How Precision Medicine Is Changing Acute Myeloid Leukemia Therapy

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overview

Pretreatment somatic mutations influence acute myeloid leukemia (AML) pathogenesis and responses to chemotherapy. Integration of cytogenetic abnormalities and molecular mutations, co-occurring and in isolation, have resulted in a more refined prognostic assessment. In addition, research performed over the last few years has led to the development of novel therapies and new drug approvals in patients with both newly diagnosed and relapsed/refractory (R/R) AML. Here we discuss the use of these newly approved therapies. Advances in AML have also occurred through development of better tools to assess response to treatment. Both multiparameter flow cytometry and polymerase chain reaction can be used to assess for the presence or absence of measurable residual disease (MRD) and increase the sensitivity of response assessment. The role of MRD assessment is gaining relevance and its integration in clinical trials and treatment decision making will be explored in the second half of this article.

INTRODUCTION

Over the last few decades, researchers have deciphered the complex biology of AML with a specific focus on how pretreatment somatic mutations influence AML pathogenesis and responses to chemotherapy. These advances have resulted in a better understanding of leukemogenesis, which has resulted in more refined prognostic assessment and allowed alterations in treatment that are improving survival.[1] Small molecule inhibitors of pathogenic mutant proteins have been studied in well-designed clinical trials and are now approved for the treatment of AML alone or in combination with chemotherapy.

Complete remission (CR) rates and duration of response differ based on patient and disease characteristics.[2,3] Until recently, the standard induction regimen for "fit" patients with AML was 7 + 3, a 7-day continuous infusion of cytarabine and a 3-day course of daunorubicin or idarubicin.[4] Older unfit patients have worse clinical outcomes because of an increased rate of unfavorable-risk cytogenetic and molecular genetic abnormalities and poor tolerance of traditional induction chemotherapy.[5] This article focuses on the major progress of the last few years—how molecular abnormalities have affected treatment decisions and the role of incorporating dynamic risk assessment in the form of MRD testing during AML treatment.

The French-American-British classification of AML was an initial attempt to categorize the prognosis of patients based on morphology and cytochemical stains. Advances in chromosomal analysis (traditional metaphase karyotyping and fluorescence in situ hybridization) led to further refinements in categorization. The revolution in molecular genetics and the affordable use of next-generation sequencing (NGS) has defined the prognosis for the approximately 50% of patients with a normal karyotype, which has resulted in the ability to comprehensively integrate genomic assessments for precise categorization of risk of relapse and death.[6,7] Clinicians are now realizing the clinical benefits of this basic biologic inquiry. The spectrum of therapies that have been developed for the treatment of AML includes options for both newly diagnosed and relapsed disease. The recent leukemia drug approvals have changed our treatment paradigms for many forms of AML, and there are now new therapeutic choices for our patients. Some of the most exciting advances have developed from the recognition of specific mutations in AML and the ability to target these mutations. This article will focus on how the molecular landscape affects current treatment decisions in AML. We will focus our review on the currently actionable mutations in AML.

FMS-LIKE TYROSINE KINASE 3 MUTATIONS

FMS-like tyrosine kinase 3 (*FLT3*), which plays a role in the proliferation and apoptosis of primitive hematopoietic stem cells, is mutated in approximately 30% of cases of AML.[8] The *FLT3*-internal tandem duplication (ITD) mutation occurs in approximately 23% of de novo AML cases and consists of duplications in the juxtamembrane domain that leads to constitutive tyrosine kinase activity.[2,8] An activating point mutation of the tyrosine kinase domain (TKD) occurs in approximately 7% of AML cases, most commonly at the D835

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PRACTICAL APPLICATIONS

- The importance of understanding leukemogenesis at the molecular level through genetic sequencing has resulted in more refined prognostic assessments and has led to the use of targeted therapies.
- Mutational analysis through the use of NGS is essential for selecting optimal therapies.
- The adoption of these targeted therapies has been rapid and their clinical use will be reviewed in this article.
- The prognostic role of MRD testing in response assessment in AML is well established.
- MRD is not yet widely established as a predictive biomarker or surrogate endpoint, and standardization of the technology for MRD assessment and its use in randomized trials are anticipated so MRD can be utilized to modify treatment interventions.

