



How close are we to incorporating measurable residual disease into clinical practice for acute myeloid leukemia?

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ABSTRACT

Assessment of measurable residual disease, also called “minimal residual disease,” in patients with acute myeloid leukemia in morphological remission provides powerful prognostic information and complements pretreatment factors such as cytogenetics and genomic alterations. Based on data that low levels of persistent or recurrent residual leukemia are consistently associated with an increased risk of relapse and worse long-term outcomes, its routine assessment has been recommended by some experts and consensus guidelines. In addition to providing important prognostic information, the detection of measurable residual disease may also theoretically help to determine the optimal post-remission strategy for an individual patient. However, the full therapeutic implications of measurable residual disease are uncertain and thus controversy exists as to whether it should be routinely incorporated into clinical practice. While some evidence supports the use of allogeneic stem cell transplantation or hypomethylating agents for some subgroups of patients in morphological remission but with detectable residual leukemia, the appropriate use of this information in making clinical decisions remains largely speculative at present. To resolve this pressing clinical issue, several ongoing studies are evaluating measurable residual disease-directed treatments in acute myeloid leukemia and may lead to new, effective strategies for patients in these circumstances. This review examines the common technologies used in clinical practice and in the research setting to detect residual leukemia, the major clinical studies establishing the prognostic impact of measurable residual disease in acute myeloid leukemia, and the potential ways, both now and in the future, that such testing may rationally guide therapeutic decision-making.

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Introduction

Acute myeloid leukemia (AML) is a heterogeneous disease, with a widely variable likelihood of cure with conventional therapies that depends on both patient- and disease-related characteristics.¹ Historically, pretreatment characteristics such as the patients' age, karyotype, and, more recently, genetic mutations have been the primary determinants of prognosis in patients with newly diagnosed AML. How well the leukemia responds to initial treatment also provides important information about chemosensitivity of an individual's leukemia that cannot always be predicted from pretreatment characteristics. One measure of treatment response is the achievement of complete remission, defined as bone marrow with <5% blasts with recovery of peripheral blood elements and no evidence of extramedullary disease.² However, among patients <60 years of age who achieve complete remission with induction and then receive consolidation chemotherapy, the cure rate is only approximately 50% (and significantly lower in older patients), suggesting that morphological assessment of the bone marrow alone is inadequate to discriminate relapse risk.³ Accurate assessment of the risk of relapse is imperative in the treatment of AML, as the decision to pursue consolidation chemotherapy rather than allogeneic hematopoietic stem cell transplantation (HSCT) in first remission is largely decided by the anticipated risk of relapse in the absence of HSCT balanced against the risk of relapse after HSCT as well as the expected transplant-related

mortality.⁴ Although improvements in genomic classification of AML have refined our current risk stratification systems, relapses are unfortunately still common even with risk-adapted treatment.

Assessment of measurable residual disease (MRD), also called “minimal residual disease,” allows for the detection and quantification of lower levels of residual leukemia that can be detected by morphological assessment alone.^{5,6} In both pediatric and adult acute lymphoblastic leukemia, MRD is routinely used to refine prognostic assessment and also allocate post-remission therapies, particularly in the pediatric population.⁷⁻¹¹ Similarly, studies utilizing various methods of MRD determination have consistently shown that MRD is highly prognostic in AML and complements (and sometimes supersedes) historically relevant pretreatment characteristics.¹²⁻¹⁸ However, many questions remain as to the optimal MRD assay, appropriate timing of MRD assessment, and whether MRD should guide treatment. Herein, we review methods of MRD detection and the prognostic impact of MRD across AML subtypes. We also offer some practical considerations for MRD testing in the clinical setting and review the available data on how MRD can potentially guide post-remission treatment decisions in AML.

Methods of measurable residual disease assessment

Several methods of MRD assessment are available in the clinical and research settings, each of which has its own advantages and disadvantages. The European

LeukemiaNet MRD Working Party has released comprehensive, consensus recommendations regarding their appropriate use and technical limitations.⁶ These various MRD methods vary in their level of sensitivity, applicability to the vast majority of patients with AML *versus* only certain subtypes, cost, and level of technical expertise needed to obtain an accurate result, all of which may influence the choice of assay for a particular patient.¹⁹ The major differences among MRD technologies are summarized in Table 1.

Karyotype analysis and fluorescence *in situ* hybridization

In patients with an abnormal pretreatment karyotype, the persistence of an abnormal leukemia-associated karyotype at remission suggests the presence of residual disease. Persistent cytogenetic abnormalities have been associated with worse survival in several studies and may also identify patients who could benefit from HSCT in first remission.^{20,21} While cytogenetic analysis at remission adds prognostic information in patients who achieve complete remission, the sensitivity of this method is relatively poor, as it can only detect one abnormal metaphase out of 20 (i.e. sensitivity ≈5%). Furthermore, up to 50% of adult patients present with cytogenetically normal AML, further limiting karyotype analysis as a universal MRD marker.¹ Fluorescence *in situ* hybridization may also be used to detect persistent cytogenetic abnormalities with slightly increased sensitivity compared to conventional cytogenetics. However, the maximum sensitivity achieved with fluorescence *in situ* hybridization is approximately 1%, which is not adequate to detect low levels of clinically relevant MRD.²²

