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Real-world heterogeneity in the prognostic value of pre-transplant flow cytometry measurable residual disease in acute myeloid leukemia in first complete remission: CIBMTR analysis

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Running head

Real-world heterogeneity of pre-HCT MFC-MRD in CR1

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Data availability

CIBMTR makes its publication analysis datasets freely available to the public for secondary analysis while safeguarding the privacy of participants and protecting confidential and proprietary data: https://cibmtr.org/CIBMTR/Resources/Publicly-Available-Datasets#.

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Conflict of interest

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Contribution:

J.M.T., C.S.H., and F.E. were responsible for conception and design, and developed the methodology; W.L., M.T.N., F.M., N.B., V.B., L.B., W.S., K.M.P., and C.S.H, were responsible for acquisition of data (eg, acquired and managed patients, provided facilities); J.M.T., M.Z., C.S.H., and F.E. analyzed and interpreted the data (eg, statistical analysis, biostatistics, computational analysis); J.M.T., M.Z., W.L., M.T.N., F.M., N.B., V.B., L.B., W.S., G.G., L.W.D., R.B.W., C.S.H., and F.E. wrote, reviewed, and/or revised the manuscript; and W.L., M.T.N., N.B., V.B., L.B., and W.S. provided administrative, technical, or material support (ie, reporting or organizing data, constructing databases).

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ABSTRACT

In this real-world, large, observational study from the Center for International Blood and Marrow Transplant Research (CIBMTR), we examined the association between pre-transplant measurable residual disease (MRD) detected by multiparameter flow cytometry (MFC) test results and outcomes after allogeneic hematopoietic cell transplantation (alloHCT) in patients with acute myeloid leukemia (AML) in first complete remission (CR1). We included 2,544 patients who underwent transplant during 2013-2019; 11% had detectable MRD prior to alloHCT. Patients' median age was 58 years. Among MRD-negative and MRD-positive groups, 48% vs 52% received myeloablative conditioning, and 37% vs 29% had matched unrelated donors, respectively. The 1-year cumulative incidence of relapse was 35% in the MRD-positive group and 25% in the MRD-negative group ($P < .001$). MRD positivity was associated with inferior overall survival (hazard ratio [HR], 1.27; 95% CI, 1.06-1.51; $P = .009$) and disease-free survival (HR, 1.31; 95% CI, 1.11-1.53; $P = .001$), and increased relapse risk (HR, 1.42; 95% CI, 1.17-1.72; $P < .001$), but not with non-relapse mortality. Notably, patients with pre-alloHCT MRD negativity remained at high risk of relapse, underscoring the limited prognostic utility of registry-reported MFC-MRD testing due to variability in methods and thresholds. Survival analyses across the 12 largest centers demonstrated substantial variability in the prognostic impact of MRD. These findings underscore that although pre-alloHCT MRD by MFC remains a clinically relevant prognostic biomarker, its reliability is contingent upon methodological standardization across centers. These findings highlight the need for standardized MRD assessment to improve risk stratification in AML.

INTRODUCTION

Measurable residual disease (MRD) in acute myeloid leukemia (AML) has emerged as an important tool for prognostication, monitoring, and assessment of response to therapy (1). Several technologies can be used for MRD monitoring in AML, including multiparameter flow cytometry (MFC) and molecular testing, including next-generation sequencing (NGS) and polymerase chain reaction (PCR). However, most MRD assays lack technical and interpretive standardization, making testing heterogeneous, and clinical interpretation challenging.

Despite these limitations, numerous studies show that MRD detection during morphological remission, following chemotherapy and prior to allogeneic hematopoietic cell transplantation (alloHCT), is associated with an increased risk of relapse and inferior survival outcomes, regardless of the testing modality (2-9). Among available techniques, MFC remains the most widely accessible and cost-effective, and least time-intensive, method for MRD testing in AML. A 2017 survey found that 69% of leukemia physicians in the United States routinely incorporated MRD assessment into the management of patients with AML, underscoring the need for standardized MRD testing (10). However, MRD assessment by MFC remains challenging (11, 12). The MFC technique relies on identifying a diagnostic leukemia-associated immunophenotype or “different from normal” aberrant immunophenotypes to track the leukemia clones, both of which are user-dependent and require an experienced hematopathologist to detect and confirm the leukemia clones, introducing potential variability. Although MRD by MFC in AML can predict survival (13, 14), its utility in guiding therapeutic decisions can be deceiving due to the complexity of the disease biology, clonal heterogeneity, and the potential for false negative results (15).

Efforts by cooperative groups such as the European LeukemiaNet (ELN) have aimed to harmonize MFC-MRD methodology and reporting criteria, but implementation remains

voluntary, and centers are not required to follow standardized protocols. As a result, considerable variability persists in how MRD testing is performed and reported across institutions (1, 11). Centralized testing, as implemented by large European trial groups, has improved reproducibility, but MRD testing in the United States remains largely decentralized, with varying methodologies and thresholds across laboratories. Furthermore, while the US Food and Drug Administration (FDA) has issued guidance on the use of MRD as a biomarker and outlined criteria for assay performance, laboratories are not required to obtain FDA clearance or approval, leaving compliance to individual laboratories (16).

