

# Test Then Erase? Current Status and Future Opportunities for Measurable Residual Disease Testing in Acute Myeloid Leukemia

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## Keywords

Measurable residual disease · Acute myeloid leukemia · Surrogate endpoint · Molecular measurable residual disease

## Abstract

**Background:** Measurable residual disease (MRD) test positivity during and after treatment in patients with acute myeloid leukemia (AML) has been associated with higher rates of relapse and worse overall survival. Current approaches for MRD testing are not standardized leading to inconsistent results and poor prognostication of disease. Pertinent studies evaluating AML MRD testing at specific times points, with various therapeutics and testing methods are presented. **Summary:** AML is a set of diseases with different molecular and cytogenetic characteristics and is often polyclonal with evolution over time. This genetic diversity poses a great challenge for a single AML MRD testing approach. The current ELN 2021 MRD guidelines recommend MRD testing by quantitative polymerase chain reaction in those with a validated molecular target or multiparameter flow cytometry (MFC) in all other cases. The benefit of MFC is the ability to use this method across disease subsets, at the relative expense of suboptimal sensitivity and specificity. AML MRD detection may be improved with molecular methods. Genetic characterization at AML diagnosis and relapse is now standard of care for

appropriate therapeutic assignment, and future initiatives will provide the evidence to support testing in remission to direct clinical interventions. **Key Messages:** The treatment options for patients with AML have expanded for specific molecular subsets such as *FLT3* and *IDH1/2* mutated AML, with development of novel agents for *NPM1* mutated or *KMT2A* rearranged AML ongoing, but also due to effective venetoclax-combinations. Evidence regarding highly sensitive molecular MRD detection methods for specific molecular subgroups, in the context of these new treatment approaches, will likely shape the future of AML care.

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## Introduction

Acute myeloid leukemia (AML) is an aggressive hematopoietic cancer with variable outcomes dependent on patient factors, baseline disease characteristics, response to therapy, access to care, and allogeneic hematopoietic cell transplantation (alloHCT) [1]. While most achieve initial complete remission (CR), only one-third are expected to survive beyond 5 years [2, 3]. For many patients, alloHCT remains the standard consolidative approach to preserve remission status with the best chance of cure; however, relapse occurs in approximately 30% of patients

and is the leading cause of post-transplant death. Measurable residual disease (MRD) detection prior to alloHCT is a strong predictor of relapse, though some patients without MRD detected by current methods will still experience relapse. CR is defined as less than 5% myeloblasts present cytomorphologically on bone marrow assessment using light microscopy, which leads to variable quantities of residual disease. Sensitive and accurate MRD testing in these patients is important for prognostication.

Baseline disease characteristics and MRD status may be used to develop risk-adapted patient tailored approaches [4, 5]. AML is characterized by multiple disease subsets harboring different molecular and cytogenetic profiles with different prognostic risk established by the European LeukemiaNet (ELN), updated in 2022 [3, 6]. Individual patients may also have multiple unique clones adding another layer of complexity. The inherent heterogeneity of AML poses a unique challenge for unified MRD assessment across disease subsets and in individual patients. The genetically diverse disease subsets not only behave differently, but they may warrant different methods for MRD assessment. Efforts are underway to establish standardized molecular methods of MRD detection of validated biomarkers that can then be integrated in therapeutic clinical trials to guide decision making. An ideal biomarker for MRD assessment would be solely present in AML clones, conserved throughout the disease course, correlate with therapeutic responses, be detectable by highly sensitive assays, and recur at the time of relapse.

The current ELN MRD guidelines established for AML in 2021 recommend MRD assessment using multiparameter flow cytometry (MFC-MRD) with a limit of detection of 0.1% analyzed using leukemia-associated immunophenotype and different from normal (Dfn) approaches [7]. The exceptions are patients with a validated molecular marker that can be followed by quantitative polymerase chain reaction (qPCR) with a sensitivity of approximately 1/100,000 including *NPM1*, t(8;21), inv(16), and t(15;17). The benefit of MFC-MRD is the ability to use this in all AML subtypes, at the expense of relatively low sensitivity and reliability. At present, MRD testing is not harmonized or regulated, leading to inconsistent results and suboptimal prognostication.

There is a strong correlation between MRD detection and higher risk of relapse with shorter survival at several timepoints including early after induction, post-induction, during consolidative chemotherapy, and before and after alloHCT [5, 8–17]. MRD is commonly encountered throughout the treatment course of AML,

with approximately 40% of patients in CR after induction therapy testing positive [18]. A systematic review and meta-analysis of over 11,000 patients with AML who underwent intensive treatment reported an estimated 5-year disease-free survival of 25% in those with MRD detected compared to 64% in those without [15].

### **MRD and Allogeneic Hematopoietic Cell Transplantation**

There is robust evidence that MRD prior to alloHCT is an independent prognostic risk factor for posttransplant relapse and decreased overall survival (OS), by various testing methods [19–24]. Potential strategies to address this population at increased risk include pretransplant therapies aimed at eradication of MRD, various intensified or targeted conditioning regimens, graft manipulation and immunotherapies (including engineered T cell products for graft versus leukemia effect, donor lymphocyte infusions, natural killer cells, vaccines), post-transplant maintenance therapy, preemptive MRD treatment with various therapies under clinical investigation, and early withdrawal of immunosuppressive agents.