Table 1. Methods of measurable residual disease assessment in acute myeloid leukemia.

| Method | Sensitivity | Advantages | Disadvantages |
|--------------------------|--|---|---|
| Conventional karyotyping | ~5% | <ul style="list-style-type: none"> Common in routine clinical practice | <ul style="list-style-type: none"> Poor sensitivity Time-consuming and labor-intensive Applicable only to patients with baseline abnormal karyotype (~50%) |
| FISH | Up to 10 ² | <ul style="list-style-type: none"> Useful for numeric cytogenetic abnormalities (i.e. gains or deletions) | <ul style="list-style-type: none"> Worse sensitivity than MFC or PCR Applicable only to patients with baseline abnormal karyotype (~50%) |
| MFC for LAIP or DfN | 10 ³ to 10 ⁵ | <ul style="list-style-type: none"> Sensitive Fast (results usually available within 24 hours) Relatively inexpensive Applicable to >90% of AML cases | <ul style="list-style-type: none"> Potential for immunophenotypic shifts (mitigated by using a DfN-based approach) Requires significant technical expertise to interpret Limited standardization across laboratories |
| RT-qPCR | 10 ⁴ to 10 ⁶ | <ul style="list-style-type: none"> Sensitive Well standardized Can be run in any laboratory with RT-qPCR capabilities | <ul style="list-style-type: none"> Appropriate molecular targets present in <50% of cases (<35% in older adults) Many mutations are not suitable for MRD detection (e.g. <i>FLT3</i>) Time-consuming and labor-intensive Results may take several days |
| NGS | Highly variable (1% to 10 ⁶) | <ul style="list-style-type: none"> Potential for very high sensitivity (depending on technology) Can test multiple genes at once | <ul style="list-style-type: none"> Low sensitivity with most commonly used platforms May be confounded by persistence of preleukemic mutations (e.g. CHIP) Results may take several days Expensive Not standardized Requires complex bioinformatics |

FISH: fluorescence *in situ* hybridization; MFC: multiparameter flow cytometry; PCR: polymerase chain reaction; LAIP: leukemia-associated immunophenotypes; DfN: difference from normal; AML: acute myeloid leukemia; RT-qPCR: real-time quantitative polymerase chain reaction; NGS: next-generation sequencing; MRD: measurable residual disease; CHIP: clonal hematopoiesis of indeterminate potential.

Multiparameter flow cytometry

Multiparameter flow cytometry (MFC) uses a panel of fluorochrome-labeled monoclonal antibodies to identify aberrantly expressed antigens on leukemic blasts. This MFC-based MRD analysis may be accomplished through either the tracking of leukemia-associated immunophenotypes in the pretreatment and remission samples or the use of “difference from normal” analysis.²² Leukemia-associated immunophenotypes consist of the aberrant expression of antigens compared to that on normal myeloid precursors, cross-lineage antigen expression (e.g. expression of lymphoid antigens on myeloblasts), over- or under-expression of antigens normally expressed, and aberrant co-expression of antigens normally found in early or late hematopoietic differentiation.⁶ In contrast, the “difference from normal” approach is used to detect any differences in the remission immunophenotype compared to the highly stereotypical normal immunophenotype distribution.²³ The advantage of “difference from normal” analysis is that it does not require knowledge of the diagnostic immunophenotype; furthermore, it may also be less susceptible to immunophenotypic shifts that can occur as a direct result of therapy or due to a shift in clonal architecture.^{24,25} Workflows that incorporate both of these methods (i.e., a leukemia-associated immunophenotype-based, “difference from normal” approach) may help to further optimize MRD assessment.⁶ Some studies suggest that addition of leukemia stem cell markers (e.g. CLL1, CD44, CD123, and CD184, among others) to flow MRD antibody panels may add additional prognostic information to standard MFC-based MRD, particularly by identifying those patients at very high risk of relapse (i.e. those who are positive for both MRD and leukemia stem cell markers).²⁶⁻²⁸

MFC-based MRD assessment can achieve a sensitivity of 10^{-3} to 10^{-5} , which is dependent on the number of cells analyzed, gating method, and number of antibody colors used; in most cases, a sensitivity of 10^{-4} is achieved. Compared to real-time quantitative polymerase chain reaction (RT-qPCR), MFC is significantly faster and less labor-intensive. It also has the advantage of being applicable to more than 90% of patients with AML, unlike other methods that rely on specific genetic or molecular targets.²² Despite these advantages, the interpretation of MFC MRD is not standardized in most countries, including the USA, and requires significant technical expertise on the part of the interpreting pathologist, which can lead to inter-laboratory discordance. Because of the complexity of interpreting flow-based MRD, there is interest in the use of machine-based learning artificial intelligence to reduce the potential for bias or other subjective errors in MRD interpretation. Such artificial intelligence-based algorithms are promising and may result in more clinically significant MRD results, although further validation of this technology is needed.²⁹