In this study, we evaluated the association between pre-alloHCT MFC clinical MRD testing results, as currently performed, and reported in routine clinical practice to CIBMTR, with post-transplant outcomes, including relapse, overall survival (OS), non-relapse mortality (NRM), and disease-free survival (DFS) in patients with AML in first complete remission (CR1). In addition, we explored inter-laboratory variability in the prognostic value of MRD across transplant centers, to assess how methodological heterogeneity affects its clinical utility.

METHODS

Data source and patient eligibility

This retrospective observational study was conducted through the Center for International Blood and Marrow Transplant Research (CIBMTR). It included adult (≥18 years old) patients with AML in CR1 who underwent their first alloHCT during 2013-2019 at 131 centers in the United States. Eligible patients had available MRD by MFC data before allogeneic HCT and ≥1-year follow-up, including those who died within the first year. MRD by MFC was assessed according to local transplant center criteria and protocols, with results reported to the CIBMTR as either MRD-negative or MRD-positive, based on the local laboratory's cutoff. This binary classification

reflects how MRD data are routinely captured in the registry and facilitates uniform analysis across centers. Patient-, disease-, and transplant-related covariates that were included in the analysis are noted in **Table S1**. CIBMTR data undergo standardized quality assurance procedures (**Supplementary Methods**). The study was approved by the NMDP Institutional Review Board.

Study endpoints

The study's primary outcomes included OS, DFS, NRM, and relapse. OS was defined as time from transplantation to death from any cause; survivors were censored at last follow-up. DFS was defined as time from transplantation to relapse or death. NRM was defined as death without prior relapse, including complications or adverse effects related to transplant complications or adverse effects of the pre-transplant conditioning regimen. OS, DFS, and NRM were assessed at 1- and 3-years post-HCT. Relapse was defined as disease detected by molecular, flow cytometric, cytogenetic, or clinical/hematologic assessment in bone marrow, blood, or an extramedullary site according to reporting center and assessed at 1-, 3-, and 5-years post-HCT. MRD positivity alone, in the absence of clinical or morphological evidence of disease progression, was not considered a relapse event unless explicitly reported as such by the center. Outcomes were also evaluated by transplant center to explore inter-laboratory variability in the prognostic impact of MRD.

Statistical analyses

Descriptive statistics were used to summarize patient, disease, and transplant characteristics. Continuous and categorical variables were compared using Wilcoxon rank-sum and Pearson chi-square tests, respectively. Survival was estimated by Kaplan–Meier and relapse by cumulative incidence.

Multivariable analysis (MVA) was performed using the Cox proportional hazards model to adjust for patient, disease, and transplant factors (**Supplementary Methods**). Clinically relevant patient, disease, and transplant variables were included in the Cox model, and retained if significant. Interactions between the main effect (MRD-status) and all covariates were tested at a significance level of $P < .05$.

To examine whether the effect of MRD on outcomes differed across transplant centers, we included an interaction term between MRD status and transplant center (treated as a fixed effect) in the Cox proportional hazards models. Model improvement was evaluated using a likelihood ratio test. To ensure stable estimation, subsequent center-level analyses were restricted to centers with ≥50 patients. For these centers, relapse, DFS, and OS were compared by MRD status, using both univariate and multivariable models adjusted for clinical covariates.

Analyses were restricted to patients with research-level data. We also examined associations between patient age, conditioning intensity, and MRD status. We categorized age at the time of diagnosis as <50 vs ≥50 based on the categories used by Gilleece et al (14).

RESULTS

Patient characteristics by MRD status

Between 2013 and 2019, a total of 3,091 patients with AML in CR1 undergoing alloHCT in the US were identified with complete follow-up (**Table S2, Table S3**). After excluding 549 patients with missing pre-transplant MFC-MRD data (**Table S3**), 2,542 patients were eligible for analysis (**Table S1**). Out of these patients, 2261 (89%) patients had MRD-negative disease (MRD-neg) and 281 (11%) had MRD-positive disease (MRD-pos). The median age of patients was 58.3 years (range 18-80.8) in the MRD-neg group and 57.6 years (range 18.2-77.1) in the MRD-pos group ($P = .40$). Most patients were male (54%), white (76%) and had a KPS of ≥ 90 (56%).

Disease characteristics by MRD status

Most patients included had de novo AML (78% in MRD-neg and 77% in MRD-pos groups) (**Table S1**). In the MRD-neg group, intermediate-risk AML by ELN 2017 was the most common (46%), whereas adverse-risk AML was the most predominant in the MRD-pos group (46%). Time to transplant was similar across the MRD groups, with a median of 4.5 months (range, 1.5-31.2) in the MRD-neg and 4.4 months (range, 1.9-17.2) in the MRD-pos groups.