### **Pretransplant Eradication of MRD**

Few studies have reported on post-remission/consolidation therapies to clear evidence of MRD and address whether to take patients testing positive for MRD directly to transplant or attempt to “erase MRD” with more therapy. There is some evidence that conversion to MRD test negativity prior to transplant with post-induction therapy is associated with lower chances of relapse, however, in patients who fail to respond to these therapies or develop complications from therapy, this may prolong time to transplant, alter their eligibility, and expose them to other complications.

Late MRD clearance has been shown to have similar benefit to other MRD negative remissions in a few series. Understanding patient and disease profiles most likely to attain late MRD negative remissions may help guide treatment approaches. In the GIMEMA AML 1310 study evaluating a risk-adapted MRD-directed approach, patients with late MRD clearance (post-consolidation negative, post-induction positive) occurred in 11% of patients – and these patients had similar 5-year OS and relapse-free survival (RFS) as those with early MRD clearance post-induction [12, 25]. Another retrospective

study reports comparable outcomes in patients who achieved early MRD-negative remissions after induction and those who achieved MRD-negativity with post-induction therapy in an analysis of molecular predictors of MFC-MRD clearance [26]. Importantly, this study reports specific cytogenetic and molecular profiles most likely to achieve MFC-MRD negative remission post-induction (core-binding factor (CBF), *CEBPA*, *NPM1*, as well as *RAS* mutations), and those who rarely achieved MRD-negativity prior to allogeneic transplant (*inv(3)*, monosomy 5/del(5q), monosomy 7/del(7q), *TP53*, *SF3B1*) even with further consolidation or salvage therapies. Patients with higher clonal diversity by single-cell sequencing also had lower rates of MRD-negativity.

Care must be taken to avoid characterizing any residual AML-associated somatic mutation as evidence of leukemic persistence or increased relapse risk ("MRD"), particularly in older patients and those with secondary AML (sAML). A study supporting this evaluated next-generation sequencing (NGS)-MRD in 192 older patients (median age 66) with AML undergoing alloHCT [27]. Most patients in this series had adverse risk genomics (52%) receiving less intensive induction regimens and conditioning therapies. The majority (79.7%) had residual detection of mutations at variant allele frequency at least 2%, with 18.3% of these patients only with *DNMT3A* or *TET2* [27]. Patients with secondary ontogeny and *TP53* mutated disease had higher levels of molecular persistence. Those with sAML had higher rates of relapse in the absence of molecular persistence, theorized to be from prior treatment selecting for more clonal diversity. This represents a distinct population of older patients with a high prevalence of adverse risk disease and requires further study with larger sample sizes.

One small study evaluating MRD as a decision point directed patients to more chemotherapy or proceeding directly to alloHCT, with no difference in outcomes detected between the two interventions [28]. Preemptive intervention of molecular failure in *NPM1*-mutated AML with chemotherapy versus direct alloHCT in 33 patients demonstrated no difference in 2-year OS (81.5% vs. 90%). Eight of these patients had hematologic relapse prior to salvage therapy with worse outcomes. This small series may be of importance particularly for *NPM1*-mutated AML, with a more sensitive qPCR MRD assay, and ability of alloHCT to eradicate lower molecular burden of favorable risk disease. A fear demonstrated here is the progression of disease some may experience while undergoing MRD-directed therapies delaying the option of alloHCT.

The benefit of MRD-negativity also expands beyond first remission. A study by the acute leukemia working

party of the European society of blood and marrow transplantation (EBMT) evaluated transplant outcomes in 1,042 adults undergoing alloHCT in second CR (CR2) with MRD positive or negative test results [29]. Two-year relapse rates were lower in patients with MRD test negativity compared to MRD positivity (24% [95 CI: 21–28] versus 40% [95 CI: 34–46],  $p < 0.001$ ). In this analysis, MRD testing was by MFC or PCR in those with a target, though a limitation was that the authors did not have access to this individual information to compare methodologies of MRD testing.

Overall, these studies support achieving MRD-test negativity prior to transplant at any time point is associated with better outcomes. Larger datasets reporting individual subsets of disease will be important for future MRD-directed approaches. The clinical utility of AML MRD testing before alloHCT in older patients, those with adverse ELN risk, and with sAML remains to be fully demonstrated.

Pretransplant MRD+ AML takeaway:

- MRD negativity prior to transplant is associated with lower relapse rates and longer OS.
- Well-designed prospective studies in MRD+ AML evaluating MRD clearance compared to proceeding with transplant are needed.
- Baseline disease characteristics may influence the potential of MRD clearance in various disease subsets.

### Conditioning Regimens and MRD

In younger fit patients, multiple lines of evidence demonstrate lower relapse rates with myeloablative conditioning (MAC) compared to reduced intensity conditioning (RIC) for patients with AML who test MRD positive prior to transplant. A registry analysis of 2,292 patients by the acute leukemia working party and EBMT assessed interactions of MRD and HCT conditioning regimen intensity in patients older or younger than 50 years of age undergoing alloHCT in first CR (CR1) [30]. Patients younger than 50 testing MRD positive benefited from MAC; RIC in this age group was associated with higher relapse (HR: 1.71) with a trend toward worse OS. There was no advantage of MAC in patients older than 50 years testing MRD-positive, however. Again, a limitation of this study was lack of access to genetic risk subgroups, MRD targets, and testing methodologies.