Polymerase chain reaction

RT-qPCR can be used to monitor recurrent genomic alterations in certain subtypes of AML. To be a useful marker for PCR-based MRD assessment, the target gene fusion or mutation should be stable throughout the disease course and its presence should reflect true persistent disease (rather than preleukemic subclones). Suitable targets that have been evaluated in large studies include *PML-RARA* in acute promyelocytic leukemia, *CBFB-*

MYH11 and *RUNX1-RUNX1T1* in core-binding factor AML, and mutant *NPM1*.^{15,30-36} In contrast, other mutations may emerge or disappear at the time of relapse (e.g., mutant *FLT3*^{337,338}) and are therefore generally unreliable MRD markers for assessing meaningful “MRD negativity,” although their persistence likely represents residual disease in most cases. One disadvantage of PCR is that appropriate, validated targets are present in less than 50% of patients with AML, and the incidence of these AML subtypes declines substantially with increasing age at diagnosis.¹ To overcome this limitation, attempts have been made to track residual disease using markers that are expressed at significantly higher levels in leukemic blasts than in normal hematopoietic cells. For example, several studies have evaluated monitoring levels of *WT1* or *EVI1* mRNA transcripts over the course of treatment as a marker of MRD.³⁹⁻⁴² While these targets may provide some prognostic information, they are generally not specific enough for residual leukemia to be used in routine practice.⁶

PCR-based MRD assessment has the advantage of being highly sensitive (sensitivity $\approx 10^{-4}$ to 10^{-6} , depending on the input of RNA/DNA) and is generally better standardized than MFC.¹⁹ To further refine relapse risk, ultrasensitive digital droplet PCR technologies have been developed in order to detect low levels of residual gene mutations.⁴³ In contrast to standard PCR, digital droplet PCR does not require calibration standards, and is thus faster and more precise and reproducible.⁴⁴ Due to its absolute quantification of DNA copy numbers, it can also provide information about clonality and subclonality. This technology may have greater sensitivity and be better able to quantify very low levels of MRD than standard PCR²²; however, whether this added sensitivity will translate into more accurate prognostic discrimination is yet to be determined.

Next-generation sequencing

Targeted next-generation sequencing (NGS) panels are commonly used at the time of diagnosis to identify prognostic gene mutations or mutations that may be therapeutically targeted (e.g. *FLT3* or *IDH1/2* mutations). Several studies have also evaluated re-using similar NGS panels at the time of remission to assess the relationship between decline in mutational burden and clinical outcomes.^{17,45,46} This methodology relies on a similar principal as PCR regarding the tracking of genomic or molecular targets, although NGS is able to target multiple genes at once, or even the entire genome, if desired.

Although there is understandable excitement about the potential for high-throughput NGS-based MRD monitoring in AML, there are several practical considerations that limit its clinical use at present. NGS MRD assessment is relatively expensive, is not standardized, and requires complicated bioinformatics. The interpretation of NGS MRD results is further complicated by the presence of preleukemic clones that may not fully clear even in patients who achieve deep, long-term remissions with chemotherapy, such as mutations associated with clonal hematopoiesis of indeterminate potential (CHIP).⁴⁷⁻⁴⁹ CHIP mutations, particularly *DNMT3A*, *TET2*, and *ASXL1*, commonly persist in patients who do not relapse, suggesting that they should not be routinely used as MRD markers.^{17,46} Furthermore, at present, the sensitivity of NGS for MRD assessment is generally $\sim 1\%$ with most commonly

used platforms, due largely to the intrinsic error rate of sequencing. This lack of sensitivity is often because the assays are designed for identification of mutations in baseline samples (for which high sensitivity is generally not needed) and then repurposed for assessment of MRD (for which adequate sensitivity is imperative). However, depending on the NGS platform used and the amount of input DNA, NGS can theoretically achieve a sensitivity of 10^{-6} , making it an attractive potential option for very sensitive MRD detection. Advances in NGS technologies, including molecular barcoding and duplex sequencing, may improve the sensitivity of NGS and allow for the identification of very low levels of residual leukemia.^{50,51}

Prognostic Impact of measurable residual disease in acute myeloid leukemia

Achievement of MRD negativity has been shown to be a powerful prognostic factor in numerous studies of patients undergoing frontline AML therapy. Based on the consistent impact of MRD on long-term outcomes across multiple studies and AML subtypes, consensus guidelines from the European LeukemiaNet support “complete remission without MRD” as an official AML response criterion.² There are also ongoing efforts in the USA and elsewhere to validate MRD as a surrogate endpoint for accelerated regulatory approval of novel drugs and combinations. Here we review some of the major studies supporting the clinical use of MRD to provide prognostic and predictive information in AML.