Transplant characteristics by MRD status

The MRD-pos and MRD-neg groups were generally balanced for transplant-related characteristics (**Table S1**), except for differences observed in donor type ($P < .01$), donor-recipient sex match ($P < .01$), donor-recipient cytomegalovirus (CMV) serostatus ($P < .01$), graft source ($P < .01$), GVHD prophylaxis ($P = .02$), and the year of alloHCT ($P < .01$).

Overall survival

Post-transplant OS was worse in the MRD-pos group vs MRD-neg group at 1 year (67%, 95% CI 61-72, vs 72%, 95% CI 70-74; $P = .045$) and 3 years (48%, 95% CI 41-54 vs 56%, CI 54-58; $P = .008$) (**Table 1**). In multivariable analysis, MRD positivity compared to MRD negativity was associated with lower OS (HR, 1.27; $P = .009$) (**Table 2 & Figure 1A**). Other factors associated with inferior survival outcomes, which were adjusted for in the MVA model, included older age (> 50 years); lower KPS (< 90%); AML transformed from MDS; ELN 2017 intermediate- and adverse-risk AML; and use of nonmyeloablative conditioning regimens.

Non-relapse mortality

NRM probabilities were similar between MRD-pos and MRD-neg groups at 1 (14%, 95% CI 10-18 vs 14%, 95% CI 12-15; $P = .8802$) and 3 years (16%, 95% CI 12-21 vs 18%, 95% CI 17-20; $P = .3315$, respectively) (**Table 1**). In multivariable analysis, we observed no significant

difference in NRM based on MRD status (HR, 0.97; P = .8312) (**Table S4 & Figure 2A**). Factors associated with increased NRM, which were adjusted for in the MVA model, included older age (> 50), AML transformed from MDS, higher HCT-CI ≥ 3, and umbilical cord blood graft source.

Disease-free survival

DFS was significantly worse for the MRD-pos group compared to the MRD-neg group at 1 year (50%, 95% CI 44-56 vs 62%, 95% CI 60-64; P < .001) and 3 years (40%, 95% CI 34-45 vs 47%, 95% CI 45-49; P = .016) post-alloHCT (**Table 1)**. In multivariable analysis, MRD positivity compared to MRD negativity was associated with a lower DFS (HR, 1.31; P = .001) (**Table 3, Figure 1B**). Patients with AML transformed from MDS had a significantly lower DFS (HR, 1.50; P < .001). Conditioning regimens also impacted DFS, as both reduced-intensity conditioning (RIC) (HR, 1.28; P < .001) and nonmyeloablative conditioning (NMA) (HR, 1.65; P < .001) had lower DFS compared to myeloablative conditioning (MAC). ELN risk category also impacted DFS, with patients with adverse ELN17 risk category having a lower DFS than those with favorable risk (HR, 1.55; P < .001). However, those with intermediate-risk ELN 2017 risk category did not show significant differences from those with favorable-risk disease (HR, 1.16; P = .091). Patients with HCT-CI ≥ 3 vs HCT-CI 0 had lower DFS (HR, 1.26; P = .004).

Relapse

Patients in the MRD-pos group had a higher probability of relapse compared to the MRD-neg group at 1 year (35%, 95% CI 29-40 vs 25%, 95% CI 23-26; P = .001) and 3 years (43%, 95% CI 38-49 vs 36% vs 36%, 95% CI 34-38; P = .019) post-alloHCT (**Table 1 and Figure 2B**). However, both groups had similar relapse rates at 5 years (45%, 95% CI 38-51 vs 41%, 95% CI 38-43; P = .239). In multivariable analysis, MRD positivity remained an independent predictor for relapse (HR, 1.42; P < .001) (**Table S5**). Relapse was also significantly higher with AML transformed from MDS vs de novo AML (HR, 1.55; P < .001) and with adverse-risk ELN 2017 vs

favorable (HR, 1.75; P < .001). The intensity of the conditioning regimen also impacted the risk of relapse, as both RIC (HR, 1.28; P = .002) and NMA (HR, 2.02; P < .001) were associated with a higher risk of relapse compared to MAC. However, despite highly significant statistical correlations noted at the cohort level, only 12% (n = 47) of those who relapsed by 1 year (n = 389) and 10% (n = 60) of those who relapsed any time after transplant (n = 620) had a positive pre-transplant MFC test result.