The randomized phase 3 clinical trial BMT-CTN 0901 supported the use of MAC when feasible, specifically for those in CR but with genomic evidence of MRD prior to

alloHCT [31]. Ultra-deep NGS MRD testing for commonly mutated genes was performed on 190 pretransplant blood samples from patients with AML in CR prior to randomization to either MAC or RIC. This study showed higher relapse rates (3-year cumulative incidence, 72% vs. 15%;  $p < 0.001$ ) and lower survival (3-year OS, 34% vs. 59%;  $p = 0.01$ ) in those with detectable AML mutations pretransplant who were randomized to receive RIC rather than MAC.

In patients with *NPM1*-mutated AML, testing MRD positive by qPCR, the 2-year OS was 50% for those receiving MAC versus 43% for those receiving RIC [32]. Conditioning intensity was not randomized and this difference was not statistically significant.

Important data on the clinical utility of flow cytometry for AML MRD testing in the peri-transplant period have come from a series of articles retrospectively describing observations from a very large cohort from a single center. These non-randomized reports have been unable to demonstrate the benefit of MAC over RIC to improve outcomes in MRD-positive disease pretransplant [33, 34], as would be expected when conditioning assignment is based on knowledge of patient and disease factors rather than by randomization to either intensity while blinded to pretransplant MRD test status. A series of 810 adults transplanted in first or second CR reported outcomes related to peri-transplant MRD dynamics (pre- and post-transplant) and impact of conditioning with MAC versus non-MAC [35]. Although pretransplant MRD positive test results were converted to a negative result posttransplant more commonly with a MAC (81.7%) than non-MAC (57.9%), when cleared by non-MAC the impact on outcomes were greater. Many patients converted to MRD test negativity in the intermediate term posttransplantation, thought to be anti-AML conditioning effect rather than graft versus leukemia. Another study reporting 279 patients from the same center undergoing MAC alloHCT for AML in CR1 or CR2 observed conversion to MFC-MRD negative disease 1 month posttransplant was not associated with improved outcomes [36].

Various levels of conditioning intensity within the umbrella term of “RIC” regimens have been described, and the effect of various intensities on MRD has been contemplated [37]. The FIGARO trial assessed a relatively intensified RIC regimen FLAMSA-Bu (fludarabine, amsacrine, cytarabine, busulfan) compared to standard fludarabine-based RIC, with no improvement in patients transplanted for AML with MFC-MRD positive disease [16]. Interestingly, having full donor chimerism at 3 months in this study appeared to abrogate the adverse impact of pretransplant MRD.

Conditioning and MRD takeaway:

- MAC, when feasible depending on patient characteristics and fitness, is favored in patients in clinical remission before transplant but with detectable MRD test positivity.
- Studies in various AML disease subsets with levels of MRD are needed to determine future recommendation on conditioning approaches in MRD+ disease.

## Novel Conditioning Approaches

Approximately 55–75% of newly diagnosed AML cases are in patients over the age of 65, with a median age of 69 at diagnosis, with survival decreasing with age [38–40]. Older adults with AML are more likely to have adverse risk disease, which may be associated with higher rates of MRD [27, 41]. Lack of disease control has been cited as one barrier to transplant [42]. These patients are generally ineligible for standard MAC regimens with high rates of non-relapse mortality (NRM), therefore novel conditioning regimens are needed to address these patients at high risk for relapse with RIC.

The ideal conditioning regimen aims to eradicate any residual malignancy and adequately provide appropriate immunosuppression to prevent graft failure. Current studies are evaluating the addition of venetoclax to standard conditioning regimens to provide further disease control. A phase I study evaluating the addition of venetoclax to FluBu2-based RIC in 22 patients with high-risk AML, myelodysplastic syndromes (MDSs) and MDS/myeloproliferative neoplasms demonstrated safety of this combination and encouraging signal of MRD clearance [43]. Similarly, venetoclax is being evaluated with MAC as well. A prospective phase II trial of MAC in 33 older patients with AML or MDS up to 70 years (median age 59) with adequate organ function using fractionated busulfan (administered over a longer period of time) with fludarabine, cladribine, and the addition of venetoclax demonstrated safety and promising early data. All patients received post-transplant cyclophosphamide and tacrolimus. The 1-year OS was 84%, with relapse incidence (RI) of 13% and NRM of 10%. Another study in younger patients (median age 25) reported outcomes of venetoclax with MAC in 31 high-risk AML patients who underwent their first alloHCT with low 600-day RI and NRM, 6.9% and 11.7%, respectively [44]. Of note, most patients received haploidentical transplant and high-risk AML was defined as refractory or relapsed AML, MRD-positive at transplantation, or adverse ELN risk.

Antibody-based conditioning regimens in the older population are also being explored, with the goal of less off-target toxicity. Monoclonal antibodies (MABs) may be coupled with radioisotopes to deliver targeted radioimmunotherapy to leukocyte progenitor cells with targets including anti-CD45 and anti-CD117, among others. JSP191 is an anti-CD117 MAB that is being studied in combination with fludarabine and low-dose TBI for patients with MDS or AML with pre-transplant evidence of MRD (NCT04429191) [45]. High rates of MRD clearance were observed in the first 17 patients, which appear to persist beyond day 30, presented at the 2022 Tandem meeting with full results awaited. The phase 3 SIERRA trial (Study of Iomab-B in Elderly Relapsed or Refractory AML) investigating the use of Iomab-B, an <sup>131</sup>I-labeled anti-CD45 MAB in patients who have lack of response to standard-of-care regimens and targeted therapy [46] demonstrated that 74.6% (44/59) of evaluable patients on the Iomab-B arm achieved initial CR/CRp compared to only 6.3% (4/64) evaluable on the conventional care arm. Durable CR rates at 6 months were 22% versus 0% (95% CI: 12.29, 34.73;  $p < 0.0001$ ) [47]. How such results will translate to the MRD context is unknown.

