now, transplantation seems the only curative option for these patients [2••].

Clinical utility of MRD determinations in older patients

The technical challenges of determining MRD in older patients have recently been reviewed [25••]. Apart from these, the major problem in applying the use of MRD techniques in patients above the age of 60 is the lack of clinical studies in this patient cohort. Both Freeman et al. [26] and Buccisano et al. [27] studied the use of MFC to distinguish prognosis in intensively treated patients above the

age of 60. In both cases, relapse rates in MRD- patients were somewhat higher (50–70%) than the 35–40% used as the discriminator for whether to proceed to an allogeneic transplant in patients in CR. Indeed, the relapse rate in MRD+ patients are discouraging (80–90%) Thus, in order to save the patient side effects and futile treatment, careful deliberation prior to proceeding to transplant in this group is warranted. Indeed, Liu et al. [28••] recently found that in (young; age 5–61) patients undergoing a matched sibling donor transplant, MFC-based MRD positivity prior to transplant resulted in a relapse rate of 67%. Results are unlikely to be better in the elderly transplanted with flow cytometric MRD+ disease.

On the other hand, if achieving MFC MRD negativity, only 12% in the Liu cohort relapsed after matched sibling donor transplant. If this number can be extrapolated to older patients remains unknown, the higher relapse rates of AML in older patients taken into account, but, even if relapse is twice as high in the older cohort, transplantation would be worthwhile in patients who achieve MRD negativity.

The importance of pre-transplant MRD negativity

"For whosoever hath, to him shall be given, and he shall have abundance; but whosoever hath not, from him shall be taken even that which he hath" (Matt 13:12)

The clinical challenge is not whether to transplant in molecular/flowcytometric CR or not. It is rather how to help patients who have not attained MRD negativity and from whom even the attained morphological CR is quite likely to be taken. It is well documented that it is of paramount importance to be MRD- before proceeding to allogeneic transplant [28••, 29]. However, in patients in CR with MRD persistence, the best treatment option will often be transplantation, except perhaps in patients with high comorbidity scores and very fragile remissions where even transplantation cannot be thought to bring a durable cure. This probably reflects the biology of leukemias who do not go into MFC or molecular remission despite several chemotherapeutic attempts to achieve this. It may also, however, represent an inability of the GvL effect of harnessing higher loads of leukemia; continuous low-level MRD positivity in transplanted patients seems to suggest this [30] as well as the fact that pre-emptive treatment (discussed below) post-transplantation seems to yield impressive results [31••]. Finally, it may simply represent the mathematical fact that, if leukemia burden is high, relapse occurs earlier, in this case before the GvL effect is established.

The correct action for achieving MRD negativity prior to transplant is not known. Based on the considerations above, treatment intensification is tempting, especially in younger patients where more intensive treatment is better tolerated. In the elderly, the lower relapse rate of a lower MRD level before transplantation may not be worth the higher mortality of the increased chemotherapy dosage. This is especially true if the lower response rates of MRD+ leukemias are more because of the biological phenomena of treatment-resistant leukemias being resistant to GvL as well rather than a simple mathematical reason of a too high tumor burden. While no evidence exists on the subject, it is currently under investigation in the UK NCRI AML18 where older (60 + years)

patients MRD+ by MFC is randomly assigned to either another course of standard-dose cytarabine and anthracycline or a combination of fludarabine, high-dose cytarabine, and anthracycline.

Treating molecular relapse: has the time for pre-emptive treatment come?

It is intuitively correct to treat leukemia relapse earlier rather than later, and, since the dawn of MRD diagnostics, pre-emptive treatment of nascent relapse has been discussed [32]. While MRD technology has been extensively tested and indeed perfected during the last 20 years and while treatment efficacy evaluation using MRD is increasingly being adopted in routine AML handling, the evidence for action upon a positive MRD determination during post-treatment leukemia surveillance is still lacking. Early concerns whether a molecular relapse was always followed by a morphological relapse has been proven unfounded; if using the right algorithms of diagnosing molecular relapse, it is inevitably followed by full-blown leukemia [21, 23, 30, 33–36]. Few randomized studies exist in this setting, and, indeed, many patients would not want to be included in the randomization of whether to treat or not when it is known that the leukemia is relapsing even if BM is still functional.

Pre-emptive treatment using intensive chemotherapy

Given the toxicities of relapse treatment, some patients will die from pre-emptive treatment months earlier than they would from a relapse of their leukemia. On the other hands, even in the age of prophylactic antifungal treatment, resistant neutropenic fever remains a major challenge only solvable when BM function regenerates. Starting treatment at a point where BM and neutrophil function is still adequate probably shortens time to neutrophil regeneration and lessens treatment onset systemic fungal burden. The questions are addressed in the NCRI AML17 and AML19 studies were patients with applicable molecular MRD markers are randomized to either MRD surveillance or not. Results are still pending.

Pre-emptive treatment using non-intensive chemotherapy

Meanwhile, interesting data on pre-emptive treatment using non-intensive strategies exists. Platzbecker et al. [31••] recently published data on the RELAZA2 study where predominantly (62%) *NPM1*+ AML patient experienced molecular relapse either after chemotherapy or after transplantation. A total of 30% of patients did not harbor any mutations with impending relapse being diagnosed using chimerism of CD34 positive cells, an MRD detection method obviously only applicable in the post-transplantation setting [37]. In a phase 2 study, patients were treated with azacitidine upon molecular recurrence of *NPM1* levels to above 1% or decline in CD34 chimerism to below 80% without concomitant morphological relapse. The strategy worked in both transplanted and untransplanted patients, with a median follow-up time of 13 months, cumulative relapse risk at 1 year of

30% in transplanted and 50% in non-transplanted patients. More relapses occurred during the second year, especially in non-transplanted patients. Hematological relapse occurred in 51% of patients with time to morphological relapse of 422 days. This should be compared to the median time from molecular relapse to morphological relapse in a historical cohort, which is much shorter (30–120 days) [21, 34]. Especially in transplanted patients, where classical relapse reinduction is less well tolerated, this could be a sound strategy. In untransplanted patients, non-intensive treatment of molecular relapse could equally well be a good strategy, but whether azacitidine is a sufficient treatment to bridge to allogeneic transplant in younger patients and whether patients that fail azacitidine treatment of their molecular relapse can be salvaged using intensive chemotherapy remain to be seen.

Conclusion

Important progress in the implementation of the use of the MRD assessments in the clinic has been made. In Europe, MRD surveillance is recommended for use in routine clinical practice [8].

Especially in the intermediate risk group, recent advances have made the segregation of patients into those who will probably benefit from transplantation and those to whom chemotherapy consolidation may be an adequate treatment. Several questions remain unanswered, however. What is the right course of action in patients in CR who shows molecular progression during therapy? No evidence exists for choosing the right treatment escalation or de-escalation. What about pre-emptive treatment? The RELAZA2 study showed clinical benefit from pre-emptive treatment but when and in what form remains undecided. Some of these questions may be addressed by the monitor vs. no monitor study of the UK NCRI AML17 and 19 studies.

In conclusion, we envision that, as techniques are being refined and evidence for how to personalize treatment based on MRD measurements evolves, MRD monitoring will become increasingly important in individualized AML treatment.

Compliance with Ethical Standards

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

Human and Animal Rights and Informed Consent
This article does not contain any studies with human or animal subjects performed by any of the authors.

Publisher's Note

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