When stratified according to ELN 2017 risk, the prognostic impact of MFC-MRD varied across risk groups. In favorable-risk AML, MFC-MRD status was not significantly associated with clinical outcomes; however, this subgroup was underrepresented in the present cohort, reflecting the lower likelihood of these patients proceeding to transplantation. In contrast, in intermediate-risk AML, MRD positivity was strongly associated with increased relapse risk and inferior disease-free survival. In adverse-risk AML, MRD positivity showed a more modest impact, primarily at early time points. Overall, these findings indicate that the prognostic value of MFC-MRD is most pronounced in intermediate-risk disease (**Table S6**).

Given that ELN guidelines recommend molecular MRD assessment in patients with established molecular markers, we performed a sensitivity analysis excluding patients with CBF rearrangements and *NPM1* mutations. In this reduced cohort, the association between MFC-MRD status and clinical outcomes remained unchanged (**Table S7**).

Inter-laboratory variability in MRD prognostic performance

To evaluate whether the prognostic association of pre-transplant MFC-MRD with post-transplant outcomes varied by transplant center, we included a center-by-MRD interaction term in the multivariable Cox models. The addition of this interaction term significantly improved model fit (likelihood ratio test *P* < .001), indicating that the impact of MRD on relapse and survival outcomes differed across centers.

Across the high-volume centers with ≥ 50 patients, MRD positivity rates ranged from 1% to 28% (median, 15.5%; *P* = .07; **Table S8**), with no significant change in MRD positivity over time (*P* = .13; **Figure S1**).

Survival analyses demonstrated considerable variability in the prognostic impact of MRD across centers (**Figure 3**). At the largest center (n = 121), MRD positivity was strongly associated with inferior outcomes: 3-year relapse risk (58% vs 26%, *P* = .006), 3-year DFS (29% vs 61%, *P* = .001; **Figure 4A-B**), and OS (36% vs 69%, *P* = .008; **Figure 4C-D**). These associations remained significant in multivariable analysis: relapse HR, 2.79; 95% CI, 1.54–5.05; *P* = .001 (**Table S9**); DFS HR, 2.57; 95% CI, 1.41–4.69; *P* = .002 (**Table S10**); OS HR, 2.02; 95% CI, 1.05–3.89; *P* = .035 (**Table S11**).

In contrast, most other centers did not demonstrate statistically significant differences in relapse or survival outcomes by MRD status. For example, at another high-volume center (n = 88), relapse (38% vs 29%, *P* = .52), DFS (45% vs 63%, *P* = .64), and OS (50% vs 77%, *P* = .25) did not differ significantly between MRD-positive and MRD-negative groups. Across the remaining 8 centers, no significant associations between MRD status and relapse, DFS, or OS were observed in either univariate or multivariable analyses.

DISCUSSION

In this large, registry-based analysis of patients in AML in CR1 undergoing alloHCT, we found that MRD by MFC, as performed in routine clinical practice, was independently associated with inferior OS, DFS, and increased relapse risk. This is consistent with previous studies, indicating that the persistence of MRD in AML prior to alloHCT is an adverse prognostic factor, whether MRD is tested with MFC, PCR, or NGS techniques (2, 7, 13, 17-20), and whether it is tested in CR1 or subsequent remissions (21). Although this represents the largest registry-based analysis of MFC-MRD in this setting to date, our study also highlights the limitations of registry collection

of MRD data and substantial inter-laboratory variability in prognostic value, which sets the stage for future efforts towards harmonization of MRD.

Compared to the patients with MRD-negative disease, patients with MRD-positive disease had significantly higher cumulative incidence of relapse and worse survival at 1- and 3-years post-transplant. This underscores the prognostic value and importance of achieving MRD negativity prior to alloHCT for AML in CR1. However, overcoming the persistence of MRD positivity before alloHCT remains an area of active research, as MRD positivity could be attributed to a drug-resistant leukemia clone, reflecting the actual biology of AML, for which additional cycles of chemotherapy could add further toxicities without additional benefit. Prior studies suggest that a MAC regimen could overcome the negative impact of the MRD positivity (9, 14, 22); however, intensive conditioning regimens can only be safely used in younger patients and on those with no significant comorbidities. While augmented RIC regimens do not improve post-alloHCT outcomes (23), melphalan-containing regimens can potentially overcome MRD persistence (9, 24). Importantly, in our study, additional analysis adjusting for conditioning regimen intensity and for melphalan-containing regimens in the multivariable model did not materially change the prognostic impact of MRD status (**Table S12)**. A retrospective study has suggested that umbilical cord blood transplants could as well overcome the persistence of MRD positivity (25), although this remains controversial (26).