### Targeted AML Therapies and MRD

As we move to more targeted treatment approaches, clinical trials with newer agents are being paired with MRD assays to establish the value of MRD as a surrogate endpoint determining survival and relapse. Potential targets of disease eradication and MRD assays are discussed below.

#### Fms-Like Tyrosine Kinase 3-Mutated AML

Mutations in *FLT3* are common in newly diagnosed patients with AML, found in approximately 30% of cases [48, 49]. Activating mutations occur as internal tandem duplications (ITDs) interfering with the regulatory function of the juxtamembrane region, and tyrosine kinase domain (TKD) mutations affecting the activation loop resulting in constitutive activation. *FLT3*-mutated AML has been associated with proliferative disease and higher propensity of relapse, even after alloHCT [50–53]. The introduction of *FLT3*-inhibitors to frontline AML therapy may lead to deeper remissions [54]. The RATIFY trial leading to the approval of midostaurin did not include MRD testing, however, patients in CR that pro-

ceeded to alloHCT on the midostaurin arm had a reduction in relapse compared to the control arm, possibly due to higher rates of MRD eradication with the addition of midostaurin [54, 55]. A separate study demonstrated patients treated with *FLT3*-inhibitors in addition to chemotherapy had lower level detection of MRD using a combined PCR-NGS assay for *FLT3*-ITD mutations on paired diagnosis and remission samples [54]. Due to the occasional loss at relapse of the *FLT3*-ITD mutation this test is associated with high-rates of relapse when positive, but may not be suitable alone for longitudinal screening for early detection of impending relapse particularly in those treated with *FLT3*-inhibitor maintenance therapy. The recent pre-MEASURE study demonstrated the ability of ultra-deep NGS DNA-sequencing MRD testing of blood at the pretransplant landmark of patients in first CR from *NPM1* and/or *FLT3*-ITD-mutated AML to predict those with the highest risk for relapse and death after first alloHCT [56]. In both discovery and validation cohorts, residual variants of either mutation were associated with higher rates of relapse at 3 years (68% vs. 21%; HR: 4.32;  $p < 0.001$ ) and decreased survival at 3 years (39% vs. 63%; HR: 2.43;  $p < 0.001$ ), validated the use of NGS-MRD testing for this specific context of use. In contrast clinical flow cytometry performed at centers, prior to transplant, had limited or no clinical utility. The clinical utility of *FLT3*-ITD MRD testing by NGS in remission prior to transplant has been confirmed by multiple groups internationally and is likely to be adopted as a new standard of care for AML MRD testing [57, 58]. In addition, studies are considering a dose-response evaluation of MRD, exemplified in two recent ASH abstracts with *FLT3*-ITD detection having a dose dependent correlation between residual levels and adverse clinical outcomes, with variant allele frequency  $\geq 0.01\%$  identifying those with the greatest risk of relapse [59, 60].

In the SORMAIN study, patients with *FLT3*-mutated AML MRD-positivity prior to alloHCT derived the most benefit from posttransplant maintenance with sorafenib [61], although the modality used to test for MRD was not consistent. Another phase III trial of patients randomized to sorafenib or placebo post-transplant maintenance demonstrated reduced risk of relapse in both MRD-positive and negative patients [62]. Due to the relatively recent FDA approvals of midostaurin and now quizartinib in the frontline setting, most patients in these two trials did not receive a prior *FLT3*-inhibitor, which may have an impact on post-transplant maintenance outcomes. It may be possible that pretransplant *FLT3*-inhibitors lead to high pretransplant MRD-negativity.

The BMT-CTN 1506/MORPHO phase 3 study (NCT02997202) of gilteritinib posttransplant maintenance for *FLT3*-ITD mutated AML presented at the 2023 European Hematology Association conference demonstrated an overall 32% reduction in the risk of relapse compared to placebo with HR 0.679,  $p = 0.0518$ , found to be not statistically significant, missing the primary end point [63]. A NGS MRD platform for *FLT3*-ITD detection was paired with this trial, at a threshold of  $10^{-6}$  tested on pre- and posttransplant bone marrow aspirates. In patients testing positive for persistence of *FLT3*-ITD, there was a 48% reduction in relapse in those who received gilteritinib compared to placebo. The RFS benefit was lower in patients with MRD-negative disease. MRD eradication was seen in 68.8% of patients with gilteritinib and 43.6% with placebo. Interestingly, the outcomes were different based on location, with patients in North America showing significantly higher RFS (HR: 0.397;  $p = 0.0022$ ) compared to Europe, Asia, and rest of the world. Patients in North America were more likely to be treated with a *FLT3* inhibitor before transplant (93.5% vs. 36.6%, respectively). The levels of MRD detection pre- and posttransplant will be of interest in these patients, as will the level of *FLT3*-ITD persistence associated with greatest benefit from gilteritinib maintenance.

#### *FLT3* takeaway:

- Achieving NGS-MRD negative status prior to transplant is associated with lower relapse rates and longer survival.
- *FLT3*-inhibitors may result in more MRD negative remissions.
- Therapy with *FLT3*-inhibitors should strongly be considered in all patients with *FLT3*-mutated AML, and particularly in those with evidence of pre- or post-transplant MRD.