Multiparameter flow cytometry-based measurable residual disease studies

MRD as assessed by MFC is highly prognostic when measured at various time points, including after induction, during and after consolidation, and peri-transplant.^{12,13,16,18,52-56} While studies have used varied cutoffs to define MRD “negativity,” many have used levels of $\leq 0.1\%$ to define negativity, both because this level of sensitivity can be reliably achieved with flow-based assays and because this level appears to provide the best discrimination for relapse risk at most time points.^{15,56} However, it is important to note that some studies have also suggested that even levels of residual leukemia below the 0.1% threshold may be associated with worse outcomes than lack of detectable MRD.^{12,53} Several studies in younger adults ≤ 60 years of age with newly diagnosed AML undergoing standard frontline chemotherapy have shown that achievement of MRD negativity is associated with a significantly lower risk of relapse and better survival.^{13,16,18} The impact of MRD status consistently adds additional prognostic information beyond that provided by pretreatment characteristics, such as cytogenetics and genetic mutations. Notably, most studies have evaluated MRD in younger adults and the data regarding the impact of MRD in patients >60 years are relatively scant. However, the available data suggest that achievement of MRD negativity in the older population may be predictive for lower relapse risk whether the patients are treated with intensive chemotherapy or hypomethylating agents.^{12,57} Because most older patients with AML will not be candidates for allogeneic HSCT, the potential therapeutic implications of persistent MRD in these patients is less clear than in their younger counterparts.

The National Cancer Research Institute AML17 trial is the largest study of MRD in AML to date and provides some key insights into the impact of MRD in AML.¹⁸ This study enrolled 1,874 adults <60 years of age with AML who received standard daunorubicin plus cytarabine-based induction, followed by risk-adapted chemotherapy consolidation, with or without allogeneic HSCT. Patients with MRD positivity (defined as $\geq 0.1\%$ by MFC assay) after cycle 1 had similar 5-year overall survival as patients who only achieved a partial response (51% vs. 46%, respectively), highlighting the poor outcomes associated with persistent MRD. Given the large size of this population, subgroup analyses were possible, including those among patients with standard-risk AML without *NPM1* mutations. Of note, this is a particularly important group to refine prognosis using MRD, since there is controversy as to whether standard-risk patients (as determined by pretreatment characteristics) should routinely undergo HSCT in first remission; it is also a population in which MFC-based MRD assessment plays an integral role given the absence of a reliable molecular MRD target (e.g. mutant *NPM1*).^{1,22} Among patients with standard-risk AML without *NPM1* mutation, the 5-year overall survival rates for patients who achieved MRD negativity after two cycles and for patients who remained MRD-positive were 63% and 33%, respectively ($P=0.003$), with the difference being driven by a significantly higher relapse rate in the MRD-positive group. Thus, MRD status after induction or in early consolidation may be able to stratify standard-risk patients according to risk of relapse and, consequently, help to determine the potential benefit from consolidative allogeneic HSCT.

Detectable MRD immediately prior to HSCT was also associated with increased risk of post-HSCT relapse and worse overall survival in several studies.⁵²⁻⁵⁵ These observations are further supported by a meta-analysis of 19 studies evaluating pre-HSCT MRD (the majority of which assessed MRD by MFC).⁵⁸ In one study of patients with AML who underwent HSCT, those in complete remission but with detectable MRD had a similar post-HSCT relapse rate and overall survival as those transplanted when not in morphological remission.⁵⁴ The 3-year overall survival rates for patients who were in complete remission but MRD-positive versus those with active AML at the time of HSCT were 26% and 23%, respectively, compared to 73% for patients who were MRD-negative. The impact of post-HSCT MRD status is, however, less clear.^{55,59} While patients with persistent or recurrent MRD after HSCT appear to have relatively poor outcomes, one analysis suggests that pre-HSCT MRD status carries relatively more prognostic information than post-HSCT MRD status.⁵⁵ In this study, only pre-HSCT MRD was independently associated with long-term outcomes. The 3-year overall survival rate for patients with pre-HSCT MRD that persisted after the transplant was 19% and only slightly higher for those with pre-HSCT MRD that cleared with transplantation (3-year overall survival rate: 29%).

Polymerase chain reaction-based measurable residual disease studies

The prognostic impact of MRD detected by PCR has been shown primarily in acute promyelocytic leukemia, core-binding factor leukemias, and *NPM1*-mutated AML, as the target alterations in these AML subtypes represent stable, foundational genomic lesions that become unde-

tectable in long-term survivors (with rare exceptions^{60,61}) and persist or re-emerge in the setting of relapsed disease.¹⁹ In acute promyelocytic leukemia, the persistence of *PML-RARA* transcripts strongly predicts for relapse, and patients with persistent transcripts invariably relapse without intervention.^{30,31,62} Among patients with acute promyelocytic leukemia treated with chemotherapy plus all-*trans* retinoic acid, initiation of arsenic trioxide for those with positive MRD prevents morphological relapse in the majority of cases.³¹ However, in this context, it is important to confirm a positive MRD assessment with repeat testing, given the potential for false positives. Consensus guidelines have therefore defined molecular remission as an important therapeutic milestone and recommend molecular assessment in routine clinical practice.⁶³ The link between MRD status and prognosis, as well as an effective salvage therapy for MRD-positive disease, makes acute promyelocytic leukemia a model for MRD-guided therapies, although it is unclear whether routine, universal monitoring is still required in the era of regimens capable of curing >95% of non-high-risk patients.^{64,65}