While our findings underscore the prognostic impact of pre-alloHCT MRD status when tested using MFC in clinical practice, they also reveal the substantial risk of relapse, even among patients with pre-transplant MRD negativity. The adjusted probabilities of OS, DFS, and cumulative incidence of relapse appear to converge over time (**Figure 1 and Figure 2)**, particularly at 5 years post-alloHCT. While MRD negativity is associated with improved outcomes in AML irrespective of the initial treatment intensity (27), this narrowing of the curves, which is partially due to late NRM, suggests that it is not a guarantee of long-term disease

control. While MRD test status remains one the strongest predictors of relapse, other factors such as age, ELN 2017, and incomplete count recovery have been previously associated with an increased risk of relapse (28). The MRD-positivity rate in this registry cohort (11%) is lower than that reported in several large prospective trials conducted during a similar time period, where MRD-positivity rates were approximately 25% (29-31). In univariate analyses of the overall cohort, MRD-positivity rates increased significantly over time. Although this temporal trend was less evident when restricted to the twelve largest centers, the overall increase may reflect evolving assay sensitivity, expanded use of higher-color panels, or other technical refinements over the study period. These observations further underscore the impact of methodological heterogeneity on real-world MRD reporting.

Importantly, we observed significant inter-laboratory variability in the prognostic performance of MRD across US transplant centers. The strength and direction of MRD's association with relapse and survival varied widely between sites, with some centers showing strong predictive value and others none at all. This heterogeneity likely reflects differences in local assay protocols, analytical thresholds, and interpretive expertise.

Such center-level variability has important clinical implications. It suggests that, in real-world US practice, decentralized MFC-MRD testing yields results that are not uniformly reliable for prognostic or therapeutic decision-making. Centralized testing, as implemented in several European cooperative group studies, has been shown to reduce variability and improve assay reproducibility. In Spain, where MFC-MRD was previously performed in a decentralized manner, evidence indicated that heterogeneity introduced by local testing weakened the association between MRD status and relapse risk, DFS, and OS (12). These observations led to the adoption of centralized testing protocols to ensure greater consistency and prognostic accuracy, which has been shown to improve reproducibility and clinical utility of MRD testing (32).

We further explored the interaction between MRD status, patient age, and conditioning intensity. Age stood out as a significant factor for increased risk of relapse, DFS, and lower OS, which could be partly explained by the more common use of lower-intensity conditioning regimens (e.g., RIC/NMA) for older patients (**Table S13 and S14**). Nevertheless, age did not significantly impact NRM. Moreover, patients who had MRD-pos disease pre-alloHCT had a higher risk of relapse, lower DFS, and OS when receiving a RIC/NMA conditioning regimen.

This study has several limitations. MRD by MFC was characterized by binary classification, and flow cytometry testing for MRD is not standardized across reporting sites. While MFC is not the only method of detecting the MRD status in patients with AML, we could not further analyze other MRD testing modalities (molecular by PCR, NGS, or fluorescence in situ hybridization [FISH], etc.) because of the limited MRD data reported to the CIBMTR. Although 41% of MRD-negative and 36% of MRD-positive patients had both molecular and MFC-MRD testing reported, the collected molecular MRD data were not comprehensive. For the inter-laboratory analysis, center volume varied widely in our study with mixed amounts of events, limiting the interpretability of results from low-volume centers. Due to the limit information regarding site specific practice, we were unable to identify specific patterns of sites with good relapse prediction compared to sites who perform poorly. Finally, we could not assess the effect of post-alloHCT maintenance therapy, which can be informative for patients with pre-alloHCT MRD-pos disease (33-35), as most patients did not receive post-alloHCT maintenance therapy.

In conclusion, pre-alloHCT MRD MFC test results, as currently performed in bone marrow transplant centers across the US, are independently associated with post-alloHCT outcomes in patients with AML in CR1. Nevertheless, the prognostic value varied substantially across centers, reflecting real-world methodological heterogeneity. Importantly, although MRD positivity identified patients at significantly higher relative risk of relapse, most relapses in absolute numbers occurred among pre-alloHCT MRD-negative patients, underscoring the need for

improved, standardized, and more sensitive MRD assessment tools. Adaption of more effective AML MRD testing is needed in clinical practice to enhance the prediction of relapse post-alloHCT. Future efforts should focus on harmonizing MRD assessment methods across centers, incorporating molecular and immunophenotypic MRD platforms, and integrating MRD findings into pre- and post-alloHCT therapeutic algorithms, including maintenance therapy and dynamic MRD monitoring.

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TABLE LEGENDS

Table 1 Adjusted cumulative incidence of relapse and NRM, and adjusted probability of DFS and OS

Table 2 Multivariate analysis of overall survival

Table 3 Multivariate analysis of disease-free survival

TABLES

Table 1 Adjusted cumulative incidence of relapse and NRM, and adjusted probability of DFS and OS