position.[9,10] Both mutations lead to activation of downstream signaling pathways, independent of ligand stimulation.[11] Because of its role in leukemogenesis, inhibition of *FLT3* has been studied as a therapeutic strategy in AML. The initial *FLT3* inhibitors, or first-generation agents (e.g., midostaurin and sorafenib), lacked specificity for the *FLT3* tyrosine kinase.[2,8] However, because of the availability of sorafenib (because of its approval for treatment of renal cell carcinoma, liver cancer, and thyroid cancer) and its known inhibition of *FLT3*, in addition to other kinases, sorafenib has been studied in R/R AML. The responses to these agents in relapsed disease have been relatively limited.

Midostaurin

Midostaurin is active against both the *FLT3*-ITD and TKD mutations.[12] Despite limited responses in R/R AML,[13] midostaurin was combined safely with induction chemotherapy in a phase Ib study.[14] These results led to the RATIFY trial, a multinational phase III randomized trial that enrolled 717 younger adults with newly diagnosed AML with a *FLT3*-ITD or TKD mutation. Midostaurin (50 mg twice daily) or placebo was given on days 8 to 21 of up to two cycles of induction chemotherapy and all cycles of high-dose, cytarabine-based consolidation. Patients who declined or were not eligible for allogeneic bone marrow transplantation received midostaurin or placebo maintenance. The 4-year overall survival (OS) was 44.3% in the placebo group compared with 51.4% in patients who received midostaurin. Although the probability of CR was not statistically different between the two groups, the median disease-free survival was significantly prolonged in the midostaurin group compared with the placebo arm (26.7 months vs. 15.5 months; p = .01). A subset analysis of the different *FLT3* mutations showed a survival benefit in both *FLT3*-TKD and *FLT3*-ITD subsets. The RATIFY trial was the first trial that demonstrated a survival benefit of a targeted therapy combined with standard chemotherapy, and, in April 2017, it led to the U.S. Food and Drug Administration (FDA) approval of midostaurin in combination with standard chemotherapy for patients with the *FLT3* mutation of AML.[15]

Giltertinib (ASP2215) has dual inhibitory action on *FLT3* and *AXL*, a member of the *TYRO3*, *AXL*, and *MER* subfamily of receptor tyrosine kinases.[2,16] In a phase I/II trial of 191 patients with the *FLT3* mutation, 70 (37%) patients achieved a composite CR. Most of these composite CRs occurred in the 69 (41%) patients who received doses of 80 mg/day or higher.[14] These results led to a randomized phase III trial of single-agent gilteritinib at 120 mg/day compared with salvage chemotherapy in patients with R/R *FLT3*-mutated AML. The rates of CR/CR with partial hematologic recovery were 21% and 31% in patients who achieved transfusion independence, respectively.[17] This clinical activity led to FDA approval of gilteritinib in November 2018 for the treatment of adult patients with R/R *FLT3*-mutated AML.

Quizartinib is a FLT3 inhibitor with activity against *FLT3*-ITD, but importantly not against the *FLT3*-TKD. In a randomized trial of quizartinib versus salvage chemotherapy in patients with R/R *FLT3*-ITD–mutated AML, quizartinib led to an improvement in median OS of 6.4 weeks. At the time of this writing, quizartinib is being considered by the FDA for approval based on the results of this study.[18]

Like gilteritinib, crenolanib inhibits both the TKD and ITD mutations.[19,20] In a phase II trial of patients with R/R AML, 23% of tyrosine kinase inhibitor–naive patients achieved CR with incomplete hematologic recovery with a median OS of 55 weeks.[21] The demonstration of tolerability and efficacy of these agents in R/R AML has led to frontline combinatorial studies.