Core-binding factor AML accounts for 20-25% of cases of AML in younger patients, but <10% in older adults.^{2,66} PCR-based MRD assessment of *CBFB-MYH11* and *RUNX1-RUNX1T1* transcripts for patients with *inv(16)* and *t(8;21)*, respectively, has been consistently associated with risk of relapse across several studies.^{32,33,67-70} In the largest study of core-binding factor AML and MRD, 278 patients [163 with *t(8;21)* and 115 with *inv(16)*] were treated.³³ A >3 log reduction in *RUNX1-RUNX1T1* in the bone marrow and *CBFB-MYH11* copy number >10 in the peripheral blood after induction were the most prognostic measures of MRD for risk of relapse. Levels of MRD above these thresholds were associated with overall survival in the *t(8;21)* group but not in the *inv(16)* group, possibly due to better salvage options for patients with the latter subtype. Thresholds of MRD were identified that could predict relapse in 100% of patients in both groups and, notably, rising MRD levels on sequential samples predicted morphological relapse, suggesting a potential role for pre-emptive therapy in this population.

Monitoring of mutant *NPM1* throughout treatment may also provide important prognostic information in AML.^{15,34-36} Mutations in *NPM1* are present in approximately 30% of younger patients with AML and in up to 60% of cases of AML with normal karyotype.⁷¹ Like *PML-RARA*, *CBFB-MYH11* and *RUNX1-RUNX1T1*, these mutations are stable at diagnosis and relapse, making them ideal targets for molecular MRD assessment.⁷² In the largest study of *NPM1*-based MRD (the National Cancer Research Institute AML17 trial), 346 patients with *NPM1*-mutated AML received intensive chemotherapy and were monitored for mutant *NPM1* levels by PCR in the bone marrow and peripheral blood after each cycle of chemotherapy.¹⁵ With a median sensitivity of 10⁻⁵, MRD negativity in the peripheral blood after two cycles of chemotherapy was achieved by approximately 85% of patients and was the most prognostic MRD measure. Lack of achievement of this MRD milestone was the only independent prognostic factor for death in multivariate analysis [hazard ratio (HR) 4.84; 95% confidence interval (95% CI): 2.57-9.15; *P*<0.001]. When MRD was assessed in the peripheral blood at this time point, the 3-year overall survival rate was 24% for those who were MRD-positive and

75% for those who were MRD-negative (*P*<0.001). Similar to the experience with acute promyelocytic leukemia and core-binding factor AML, rising levels of mutant *NPM1* transcripts on sequential testing reliably predicted relapse. In a separate study, patients with *NPM1* transcripts detectable by PCR immediately prior to HSCT had significantly worse 5-year overall survival rates than those who were MRD-negative (40% vs. 89%; *P*=0.007); furthermore, the overall survival of those in complete remission prior to HSCT but with detectable *NPM1* MRD was similar to that of patients transplanted with active disease.⁷³ Together, these studies suggest that PCR-based MRD for mutant *NPM1* is prognostic across clinical contexts, both after induction/early consolidation and before HSCT.

Next-generation sequencing-based measurable residual disease studies

Compared to both MFC and PCR, the use of NGS as an instrument for detecting MRD is relatively new and consequently there are fewer studies to guide its use in clinical practice.^{17,45,46} In a study of 50 patients who underwent paired whole genome or exon sequencing at diagnosis and remission, 48% had persistent leukemia-associated mutations in at least 5% of bone marrow cells (i.e. variant allelic frequency $\geq 2.5\%$).⁴⁵ These patients with persistent mutations had shorter event-free survival (median: 6.0 vs. 17.9 months, *P*<0.001) and overall survival (median: 10.5 vs. 42.2 months, *P*=0.004), compared to those of patients with lower post-remission mutation burden. In a study from the MD Anderson Cancer Center of 131 patients undergoing induction for newly diagnosed AML, complete molecular clearance (i.e. absence of detectable mutations by NGS) was independently associated with decreased risk of relapse and better event-free survival even when MFC-based MRD status was also available.⁴⁶ These results were strengthened by removing mutations associated with CHIP (i.e. *DNMT3A*, *TET2*, and *ASXL1*, or "DTA" mutations) from the analysis, suggesting that persistence of these preleukemic mutations may not be associated with worse outcomes. In the largest study of NGS MRD in AML, 482 patients were evaluated with NGS at diagnosis and remission, 430 (89.2%) of whom had a leukemia-associated mutation detected at baseline.¹⁷ Mutations persisted in 51% of patients at variant allelic frequencies ranging from 0.02-47%. CHIP-associated DTA mutations were not associated with relapse. When DTA mutations were excluded from analysis, residual disease, as detected by NGS, was independently associated with risk of relapse, relapse-free survival and overall survival. Importantly, NGS MRD added prognostic information to MFC MRD status. Four-year relapse rates were similar among patients who were MRD-positive by NGS/MRD-negative by MFC and those who were MRD-negative by NGS/MRD-positive by MFC (52.3% vs. 49.8%, respectively), whereas patients who were MRD-positive or MRD-negative by both assays had highly divergent risks of relapse (73.3% vs. 26.7%, respectively). Together, these studies suggest that NGS-based MRD may complement MFC MRD assays, as these two approaches evaluate different targets (i.e. leukemia-associated mutations and aberrant surface protein expression), and also that reduction and/or clearance of mutations associated with CHIP may not be necessary for cure.^{17,46}