		MRD-negative		MRD-positive		
	Time	**N**	**Prob. (95% CI)**	**N**	**Prob. (95% CI)**	**P[a]**
OS						
	1 year	1591	72 (70-74)	178	67 (61-72)	.045
	3 year	895	56 (54-58)	75	48 (41-54)	.008
	5 year	387	48 (46-50)	27	45 (39-51)	.336
NRM						
	1 year	1367	14 (12-15)	133	14 (10-18)	.880
	3 year	777	18 (17-20)	63	16 (12-21)	.332
	5 year	342	22 (20-25)	23	17 (12-22)	.029
DFS						
	1 year	1367	62 (60-64)	133	50 (44-56)	<.001
	3 year	777	47 (45-49)	63	40 (34-45)	.016
	5 year	342	40 (38-43)	23	38 (32-44)	.461
Relapse						
	1 year	1367	25 (23-26)	133	35 (29-40)	.001
	3 year	777	36 (34-38)	63	43 (38-49)	.019
	5 year	342	41 (38-43)	23	45 (38-51)	.239

Abbreviations: DFS, disease-free survival; MFC, multiparameter flow cytometry; MRD, measurable residual disease; NRM, non-relapse mortality; OS, overall survival.

[a] Pointwise test

Table 2 Multivariate analysis of overall survival

Overall survival	N	HR (95% CI)	P
MRD-positive vs -negative	281 vs 2261	1.27 (1.06-1.51)	.009
Age ≥ 50 vs < 50	1765 vs 777	1.40 (1.21-1.62)	<.001
AML type			
De novo	1985	1	<.001[a]
Transformed from MDS	353	1.52 (1.31-1.77)	<.001
Therapy-related	204	1.21 (0.99-1.48)	.066
Conditioning			
MAC	1236	1	<.001[a]
RIC	832	1.11 (0.97-1.27)	.144
NMA	474	1.37 (1.17-1.60)	<.001
ELN risk			
Favorable	375	1	<.001[b]
Intermediate	1148	1.27 (1.04-1.54)	.020
Adverse	962	1.68 (1.38-2.05)	<.001
Not reported	75	1.56 (1.10-2.21)	.013
HCT-CI			
0	434	1	.006[b]
1-2	799	1.05 (0.87-1.26)	.637
≥ 3	1285	1.28 (1.08-1.52)	.005
Not reported	24	1.16 (0.66-2.04)	.618
Karnofsky Performance Status			
< 90	1103	1	.026[a]
≥ 90	1411	0.85 (0.76-0.96)	.007
Unknown	28	0.98 (0.58-1.68)	.943

Abbreviations: AML, acute myeloid leukemia; ELN, European LeukemiaNet 2017; HCT-CI, Hematopoietic Cell Transplantation Comorbidity Index; HR, hazard ratio; MAC, myeloablative conditioning; MDS, myelodysplastic syndrome; NMA, nonmyeloablative conditioning; RIC, reduced-intensity conditioning.

[a] 2 degrees of freedom test

[b] 3 degrees of freedom test

Table 3 Multivariate analysis of disease-free survival

Disease-free survival	N	HR (95% CI)	P
MRD-positive vs -negative	281 vs 2261	1.31 (1.11-1.53)	.001
AML type			
De novo	1985	1	<.001[a]
Transformed from MDS	353	1.50 (1.30-1.72)	<.001
Therapy-related	204	1.08 (0.89-1.31)	.441
Conditioning			
MAC	1236	1	<.001[a]
RIC	832	1.28 (1.14-1.45)	<.001
NMA	474	1.65 (1.44-1.89)	<.001
ELN risk			
Favorable	375	1	<.001[b]
Intermediate	1148	1.16 (0.98-1.38)	.091
Adverse	962	1.55 (1.30-1.84)	<.001
Not reported	75	1.28 (0.92-1.78)	.142
HCT-CI			
0	434	1	.014[b]
1-2	799	1.09 (0.92-1.28)	.323
≥ 3	1285	1.26 (1.08-1.47)	.004
Not reported	24	1.24 (0.73-2.10)	.424

Abbreviations: AML, acute myeloid leukemia; ELN, European LeukemiaNet 2017; HCT-CI, Hematopoietic Cell Transplantation Comorbidity Index; HR, hazard ratio; MAC, myeloablative conditioning; MDS, myelodysplastic syndrome; MRD, measurable residual disease; NMA, nonmyeloablative conditioning; RIC, reduced-intensity conditioning.

[a] 2 degrees of freedom test

[b] 3 degrees of freedom test

FIGURE LEGENDS

Figure 1 Adjusted probability of overall survival and disease-free survival

Figure 2 Adjusted cumulative incidence of non-relapse mortality and relapse

Figure 3 Relapse, disease-free survival, and overall survival, at largest centers

Note: Forest plots include patients at the 12 largest centers, those with more than 50 patients who underwent their first transplant for acute myeloid leukemia. (Left) MRD positivity was significantly associated with an increased risk of relapse at Centers 1 and 3 (Center 1: hazard ratio [HR], 2.97; 95% CI, 1.54-5.73; P < .01; Center 3: HR, 2.55; 95% CI, 1.11-5.86; P = .03). (Middle) Disease-free survival was worse for MRD-positive patients in the largest and third-largest centers. (Right) overall survival was significantly worse for MRD-positive patients at the largest center (HR, 2.02; 95% CI, 1.05-3.89; P = .04).