Giltertinib, quizartinib, and crenolanib have all been shown to be safely combined with standard intensive chemotherapy in patients with newly diagnosed AML. A randomized trial of 7 + 3 with quizartinib versus placebo is ongoing, as is a randomized trial of 7 + 3 with crenolanib or midostaurin. Likewise, a randomized trial of 7 + 3 with gilteritinib or midostaurin is planned. Table 1 summarizes the completed and ongoing studies of these next-generation FLT3 inhibitors.[22-24] Furthermore, trials combining lower intensity chemotherapy (i.e., hypomethylating agents) and FLT3 inhibitors are ongoing. The results of the randomized trials of these agents in combination with chemotherapy are greatly anticipated.

Because of the high relapse rate in patients, early transplantation referral is recommended for most patients with

TABLE 1. Emerging FLT3 Inhibitors

Agent	Half-Life (Dosing)	D835 Activity	Relapsed, Single-Agent Trial	Frontline Combination With Chemotherapy	Post-HCT Maintenance
Gilteritinib	Long (once daily)	Yes	Phase III completed; approved Nov. 2018	Phase I ongoing; randomized phase III planned	Phase III recruiting
Quizartinib	Long (once daily)	No	Phase III completed and met primary endpoint	Phase III completed; phase I ongoing	Pilot study completed; included in phase III
Crenolanib	Short (3 times daily)	Yes	Phase II completed	Randomized phase III ongoing	Pilot completed; Phase II ongoing

Abbreviation: HCT, hematopoietic cell transplantation.

FLT3-ITD–mutated AML. Despite stem cell transplantation, patients remain at risk for recurrent disease; because of this, randomized trials of FLT3 inhibition postallogeneic transplantation are in progress. A randomized trial of sorafenib versus placebo was conducted and was recently presented. With a median follow-up of more than 40 months, the median relapse-free survival (RFS) was 30.9 months in the placebo group and not reached in the sorafenib arm, corresponding to a 2-year RFS of approximately 53% in the placebo arm compared with 85.0% in the sorafenib arm.[25] These early results are encouraging. A randomized trial of gilterinib versus placebo maintenance post-transplantation is ongoing.

ISOCITRATE DEHYDROGENASE MUTATIONS

The development of isocitrate dehydrogenase (IDH) inhibitors has paralleled that of the FLT3 inhibitors. IDH1 and IDH2 are metabolic enzymes responsible for converting isocitrate to α-ketoglutarate, an oxidative decarboxylation reaction that takes place in the cytoplasm and mitochondria.[26] Point mutations in the IDH enzymes lead to aberrant proteins that dimerize and catalyze the conversion of α-ketoglutarate to the metabolite 2-hydroxyglutarate (2HG).[27] The accumulation of 2HG interferes with epigenetic regulatory processes responsible for cellular differentiation through DNA hypermethylation.[28,29]

Ivosidenib (AG-120) is an *IDH1* inhibitor evaluated as monotherapy in patients with *IDH1*-mutated AML in a dose-escalation and expansion study. Among 179 patients with R/R AML, treatment-related adverse events of grade 3 included QT prolongation, IDH differentiation syndrome, anemia, and thrombocytopenia. The overall response rate was 41.6%, with a rate of CR or CR with partial hematologic recovery of 30.4%. The median overall survival in the entire efficacy population in this single-arm study was 8.8 months.[30] These results led to FDA approval of ivosidenib for the treatment of adults with R/R IDH1-mutated AML.

Enasidenib (AG-221) is a selective *IDH2* inhibitor that was evaluated in a phase I/II trial that enrolled patients with IDH2-mutated AML; most of the patients had relapsed disease. The overall response rate in patients with R/R AML was 38.8%, with a 19.6% CR rate and a median overall survival of 8.8 months. The most common grade 3-4 treatment-related adverse events were hyperbilirubinemia, thrombocytopenia, and IDH differentiation syndrome.[31] Furthermore, the estimated median OS was 22.9 months for the 42 patients with R/R AML who achieved a CR, 10.6 months for those who had a non-CR response, and 5.6 months for nonresponders.[31] These results led to the FDA approval of enasidenib in August 2017, for patients with R/R AML characterized by an IDH2 mutation.[4]

Similar to the trials with *FLT3* inhibitors, IDH inhibitors are being studied in combination with standard intensive induction chemotherapy and hypomethylating agents in adults with newly diagnosed AML.