### Isocitrate Dehydrogenase-Mutated AML

Approximately 15–20% of patients with AML will have a somatic mutation in Isocitrate Dehydrogenase (*IDH1* or *IDH2*) [64, 65]. These mutations result in neomorphic enzyme activity resulting in the production of an oncometabolite, 2-hydroxyglutarate, which leads to global hypermethylation blocking cellular differentiation and increased self-renewal [66–68]. The FDA approval of *IDH*-inhibitors ivosidenib and enasidenib for *IDH1* and *IDH2* mutations, respectively, has expanded our repertoire of available therapies. These agents are active as single agents and may be able to produce deeper remissions in combination with other therapies. Previous

work has shown therapy with hypomethylating agents and venetoclax (HMA + VEN) has shown preferential activity in patients with *IDH1/2* mutations, with an ORR in one study of 79% in either the newly diagnosed (ND) or relapsed/refractory (R/R) setting [69, 70]. Interestingly, 90% of patients with CR or complete response with incomplete count recovery (CRi) had MRD-negative status by MFC-MRD, while only 52% were negative by NGS-MRD at the time of best response [69]. Patients with the presence of *RAS*-pathway or *TP53* mutations may confer resistance.

In those with *IDH1*-mutated advance myeloid malignancy, combination therapy with ivosidenib with venetoclax with or without azacitidine in the ND or R/R setting is associated with high rates of composite CRs (CRc) in a phase Ib/II study [71]. In patients receiving triple therapy, the CRc rate was 90% compared to 83% in those receiving ivosidenib with venetoclax. MFC-MRD negative status was reached in 63% of evaluable patients, with increased rates in patients receiving triplet therapy though not statistically significant due to small cohort. *IDH1* clearance occurred in 64% of patients receiving five or more cycles by digital droplet PCR.

In patients with *IDH2*-mutated AML, combination of enasidenib with azacitidine with or without venetoclax in the ND or R/R setting is associated with high rates of CRc in a single center phase II study [72]. In the ND cohort, all 7 patients achieved MFC-MRD negative CRc and 4/7 received triplet therapy. In the R/R cohort among 18 patients, the CRc rate was 61% with only 22% achieving MFC-MRD negative status; 6/7 patients who received triplet therapy achieved CRc.

In similar fashion, a phase I study evaluated the combination of ivosidenib or enasidenib with intensive chemotherapy (IC) for newly diagnosed *IDH1/2*-mutated AML [73]. In those with *IDH1*, ivosidenib-chemo at the end of induction led to CRc in 72%; and in those with *IDH2*, enasidenib-chemo led to CRc in 63%. Of patients who achieved CR, molecular clearance of *IDH1* or *IDH2* was observed in 39% and 23%, respectively, by digital PCR.

To determine if pretransplant clearance of *IDH* mutations was associated with survival outcomes in 56 patients *IDH*-mutated AML undergoing nonmyeloablative alloHCT, digital droplet PCR (ddPCR) or NGS MRD testing were used to detect *IDH1/2* mutations [74]. Contrary to results seen with *FLT3*, the presence of pre- or posttransplant *IDH* MRD was not prognostic of leukemia relapse. This was a relatively small series, and several patients received *IDH*-inhibitors pre- or posttransplant. Another small series that assessed MRD detection of

*IDH1/2* pretransplant with ddPCR or NGS in 44 patients demonstrated specific mutation hotspots detected prior to transplant of *IDH1* R132 and *IDH2* R172 associated with higher relapse rates [64].

The largest series of pretransplant MRD testing for patients with *IDH1* mutated AML ( $n = 148$ ), however, was unable to detect any association between the persistence of *IDH1*mut and inferior posttransplant outcomes. No posttransplant differences were observed between those testing *IDH1*m positive ( $n = 53$ , 36%) and negative pretransplant (OS:  $p = 0.4$ ; relapse:  $p = 0.5$ ). For patients with *IDH1* mutated AML co-mutated with *NPM1* and/or *FLT3*-ITD, only detection of persistent mutated *NPM1* and/or *FLT3*-ITD was associated with significantly higher rates of relapse ( $p = 0.01$ ) confirming the utility of these NGS-MRD targets [75]. Another evaluation of pretransplant MRD testing performed in 257 patients with *IDH2* mutated AML showed *IDH2*mut persistence was predictive of increased relapse rates and worse OS – however, only in the absence of concurrent *NPM1* or *FLT3*-ITD mutations [76]. In patients with *IDH2* mutations that have concurrent mutations in *NPM1* and/or *FLT3*-ITD, the latter two genetic markers were more predictive of relapse and OS by NGS-MRD. This may be due to differentiated cells maintaining *IDH* mutations in the absence of residual disease.

Posttransplant maintenance studies for *IDH*-mutated AML are still in their infancy, with phase I data available demonstrating tolerability posttransplant [77, 78]. Larger studies of peri-transplant *IDH* NGS-MRD testing and posttransplant maintenance studies are needed.

*IDH1/2* takeaway:

- *IDH*-mutation persistence may not be indicative of leukemic persistence or increased relapse risk.
- Additional studies are needed to determine the role of NGS-MRD in *IDH*-mutated AML in those treated with less-intensive regimens, those who do not undergo transplant, and those receiving posttransplant maintenance therapy.
- *IDH*-inhibitors may improve clinical outcomes but the relationship between MRD status and outcomes is unknown in this context.