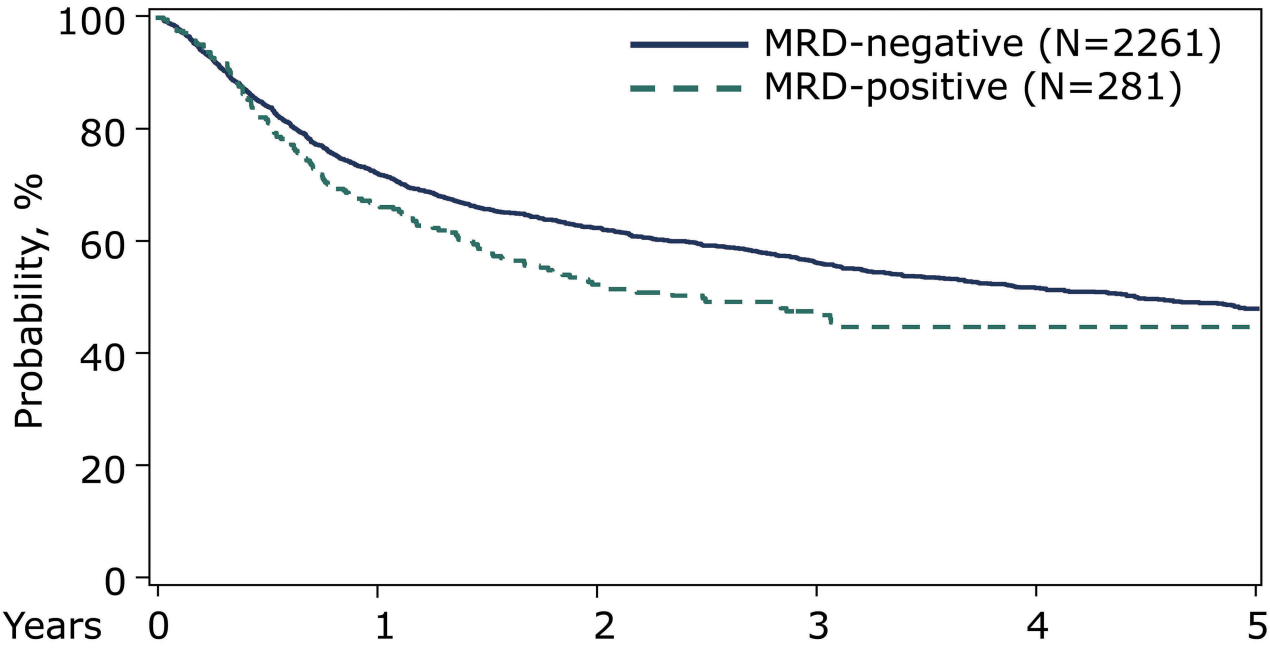
Abbreviations: AML, acute myeloid leukemia; MRD, measurable residual disease.

Figure 4 Disease-free survival by MRD status at largest vs fourth-largest center

Note: Multiparameter flow cytometry was used for measurable residual disease (MRD). At the largest site, Figure 4A, disease-free survival (DFS) at 3 years for MRD-positive vs -negative patients was 61% vs 29%, P = .001. At the largest site, Figure 4B, overall survival (OS) at 3 years for MRD-positive vs -negative patients was 69% vs 36%, P = .008. At the fourth-largest site, Figure 4C, DFS at 3 years for MRD-positive vs -negative patients was 45% vs 63%, P = .64. At the fourth-largest site, Figure 4D, OS at 3 years for MRD-positive vs -negative patients was 50% vs 77%, P = .25.

Abbreviations: DFS, disease-free survival, MRD, measurable residual disease; OS, overall survival.