Due to the background error rate of commonly used

NGS-based technologies, the sensitivity of these assays is generally no better than ~1%, limiting their discrimination for small amounts of residual disease. In a study of patients with AML undergoing HSCT, an error-corrected, molecular barcoded NGS MRD assay was able to detect residual mutations prior to HSCT with a median variant allelic frequency of 0.33% (range, 0.016%-4.91%), suggesting that such an assay can detect lower levels of MRD than can standard NGS MRD assays.⁵¹ Among the 96 evaluable patients, 45% were MRD-positive prior to HSCT using this approach. The 5-year cumulative incidence of relapse was 66% for those who were MRD-positive versus 17% for those who were MRD-negative (HR 5.58; $P < 0.001$). On multivariate analysis, MRD remained an independent predictor of relapse and also overall survival (HR 3.0; $P = 0.004$). Further studies of these more sensitive, error-corrected NGS technologies in other contexts are warranted.

Practical considerations for the evaluation of measurable residual disease in clinical practice

While MRD response has been reliably shown to be predictive for better outcomes, there is significant heterogeneity across studies depending on the specific population of patients, regimen used, AML subtype, MRD method and target, timing of MRD assessment, and threshold to define adequate response, among other factors. All of these variables must be considered when interpreting study results and attempting to generalize to a patient in clinical practice. Despite these challenges, we routinely assess MRD in our clinical practice, as this information helps to refine prognostic assessment and, in some cases, informs therapeutic decisions (e.g. identifies patients who may be candidates for MRD-directed clinical trials or HSCT). Consensus recommendations from the European LeukemiaNet support this approach of routine MRD assessment in AML.⁶ We also routinely discuss the findings with our patients, particularly when this information is used for clinical decision-making. However, despite the strong association of MRD with clinical outcomes, it is imperative to remember (and to convey to the patient) that “MRD-negativity” does not equate to “cure,” as cumulative incidences of relapse of approximately 20-50% are still observed in “MRD-negative” patients, depending on the assay and clinical context. In contrast, while “MRD-positive” patients almost invariably relapse without subsequent therapy, this is also not an absolute certainty. Thus, an acknowledgment of the limitations of our current MRD technologies is required by the clinician to avoid “over-interpreting” MRD information.

When using MRD in clinical practice, it is important to choose a complementary MRD assay tailored to the AML subtype of a particular patient. For patients with an appropriate target (e.g. those with acute promyelocytic leukemia, core-binding factor AML or *NPM1*-mutated AML), PCR is preferred to MFC since the clinical data in these populations are more robust for the former and it also has a higher sensitivity.^{2,6,22} When MFC is used, it is imperative that it is performed in a laboratory with extensive experience in interpretation to ensure accuracy of the assessment.

Whether peripheral blood can replace bone marrow for MRD testing is an area of controversy, although it is generally agreed that an accurate and sensitive peripheral

blood-based MRD assay would be welcome to both clinicians and patients, given the less invasive nature of this approach. While some studies have suggested similar findings with these two sources of assay material,^{74,75} most of the available clinical data on the prognostic and predictive impact of MRD were derived from bone marrow specimens. Furthermore, bone marrow likely provides additional sensitivity, with detectable MRD levels approximately 1-log higher than those in the peripheral blood.⁶ For example, in core-binding factor AML, a “negative” peripheral blood MRD assay could miss positive bone marrow MRD in up to 40% of cases during therapy and 10-15% of cases during follow up.³³ Conversely, in a study of PCR-based MRD in *NPM1*-mutated AML, discrimination for survival was better when peripheral blood was used than when bone marrow was used.¹⁵ Thus, the optimal source for MRD assessment is likely dependent on assay, regimen, and time. Future studies of peripheral blood MRD monitoring are needed across MRD technologies and treatment contexts, and should include investigation of the potential use of circulating cell-free DNA as a source for MRD assays similarly to what has been achieved for some solid tumors.^{76,77} Initial efforts are already underway in AML.^{78,79}

While the optimal prognostic time point of MRD assessment varies slightly based on the specific regimen and other factors, most studies have shown that detectable MRD is associated with worse outcomes regardless of when it is measured. For patients treated with intensive chemotherapy in the frontline setting, we routinely assess for MRD, at a minimum, at the end of induction and consolidation. It is reasonable to assess MRD at the end of the first cycle of consolidation as well, particularly in patients who remained MRD-positive after induction. In several studies, this time point (i.e. after 2 cycles of chemotherapy) is the most discriminatory for relapse and long-term survival.^{15,18} MRD status before transplantation is also highly associated with risk of post-HSCT relapse and may identify patients who may be candidates for investigational post-HSCT maintenance strategies.⁵²⁻⁵⁵