### Hypomethylating Agents with Venetoclax and MRD

The standard induction therapy for patients unfit for IC consists of hypomethylating agents with venetoclax (HMA/Ven). MRD status by MFC has demonstrated prognostic significance regardless of induction with IC or HMA/Ven [79, 80]. In the VIALE-A trial of azacitidine with venetoclax (AZA/Ven) in treatment naïve patients with



















AML, MRD negativity by MFC occurred in 41% of evaluable patients in CR, strongly correlating with duration of response and survival outcomes [80]. This included patients at any time point in CRc achieving MRD-negativity. The OS benefit of MFC-MRD negative responses was also seen in patients' sAML, and the median OS was not reached in patients with MFC-MRD negativity even with intermediate and poor risk cytogenetics. Similar findings were observed in a study evaluating response of 10-day decitabine with venetoclax (DEC/Ven) frontline with 54% of those in CRc or MLFS achieving MRD-negative remissions by MFC [80]. MRD negative responses were less common in patients with sAML, therapy-related AML, and adverse-risk cytogenetics (33–53%). Patients with ELN adverse risk disease achieving MRD-negativity by MFC had longer median event-free survival than those who never achieved MRD negativity (13.5 months vs. 5.8 months;  $p = 0.001$ ). In this study, the benefit of MRD-negativity on OS was similar at across various time points between one and 4 months, demonstrating prognostic value of late MRD clearance [81]. In another retrospective series, of patients who achieved CR with either HMA/Ven or IC, MRD negativity was achieved in approximately 80% of patients in both arms [79].

Molecular predictors of response to HMA/Ven have been explored [80, 82]. In the VIALE-A trial, of patients who achieved CRc, MRD-negative response rates were 50% (10/20) in patients with *FLT3*-mutations, 49% (21/43) in patients with *IDH1/2*-mutations, 30% (6/20) in patients with *TP53*-mutations, and 88% (15/17) in patients with *NPM1*-mutations. One series reports favorable response in patients with *ASXL1* mutations with CRc in 83% (15/18) patients, however 60% of these patients had MRD, with relapse occurring in 73% (11/15) [82].

In patients' ineligible for alloHCT after IC in CR1, oral azacitidine (CC-486) is approved as maintenance based on the phase 3 QUAZAR AML-001 trial [83]. Oral azacitidine improved OS and RFS independent of baseline MRD status [84]. In this study, 37% of patients had MRD at baseline, with only 19% remaining positive while on CC-486; conversion to MRD negativity was associated with longer OS and RFS. The duration of MRD-negativity was also extended by 6 months compared to placebo. CC-486 maintenance posttransplantation has also been studied with infrequent grade 3/4 adverse events [85]. The phase 3 trial of oral azacitidine (CC486) maintenance (AMADEUS; NCT04173533) after alloHCT is currently recruiting.

The RELAZA2 phase II trial evaluated the preemptive use of subcutaneous azacitidine in patients with detectable MRD after chemotherapy or alloHCT using by qPCR for *NPM1*-mutated disease or fusion detection or by falling

**Table 1.** Possible outcomes of attempts to “Erase MRD”

| Intervention | MRD test result  | Impact on relapse rate   | Impact on death rate   | Overall  |
|--------------|--|--|--|--|
| Therapy A:   |   |   |   | Ideal MRD marker and effective therapy                                 |
| Therapy B:   |   |   |   | MRD test result decreases after therapy but clinical outcome unchanged |
| Therapy C:   |   |  or  |   | Toxic therapy (“risk greater than benefit”)                            |
| Therapy D:   |   |   |  or  | Ineffective therapy  |
| Therapy E:   |  or  |   |   | Outcomes improved by therapy, but not reflected by MRD test            |

As we move toward NGS-MRD testing, various genetic markers will be assessed for their association with disease status, relapse, and death to determine their role as a surrogate endpoint. It is appealing to think that additional treatment of a patient testing MRD positive would convert a test result to negative and that would be associated with lower relapse and death (“MRD erasing”). This ideal scenario is displayed with therapy A; however, other potential outcomes are shown from B–E. A: MRD test results levels decrease with therapy A correlating with reduced relapse rates and death. B: MRD test levels decrease with therapy B with no impact on relapse or death rates. C: MRD test levels decrease with therapy C with decreased relapse but increased death rates. D: Therapy is ineffective and does not change MRD test result levels or clinical outcome. E: MRD test result levels are unchanged or increased with therapy but associated with decreased relapse and death rates.

chimerism [86]. In this study 58% of patients had a response with 36% achieving MRD-negativity with 2-year RFS of 46%. The ongoing phase 3 VIALE-T study is examining the safety and efficacy of maintenance azacitidine combined with venetoclax (NCT 04161885).

HMA/Ven and MRD takeaway:

- Achieving MRD-negativity appears correlated with duration of response and survival outcomes.
- MRD negative responses were less common in those with adverse risk cytogenetics, *TP53* mutation, secondary or therapy-related AML.
- Oral azacitidine maintenance may convert some MRD test positive patients to MRD test negativity, which was associated with longer OS and RFS.

### Future Targets

With growing understanding of the molecular pathogenesis of AML and molecular predictors of therapeutic response, more targeted approaches will likely be available in the future. The phase I AUGMENT-101 trial showed

favorable tolerability and promising results of SNDX-5613 (revumenib) in *KMT2A*-rearranged and *NPM1*-mutated AML, with an overall response rate of 53%, with 78% (18/23) achieving MRD-negativity by flow cytometry [87]. Both molecular targets may warrant their own MRD assays by qPCR or NGS-MRD with deeper sensitivity than MFC-MRD. There is prior data demonstrating early achievement of real-time qPCR negativity in patients with t [9, 11] AML associated with longer remissions [88]. This is one example where a correlative study with ongoing clinical trials may help validate new MRD assays of these stable targets and determine the use of MRD as a surrogate endpoint of meaningful clinical outcomes.