It is worth detailing the risk of IDH differentiation syndrome (DS) with IDH inhibitors. Ivosidenib and enasidenib carry black box warnings about DS, a capillary leak-type syndrome caused by differentiation of leukemic blasts, which can be fatal if not recognized and not treated. The incidence of DS with these agents ranged from 11% to 14% based on investigator report or review-committee determination. However, the FDA suspected that episodes of DS might have been underreported because both trials were first-in-human trials, and there was no adverse event term for DS outside of the context of acute promyelocytic leukemia. Thus, FDA conducted a systematic analysis of DS cases and found a rate of 19% with both agents.[32]

BEYOND SPECIFIC TARGETED THERAPIES

In addition to the previously mentioned agents, there have been other therapies recently approved for the treatment of AML. These options include hypomethylating agents or low-dose cytarabine with venetoclax or low-dose cytarabine with glasdegib for the treatment of AML in patients who are age 75 or older. They can also be used in patients who have comorbidities that preclude use of intensive induction chemotherapy and Vyxeos (Jazz Pharmaceuticals, Dublin, Ireland) for the treatment of secondary AML or AML with myelodysplasia-related changes in patients who are fit for intensive induction therapy.[33-35] Because these therapies are being adopted into practice, it is of emerging interest

and relevance to determine which subgroups of patients have the greatest response to each therapy, and it is important to try to identify which patients are unlikely to respond so they can be spared toxicities.[36]

Looking forward, there are a number of agents being studied with the goal of increasing response rates particularly in patients with R/R AML or disease that is not predicted to be sensitive to chemotherapy. Agents on the horizon include specific pathway inhibitors and antibody-based therapies. One interesting agent is APR-246, which is a novel selective molecule that induces apoptosis in cancer cells with mutated *TP53* by reinstating the wild-type conformation of the protein. In a multicenter phase Ib trial, APR-246 was combined with azacitidine in adults with myelodysplastic syndrome (MDS) or AML with 30% or fewer blasts. The early results have demonstrated a CR rate of 82%.[37] Our patients are reaping the benefits of decades of research. As we look toward the future, we can hope for not only continued development of new agents but also learning how to sequence these agents and select the right treatment for individualized patients based on disease and patient characteristics. Furthermore, our assessment of response to treatment has become more refined as we better understand how to assess for and discover the relevance of (MRD in AML.

APPLICATION OF MRD TESTING IN ASSESSING RESPONSE TO TREATMENT OF AML

How Can MRD Assessment Help in the Treatment of AML Patients?

Cytogenetic and molecular genetic alterations at the time of the diagnosis of AML are used to prognosticate patient outcomes. Based on large retrospective studies, mutations in a variety of genes, including *NPM1*, *CEBPA*, *FLT3*, *KIT*, *RUNX*, *ASXL1,* and *TP53* stratify those patients with favorable, intermediate, or unfavorable risk of disease. However, this form of prognostication is static. Early measurement of treatment response is increasingly recognized as an important tool to predict final treatment outcome. The European LeukemiaNet (ELN) 2017 treatment guidelines recommend refining response assessment through the addition of a CR MRD negative (CR_{MRD}–) category.[3] However, the National Comprehensive Cancer Network AML guidelines do not recommend routine MRD analysis in clinical practice.[38]