Therapeutic implications of measurable residual disease in acute myeloid leukemia

While it is important to obtain MRD information for prognostic purposes, ultimately the goal of developing MRD assays that can more accurately determine risk of relapse in an individual patient with AML is so that post-remission therapies can be tailored accordingly. Currently, risk stratification in AML is largely based on cytogenetic and molecular alterations present at the time of diagnosis; these factors are currently the primary disease-related considerations for deciding to pursue HSCT in first remission.² In contrast, in acute lymphoblastic leukemia, the decision to pursue HSCT in first remission is determined in large part by MRD status, particularly in the pediatric population.⁷⁻⁹ Furthermore, the CD3-CD19 bispecific T-cell engaging antibody blinatumomab is highly effective in eradicating MRD in patients with acute lymphoblastic leukemia and is approved by the United States Food and Drug Administration for this use.⁸⁰ While some data support the use of MRD to guide therapeutic decisions in AML, the role of MRD-guided

Table 2. Ongoing measurable residual disease-based clinical trials in adults with acute myeloid leukemia.

| Agent(s) | Study Phase | Timing of MRD positivity | Major inclusion/exclusion criteria | ClinicalTrials.gov identifier |
|--|-----------------|---------------------------------|---|-------------------------------|
| Autologous WT1-TCR α 4 gene-transduced CD8 ⁺ T cells | I/II | First CR/CRi | <ul style="list-style-type: none"> • Must have HLA-A*02:01 expression and elevated baseline WT1 expression • Excludes patients with prior allogeneic HSCT | NCT02770820 |
| Azacitidine + avelumab | I/II | Any time | --- | NCT03699384 |
| BL-8040 (CXCR4 antagonist) + atezolizumab | Ib/II | First CR/CRi | <ul style="list-style-type: none"> • Excludes patients with prior allogeneic HSCT | NCT03154827 |
| DCP-001 (dendritic cell vaccine) | II | First CR/CRi | <ul style="list-style-type: none"> • Excludes patients with prior allogeneic HSCT | NCT03697707 |
| FLYSYN (anti-FLT3 monoclonal antibody) | I | Any time | <ul style="list-style-type: none"> • Must have <i>NPM1</i>-mutated AML and FLT3 expression on leukemic cells • Excludes patients with prior allogeneic HSCT | NCT02789254 |
| Gemtuzumab ozogamicin | II | Any time | <ul style="list-style-type: none"> • Must be CD33⁺ | NCT03737955 |
| Guadecitabine (SGI-110) + donor lymphocyte infusion | II | Before or after allogeneic HSCT | --- | NCT02684162 |
| Lenalidomide | II | First or second CR/CRi | --- | NCT02126553 |
| Lenalidomide | I | After allogeneic HSCT | <ul style="list-style-type: none"> • MRD detected by CD34⁺ chimerism assay | NCT02370888 |
| Nivolumab | II | First or second CR/CRi | --- | NCT02126553 |
| Nivolumab (<i>vs.</i> observation) | II (randomized) | First CR/CRi | --- | NCT02275533 |
| Tagraxofusp (SL-401) | I/II | First or second CR/CRi | --- | NCT02270463 |

MRD: measurable residual disease; CR: complete remission; CRi: complete remission with incomplete hematologic recovery; HSCT: hematopoietic stem cell transplantation; AML: acute myeloid leukemia.

therapies is less clear in AML than in acute lymphoblastic leukemia. With the possible exceptions of allogeneic HSCT and hypomethylating agents, effective treatments for MRD-positive disease have not been established in AML. It has yet to be conclusively shown that eradication of MRD that persists or recurs after initial treatment is associated with improved outcomes in AML; however, given the available evidence (as discussed in detail below), we do frequently use HSCT or hypomethylating agents for patients with MRD-positive disease. Enrollment of these patients in MRD-directed clinical trials with novel agents and combinations is also imperative (Table 2).

Measurable residual disease-guided allogeneic hematopoietic stem cell transplantation

While it is common practice to refer patients who are MRD-positive to HSCT, robust data supporting this practice are lacking. Nevertheless, while the outcomes of MRD-positive patients are relatively poor regardless of whether HSCT is or is not performed, several studies suggest that outcomes may be improved with HSCT.^{14,18,70,81} In a multicenter study of 116 patients with AML harboring t(8;21), patients received either allogeneic HSCT or chemotherapy, with or without autologous HSCT, based on MRD response.⁷⁰ High-risk patients were defined as those who did not achieve a major molecular response (i.e., a >3-log reduction in *RUNX1/RUNX1T1* transcript levels in bone marrow, determined by PCR analysis) after the second consolidation, or who lost the major molecular response within 6 months of achieving it. Among patients in the high-risk group, those who underwent allogeneic HSCT had a lower cumulative incidence of relapse compared to that of the patients who did not

undergo allogeneic HSCT (22.1% *vs.* 78.9%, respectively; $P < 0.001$), which translated into an improvement in overall survival (71.6% *vs.* 26.7%, respectively; $P = 0.007$). Conversely, the overall survival rate was lower in low-risk patients who underwent allogeneic HSCT (75.7% *vs.* 100%; $P = 0.013$). These data suggest that MRD could reasonably be used to assign patients with t(8;21) to post-remission therapies. Data for similar approaches in core-binding factor AML with inv(16) are lacking.