The updated 5th edition of the World Health Organization Classification of AML reports defining genetic abnormalities including *PML::RARA*, *RUNX1::RUNX1T1*, *CBFB::MYH11*, *DEK::NUP214*, *RBM15::MRTFA*, *BCR::ABL1*, *KMT2A* rearrangement, *MECOM* rearrangement, and *NUP98* rearrangement [89]. These fusions are all potential targets for MRD monitoring of AML; however, the non-favorable risk fusions are yet to be validated as MRD markers.

**Table 2.** Ongoing or upcoming AML MRD clinical protocols

| Protocol                                 | Design/phase/aim   | MRD assay  | Endpoints   | Trial number/status  |
|--|--|--|---|--|
| AML26<br>INTERCEPT(102)                  | Australasian leukemia and lymphoma group Platform, phase 1B/2<br>Evaluating MRD monitoring in CR1/2 and novel biomarker-directed MRD treatment interventions   | <i>FLT3</i> -ITD, <i>t</i> (8;21) <i>inv</i> (16), <i>NPM1</i> , <i>KMT2A::X</i> and MFC   | Primary: MRD response<br><br>Secondary: RFS, OS, time to MRD response, duration of response | ANZCTR: ACTRN12621000439842<br>Multicenter<br>Non-randomized.<br>Recruiting<br>N = 500 |
| myeloMATCH                               | NCI precision medicine initiative – Umbrella protocol – 4 tiers (induction, consolidation/MRD eraser, transplant, maintenance)<br>Evaluating genomically assigned frontline treatments for myeloid disorders in tier 1; in later tiers, the stated focus is to target residual disease precisely | Baseline centralized rapid karyotyping and NGS with 72-h turn-around; centralized response assessment by MRD testing by MFC and duplex NGS | Unknown (may include MRD endpoints)   | NCT05564390<br>Multicenter<br>Randomized?<br>Not yet recruiting<br>N = 750             |
| MEASURE                                  | CIBMTR/NMDP.<br>Prospective, multicenter. Non-randomized.<br>Determining the clinical utility of molecular monitoring of MRD in patients with AML in CR undergoing alloHCT   | NGS-based monitoring centralized at NIH  | Primary: OS, CIR<br><br>Secondary: Biology of relapse                                       | NCT05224661<br>Multicenter.<br>Non-randomized.<br>Recruiting<br>N = 1,000              |
| Washington University School of Medicine | "Improving Risk Assessment of AML With a Precision Genomic Strategy to Assess Mutation Clearance."<br>Investigator's choice consolidation (HiDAC or alloHCT) for patients with persistent leukemia-associated mutations $\geq 2.5\%$ , HiDAC for those $< 2.5\%$                                 | Persistent leukemia-associated mutations, defined as leukemia associated mutations VAF $\geq 2.5\%$  | Primary: RFS compared to historical control<br><br>Secondary: OS                            | NCT02756962<br>Single center.<br>Non-randomized.<br>Recruiting<br>N = 110              |

VAF, variant allele frequency.

### Differentiating Clonal Hematopoiesis from MRD

Multiple genomic abnormalities can occur in a stepwise fashion leading to the emergence of AML. Clonal hematopoiesis of indeterminate potential (CHIP) is frequently found in older individuals, often involving mutant epigenetic regulators *DNMT3A*, *TET2*, and *ASXL1* (*DTA* mutations) resulting in subclinical clonal expansion, which may be present many years before disease occurs [90]. These mitotically active initial clones are at risk for subsequent mutational

acquisition, possibly by unrepaired replication errors. A distinctive pathway that has been described leading to AML involves a mutation in epigenetic regulators, followed by a mutation in *NPM1* or transcription factors, followed by a signaling pathway mutation in *FLT3* or *RAS* [91]. These "pre-leukemic" mutations may persist in patients in remission, and their significance has been debated. Some studies show increased risk of relapse after induction with persistence of preleukemic mutations and others show no impact [24, 92–94]. Patients with persistent *DTA* mutations pretransplantation also did not differ in outcomes with MAC

versus RIC [31]. Current ELN MRD guidelines recommend exclusion of *DTA* mutations in MRD analysis.

Several other preleukemic expanding lesions have been proposed including *IDH2*, *SF3B1*, *U2AF1*, *TP53*, *JAK2*, and translocations *RUNX1::RUNX1T1*, *CBF*, and *MLL* translocations [91]. As previously described, hotspot mutations in *IDH1/2* have shown differential impact on prognosis with low levels of persistent *IDH1* R132 and *IDH2* R172 associated with relapse and higher levels of persistent *IDH2* R140 not associated with relapse [64].

MRD status in patients with sAML (often with multiple mutations) has not been as prognostically informative, possibly due to similar difficulty differentiating clonal hematopoiesis and precursor clones from persistent leukemia capable of causing relapse [95, 96]. One study shows MRD by MFC was uninformative of outcomes after induction therapy, and that patients with sAML and two or more mutations had a survival benefit undergoing transplant, however, pretransplant MRD was not reported [97]. A separate series by the ALWP and EBMT of 318 patients with sAML undergoing alloHCT, reported MRD by MFC or PCR-NGS was not informative of posttransplant outcomes [18].