The prognostic role of MRD is widely accepted, with a 3- to 4- year cumulative incidence of relapse (CIR) of 6.5%–41% in patients who are MRD-negative and of 53%–82% in patients who are MRD-positive.[39-43] However, the predictive value of MRD in guiding treatment decisions has not yet been established by prospective studies. Therefore, the ELN MRD working group has issued a caution against the use of MRD as a predictive marker for therapy decisions and recommends using only a 10-fold or more increase of MRD as a clear indication for therapeutic action.[44] However, recent, but mostly retrospective, studies suggest that MRD assessment may be useful in treating patients with AML (summarized in the following and in Table 2[45-48]). In 2018, the NCRI and HOVON/SAKK study groups evaluated the value of flow cytometry (FCM)–based MRD in patients with more than or equal to 5% blasts after induction chemotherapy by cytomorphology but who had a negative MRD result.[45] They found comparable outcomes in patients with morphologically refractory disease who were MRD-negative with patients who were in morphologic CR- and MRD-negative, and thus proposed that FCM MRD analysis was superior to morphologic response assessment, particularly in patients with a borderline blast count. Several study groups also evaluated whether MRD assessment could improve the allocation of allogeneic hematopoietic cell transplantation (alloHCT) to ELN favorable and intermediate-risk patients. Zhu et al[46] prospectively compared alloHCT and chemotherapy in patients with AML who were *RUNX1-RUNX1T1*–positive. Consolidation treatment with alloHCT reduced the relapse rate and improved survival compared with chemotherapy in patients who were MRD-positive, whereas treatment with chemotherapy was associated with a low relapse rate and improved disease-free survival compared with alloHCT in patients who were MRD-negative.[46] MRD was measured after the second consolidation cycle and up to 6 months after the end of treatment. However, the allocation to alloHCT and chemotherapy groups was based on patient decision and not randomization, and therefore, required validation by a randomized trial. Balsat et al[47] evaluated the role of MRD in ELN nonfavorable NPM1-mutated AML patients (*NPM1* mutation with a *FLT3*-ITD mutation or abnormal karyotype). Patients who did not achieve a 4-log MRD reduction in peripheral blood after one to two induction cycles had a higher CIR and a shorter OS.[47] Disease-free survival and OS were improved by alloHCT in these patients but not in those with a greater than 4-log reduction in peripheral blood MRD. However, this study requires independent, ideally prospective, validation before such an approach can be recommended.

The ELN 2017 treatment guidelines recommend alloHCT for consolidation of intermediate-risk patients, if feasible. Some study groups have evaluated whether treatment intensity can be reduced in intermediate-risk patients who are MRD-negative and consolidated their patients with autologous HCT.[49] However, there are currently no prospective data to support this approach. A recent study compared alloHCT and chemotherapy consolidation in younger patients with AML in CR1 depending on MRD status, as assessed by FCM.[50] This analysis showed that relapse-free survival (RFS) was improved by alloHCT compared with chemotherapy in patients who were MRD-negative and

TABLE 2. Clinical Scenarios in Which MRD Assessment Has Been Suggested as a Diagnostic or Predictive Biomarker for Patients With AML

Clinical Setting	MRD Method	Biomarker	Type of Biomarker	Expected/Predicted Outcome
Response assessment after induction chemotherapy	FCM	MRD negativity in cytomorphologically refractory patients	Diagnostic	FCM-MRD is superior to morphologic response assessment and is associated with assessment
RUNX1-RUNX1T1 AML	qRT-PCR	MRD negativity	Predictive	Improved OS with chemotherapy consolidation compared with alloHCT
RUNX1-RUNX1T1 AML	qRT-PCR	MRD positivity	Predictive	Improved OS with alloHCT consolidation compared with chemotherapy
ELN nonfavorable NPM1 mutated AML	qRT-PCR	MRD reduction by less than 4-log10 after 1-2 induction courses	Predictive	Improved OS with alloHCT consolidation compared with chemotherapy
Patients undergoing alloHCT (including ALL and MDS)	FCM	MRD positivity	Predictive	Improved RFS and by trend OS with umbilical cord blood donor alloHCT compared with matched-unrelated or mismatched-unrelated donor alloHCT
Patients undergoing alloHCT	FCM	MRD positivity	Predictive	Lower CIR and improved OS with haploidentical donor alloHCT compared with matched-related donor alloHCT

Abbreviations: MRD, measurable residual disease; AML, acute myeloid leukemia; FCM, flow cytometry; qRT-PCR, quantitative real-time polymerase chain reaction; OS, overall survival; alloHCT, allogeneic hematopoietic cell transplantation; ELN, European LeukemiaNet; RFS, relapse-free survival; ALL, acute lymphoblastic leukemia; MDS, myelodysplastic syndrome; CIR, cumulative incidence of relapse.