The role of allogeneic HSCT in patients with intermediate-risk AML is controversial, and it is possible that MRD may be able to guide post-remission therapeutic decisions in this group.²⁴ In a study of younger patients (age 18-60 years) with newly diagnosed *NPM1*-mutated AML, HSCT was associated with better disease-free survival and overall survival in patients with non-favorable risk disease who had a <4-log reduction in mutant *NPM1* in the peripheral blood at the time of remission (disease-free survival: HR, 0.25; 95% CI: 0.06-0.98; $P = 0.047$; overall survival: HR, 0.25; 95% CI: 0.06-0.98; $P = 0.047$), whereas HSCT did not improve outcomes for patients with a >4-log reduction.¹⁴ Similarly in the National Cancer Research Institute AML17 study of standard-risk patients without *NPM1* mutations, HSCT appeared to preferentially benefit those who remained MRD-positive by MFC after the second cycle of chemotherapy but not those who were MRD-negative, although the interaction between HSCT and MRD status did not reach statistical significance ($P = 0.16$), possibly because of the small number of patients in the cohort who underwent HSCT in first remission.¹⁸ Although definitive evidence is lacking, collectively these studies provide some support for the use of MRD to guide post-remission therapies in patients with intermediate-risk AML.

Hypomethylating agents for measurable residual disease-positive acute myeloid leukemia

Although HSCT has historically been the preferred consolidation option for patients with high-risk AML, there is also interest in the use of hypomethylating agents for these patients, including those with detectable MRD. Several studies have suggested that treatment with hypomethylating agents (azacitidine or decitabine) may be beneficial for patients with AML and persistent or recurrent MRD.⁸²⁻⁸⁵ The RELAZA2 trial was the largest prospective study to assess this issue.⁸⁴ One hundred and ninety patients with AML or myelodysplastic syndrome who achieved complete remission after chemotherapy or HSCT were monitored for MRD for 24 months with either PCR for mutant *NPM1*, leukemia-specific gene fusions (*DEK-NUP214*, *RUNX1-RUNX1T1*, or *CBFβ-MYH11*) or donor chimerism in flow cytometry-sorted CD34⁺ cells (for patients who had undergone prior allogeneic HSCT). Patients who developed detectable MRD received a standard dose of azacitidine for up to 24 cycles. Among the 53 evaluable patients who became MRD-positive and received treatment with azacitidine, 19 (36%) achieved MRD-negativity after 6 months of treatment. Median relapse-free survival and overall survival times for these MRD-positive patients who received azacitidine was 10 months and 31 months, respectively. Importantly, MRD response was associated with improved relapse-free survival (HR, 0.2; 95% CI: 0.1-0.5; $P < 0.0001$) and a trend towards improved overall survival (HR, 0.4; 95% CI: 0.1-1.3; $P = 0.112$). These data suggest that hypomethylating agents may be a reasonable option for patients with MRD-positive AML, particularly those who are not suitable candidates for allogeneic HSCT. Other studies have, however, failed to demonstrate a benefit for the routine use of hypomethylating agents in the maintenance setting.^{86,87} A phase III randomized study of an oral formulation of azacitidine as maintenance therapy in patients with AML in remission will likely provide further important information in this area (NCT01757535).

Conclusions

Despite substantial heterogeneity across studies of MRD in AML (e.g. variations in AML subtype, MRD methodology and target, cutoff, regimen, and timing of assessment), achievement of MRD negativity has been reliably shown to predict risk of relapse and long-term outcomes. More sensitive assays are still needed, however, as up to 50% of patients who are “MRD-negative” according to commonly applied MRD technologies still relapse. Beyond the important prognostic information that MRD provides, the ultimate goal of assessing MRD is to use this information to appropriately guide decisions regarding post-remission therapies. This approach is, however, strongly dependent on the availability of therapies that are effective in eradicating small amounts of residual leukemia (as with blinatumomab in acute lymphoblastic leukemia). While some studies suggest that allogeneic HSCT or hypomethylating agents may provide benefit for some patients with persistent or recurrent MRD, long-term outcomes with these approaches are largely disappointing. Innovative treatments are therefore needed for these patients. There are several ongoing MRD-directed clinical trials that are evaluating novel immune-based strategies (e.g. checkpoint inhibitors, vaccines, and T-cell-based therapies such as bi-specific T-cell engaging antibodies and chimeric antigen receptor T cells) which may be able to overcome the chemoresistant phenotype associated with MRD-positive disease. Novel combinations with Bcl-2 inhibitors (e.g. venetoclax) may also be promising in this setting. Ultimately, enrollment of high-risk patients into these trials is imperative in order to advance our understanding of how to use MRD information to drive clinical decisions and to improve outcomes of patients with AML.

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