### Improvements in MRD Testing

There are many patients without pretransplant MRD by MFC who relapse posttransplant [30]. Deeper sensitivity molecular MRD methods may better predict patients at risk for relapse [24, 31]. NGS MRD testing is prognostically important at various time points, including post-induction and pretransplantation, in patients with AML [24, 31, 98]. Early studies evaluating MRD by NGS in addition to MFC showed these methods to be generally concordant with worse outcomes among patients who tested positive by more than one method [24, 32, 93, 99, 100]. More recent data suggests MFC-MRD adds little to NGS-MRD testing in specific molecular subsets. NGS-MRD is able to detect the majority of cases with MFC-MRD+ disease who relapse and many relapsing patients are MFC-MRD negative [99, 101]. The most important prognostic time points are still being discerned. In one series, patients who cleared mutations earlier, post-induction rather than post-consolidation, had significantly improved OS [99]. Yet another study shows patients who achieved late NGS-MRD negative disease post-consolidation had similar good prognoses as those who were MRD negative after induction [100].

There are various sequencing approaches being evaluated for molecular MRD detection [101, 102]. A digital targeted

RNA-sequencing-based approach was able to detect all ELN defined MRD targets, and two-thirds of patients in a single standardized assay with a sensitivity of 1/100,000 events, similar to that seen with qPCR [101]. Subsequently, duplex sequencing was tested on post-induction patient samples from the randomized phase 3 SWOG-S0106 clinical trial [101]. Duplex sequencing, which generates double-stranded consensus sequences to reduce false positive errors, outperformed MFC-MRD testing showing 35% of patients with MRD compared to 16% detected by MFC, and association with higher relapse rates [101].

Further large-scale studies comparing centralized high quality MFC and ultrasensitive NGS approaches are needed. To address this need, the multicenter national prospective MEASURE (Molecular Evaluation of AML Patients After Stem Cell Transplant to Understand Relapse Events) study (NCT05224661), sponsored by the Center for International Blood and Marrow Transplant Research, is currently recruiting at 18 major transplant centers across the USA. This study will assess genomic sequencing from diagnostic samples, and subsequent pre- and post-transplant samples for MRD with molecular detection methods at a centralized site. Efforts to harmonize current MRD testing approaches are underway by the ELN and FNHI AML MRD Biomarkers Consortium aimed at validating new methods of molecular MRD detection in AML.

### MRD as a Surrogate Endpoint

A surrogate endpoint must have substantial evidence supporting its prediction of a clinical outcome. After this is demonstrated that endpoint may be used for both accelerated and traditional regulatory approval. Data from prospective clinical trials incorporating MRD are necessary to build evidence of MRD as a surrogate endpoint for relapse and survival in various subsets of disease with highly sensitive, standardized approaches. MRD may be used in the future to pair disease subsets with various therapeutic approaches and to guide post-remission therapies. Demonstration of the depth of response seen with certain therapeutics may show specific subsets of disease that are highly sensitive to different approaches. The MRD responses can then be assessed for correlation with survival outcomes and relapse to best pair patients with targeted therapeutics. Second, patients who have MRD-positivity after standard treatments are at increased risk for relapse and death representing a population with an unmet medical need. Ultimately, well-designed randomized clinical trials are needed to determine the best management of these patients.

## MRD “Erasers”

Due to the inherent complexity of AML with multiple clones and genetic diversity, a single approach to identify and subsequently “erase” or target MRD across all AML subsets has yet to be identified. The successful MRD erasing therapy, blinatumomab in acute lymphoblastic leukemia (ALL), targets a single cell marker (CD19) using a bispecific T cell engager – and use before transplant to achieve MRD-negativity correlates with improved outcomes. Thus far immunotherapeutic approaches in AML have not been as promising, however, evolving efforts persist [103, 104]. The various disease subsets in AML may have different sensitivities to therapeutic approaches, and therefore likelihood of achieving MRD-negativity [26]. We know that on average patients with evidence of MRD who undergo alloHCT with MAC will have longer term survival after transplant than if they received RIC, however, the toxicity of MAC must be considered with high non-relapse mortality seen in older adults [30, 31]. Even if therapeutic “MRD erasing” strategies could be identified for AML, the key consideration is the association with biomarker clearance and subsequent clinical outcomes.

Detection of some residual mutations or abnormal immunophenotypes may not represent a persistent AML – persistence of such features may not be representative of relapse. Conversely, treatment may make the result of a MRD test turn from positive to negative (“MRD-erasing”); however, the patient may still experience a relapse. Examples of these scenarios are highlighted in Table 1. Rigorously designed randomized studies of interventions with robust MRD monitoring must be designed by test the hypothesis that additional intervention on patients testing MRD positive can improve OS. Current large-scale initiatives aiming to generate evidence supporting the use of MRD testing in the clinical care of patients with AML are listed in Table 2 [105].

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## Conclusion

The current use of MRD in clinical daily practice is informed by ELN MRD consensus guidelines from 2021 recommending MRD testing with MFC and qPCR for validated molecular markers (*NPM1*, t(15;17), t(8;21), inv (16)) The studies mentioned in this review guide how we interpret MRD results in various disease subsets. The emergence of NGS-MRD will likely improve our ability to accurately understand therapeutic responses in multiple disease subsets of AML. The genetic diversity seen in patients with AML poses great challenge for a unified approach for MRD testing. Standardized MRD testing approaches paired with randomized clinical trials of potential interventions will be necessary to ultimately improve our understanding and treatment of this disease.

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## Author Contributions

Amanda L. Blackmon wrote the manuscript. Christopher S. Hourigan edited the manuscript.

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