MRD-positive. However, OS was improved by alloHCT only in the MRD-positive group, whereas alloHCT and chemotherapy consolidation resulted in comparable OS in the MRD-negative group.[50] Although alloHCT is the logical next therapeutic step in patients who are MRD-positive, MRD positivity remains one of the strongest risk factors for poor outcome in alloHCT.[51-53] Buckley et al compared the conditioning intensity in a meta-analysis that included 19 trials and found that myeloablative conditioning did not improve the outcome of patients who were MRD-positive compared with patients who received reduced intensity conditioning.[54] Instead of increasing the chemotherapy intensity, it was suggested that grafts from alternative donors might increase the graft versus the leukemia effect and might be more effective in patients who were MRD-positive. RFS, and, by trend, OS, was better in patients who were MRD-positive, but not in patients who were MRD-negative and who received umbilical cord blood grafts compared with recipients of HLA-matched or HLA-mismatched grafts.[48] In addition, patients who underwent haploidentical donor HCT showed lower CIR and improved OS compared with matched-related donor HCT if MRD was positive before HCT. However, donor type had no impact on outcome in patients who were MRD-negative.[55,56] Upon confirmation of these studies, MRD may guide donor selection in the future and may promote haploidentical donor HCT.

The concept of preemptive treatment of patients with molecular relapse has been pioneered in acute promyelocytic leukemia. Early treatment with arsenic trioxide prevented hematologic relapse and improved outcome in comparison with a historical control.[57] Preemptive treatment of patients with positive MRD has also been evaluated in the post-transplantation setting using donor lymphocyte infusions,[58,59] azacitidine,[60] or interferon-alpha,[61] although randomized studies are lacking.

Who Should Be Tested for MRD and At Which Time Points?

Recommendations of the ELN MRD Working Group on AML specify the clinical use and technical requirements of MRD assessment (Fig. 1).[44] To establish the MRD marker, patients must be investigated at diagnosis, or at least a diagnostic specimen of viable cells should be stored for later analysis. In patients treated with standard induction and consolidation chemotherapy, MRD assessment is recommended after two cycles of chemotherapy and at the end of consolidation. If patients undergo alloHCT, MRD should be assessed within 4 weeks before transplantation. MRD monitoring during follow-up is currently only recommended if the patient is monitored by a molecular technique.[44] Bone marrow and peripheral blood should be monitored during follow-up every 3 months for the first 2 years. After 2 years, the decision to continue MRD monitoring should be assessed on an individual basis. Alternatively, MRD may be monitored in peripheral blood every 4 to 6 weeks for 2 years, with the monitoring interval informed by the relapse kinetics of the underlying disease and/or MRD marker. For example, the median time from molecular to clinical relapse has been reported as 1 month in MLL-translocated AML, 2 to 3 months in patients who are *RUNX1-RUNX1T1*-, *NPM1*-mutated/*FLT3-ITD*–positive and in patients who are *DEK-NUP214*-mutated,

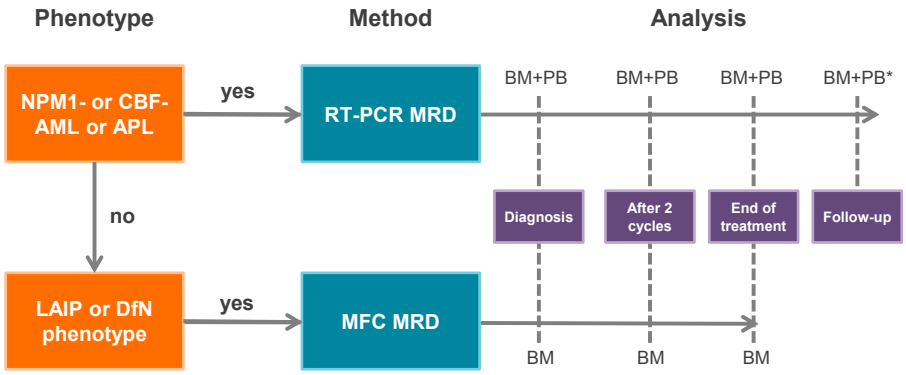

FIGURE 1. ELN Recommendation for MRD Analysis in Patients With AML Undergoing Standard Induction and Consolidation Chemotherapy

Abbreviations: AML, acute myeloid leukemia; BM, bone marrow; ELN, European LeukemiaNet; MFC, multicolor flow cytometry; MRD, measurable residual disease; PB, peripheral blood; RT-PCR, real-time polymerase chain reaction.

* During follow-up PB may be investigated every 4-6 weeks instead of bone marrow.

and 4 to 6 months in patients who are *CBFB-MYH11* and *NPM1*-mutated/*FLT3-ITD*–negative.[62]

Which Method Should Be Used for MRD Assessment?

ELN recommends assessing MRD by quantitative real-time polymerase chain reaction in patients who are positive for mutant *NPM1*, *RUNX1-RUNX1T1*, *CBFB-MYH11*, and *PML-RARA* fusion genes, which covers approximately 40% of all patients with AML. In all other patients, MRD should be assessed by multicolor FCM, which relies on antigens aberrantly expressed by leukemic cells that are present in more than 90% of patients with AML (Fig. 1).[44] Wilms tumor 1 (WT1) expression in peripheral blood is used by several study groups for MRD assessment, often in the post-transplantation setting. Giving value to leukemia specificity, sensitivity, standardization, developmental potential, and anticipated acceptance by regulators, the ELN recommendations on MRD favor leukemia-specific molecular and flow cytometric MRD approaches over gene expression–based approaches like WT1, although WT1 may be used as a MRD marker if no other MRD tests are available.

Several important technical details are specified in the ELN recommendations. The first pull from bone marrow aspirations should be used for MRD assessment to avoid hemodilution. Molecular MRD assessment may be performed from peripheral blood or bone marrow, although the sensitivity is approximately 10-fold lower in peripheral blood. In addition, the ELN recommends a multicolor FCM MRD positivity cutoff at equal to or greater than 0.1%, whereas any copy number measured by molecular MRD is considered positive based on the specific quality criteria specified by the ELN recommendations. However, patients with *NPM1* copy numbers less than 1% to 2% after the end of treatment have a very low relapse risk, which is termed molecular persistence at low copy number with a relapse risk comparable to CR MRD-negative patients.[44]

Rather than using absolute cutoff levels, a dynamic assessment by log reduction has been proposed for MRD assessment as a better indicator of chemosensitivity. Thus, molecular relapse is defined as two positive MRD measurements with a 10-fold increase of copy numbers to distinguish patients with molecular persistence at low copy numbers from patients with progressive disease. Nevertheless, further standardization of cutoffs and assessment time points is required.

Can MRD Be Quantified by Next-Generation Sequencing?

Next-generation sequencing MRD assessment has been pioneered by the introduction of molecular barcodes and error-corrected sequencing.[63-66] It is expected that NGS-MRD analysis is less dependent on experience and more widely available than other MRD technologies. The prognostic power of NGS-MRD has been proven by several studies at the end of induction and consolidation, and before and after alloHCT.[53,67,68] However, NGS-MRD is currently less standardized than other approaches, and efforts are underway to develop evidence-guided recommendations for standardized NGS-MRD analysis.

Because NGS-MRD measures the variant allele frequency of mutations that are present at diagnosis, this technology can only be applied in patients with at least one somatic mutation. If the variant allele frequency does not decline in a remission sample, it suggests that this mutation is associated with clonal hematopoiesis or with germline origin, and should be excluded from MRD analysis. Jongen-Lavrencic et al showed that mutations associated with clonal hematopoiesis, like *DNMT3A*, *ASXL1*, and *TET2*, are not prognostic at MRD assessment.[68] Mutations in genes involved in signal transduction (e.g., FLT3, NRAS) often disappear at relapse and may limit their use for MRD analysis. The interpretation of NGS-MRD needs a high level of expertise, and guidance on the use of suitable genes and procedures must be developed. Therefore, it is currently too early to recommend the clinical use of NGS-MRD.

How Should MRD Assessment Be Integrated in Future Trials?

Despite the long history of MRD assessment, there are little prospective data that have evaluated whether MRD is

a predictive marker for specific interventions. Currently, it is even more difficult to address this question because of the wide availability and use of MRD data in clinical practice. To overcome this challenge in future trials, separate trials should be designed for patients who are MRD-positive and MRD-negative, with allogeneic transplantation as the comparator arm for patients who are MRD-positive and consolidation chemotherapy or autologous HCT as the comparator arm for patients who are MRD-negative. A successful new treatment in patients who are MRD-positive may then be evaluated in a subsequent study in patients who are MRD-negative.

There is a great interest in the use of MRD as an efficacy response biomarker that can be used as a surrogate endpoint of a clinical trial. The FDA has developed draft guidance for how MRD may be incorporated as a predictive or efficacy response biomarker in future clinical trials.[69] These recommendations include the requirement for a meta-analysis that validates MRD as a surrogate endpoint in the clinical setting in which a new drug will be evaluated to justify its use in a prospective trial. In addition, this meta-analysis should include drug products with varying mechanisms of action to provide a basis for predicting patient responses to new drug products with novel mechanisms of action. The FDA further recommends that MRD analysis should be based on the intent-to-treat population, including all patients who do not achieve CR, with any patient without a MRD assessment considered as nonresponsive to treatment. In addition, it is recommended to use a single technology to assess MRD. However, if multiple technologies must be combined, the methodology for combining the test results should be prespecified. Finally, the sensitivity of the MRD assay should be at least 10-fold below the technical cutoff of the MRD test. Importantly, FDA views MRD as a biomarker that is a reliable quantitation of tumor burden, independent of the assay, and does not foresee the need for co-development of a certified MRD assay with the drug product. This strengthens academic laboratories, which can provide their services when their tests are fully validated.

The prognostic value of MRD assessment in AML is well established. In addition, the standardization of MRD technologies has been initiated with the publication of the ELN MRD recommendations. However, MRD is not yet established as a predictive biomarker and as surrogate endpoint for patient-relevant outcomes. International efforts of academic groups, industry, regulators, and funding agencies should be combined to standardize the technology for MRD assessment and determine the value of MRD as a predictive marker for current and future therapeutic interventions in patients with AML.

CONCLUSION

The tremendous gains in the treatment of AML have been due, in great part, to an understanding of the prognostic role of specific mutations and development of treatments targeting these mutations. Targeted small molecule inhibitors against mutant *FLT3* have led to improved overall survival in patients with newly diagnosed and R/R *FLT3*-mutated AML. Similarly, the remission rates of patients with R/R IDH1/2 AML who receive IDH1/2 inhibitors appear robust with minimal toxicity. In concert with the continued development of novel treatments, refining our understanding of which disease characteristics predict response to treatment is needed. Moving forward, MRD assessment may help drive more rapid approval of therapies. MRD assessment allows for the potential for early treatment modification in patients who do not respond and early intervention in those with evidence of early recurrence. Well-designed, prospective clinical trials will be able to determine whether interventions to prevent hematologic relapse in patients who are MRD-positive are able to change the outcomes of patients with AML.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST AND DATA AVAILABILITY STATEMENT

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