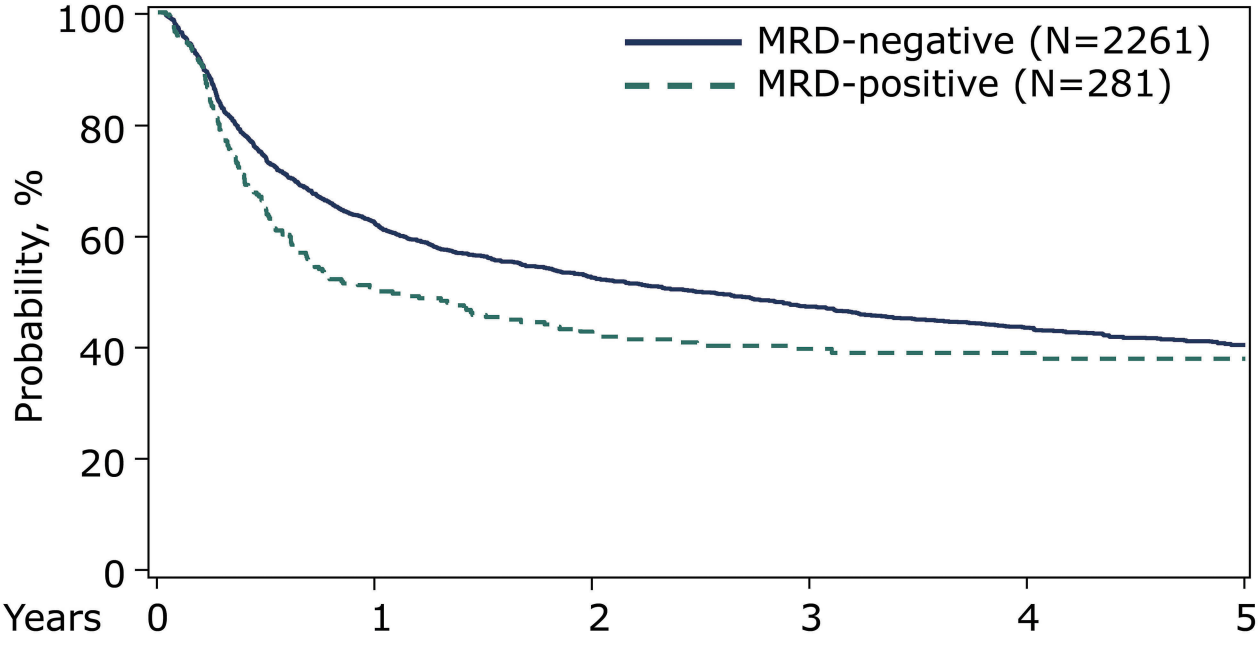
Figure 1A
Adjusted probability of overall survival



No. at risk						
MRD-negative	2261	1591	1193	895	617	387
MRD-positive	281	178	119	75	48	27

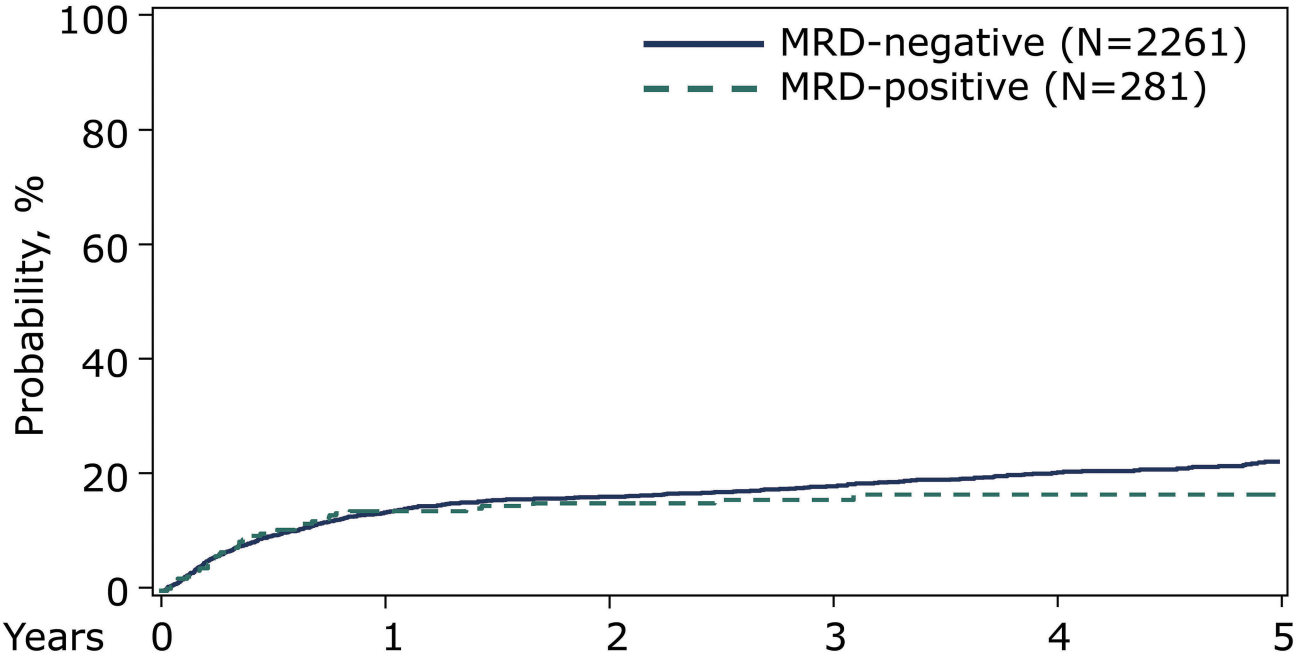
Abbreviation: MRD, measurable residual disease

Figure 1B
Adjusted probability of disease-free survival



No. at risk						
MRD-negative	2261	1367	1019	777	542	342
MRD-positive	281	133	96	63	41	23

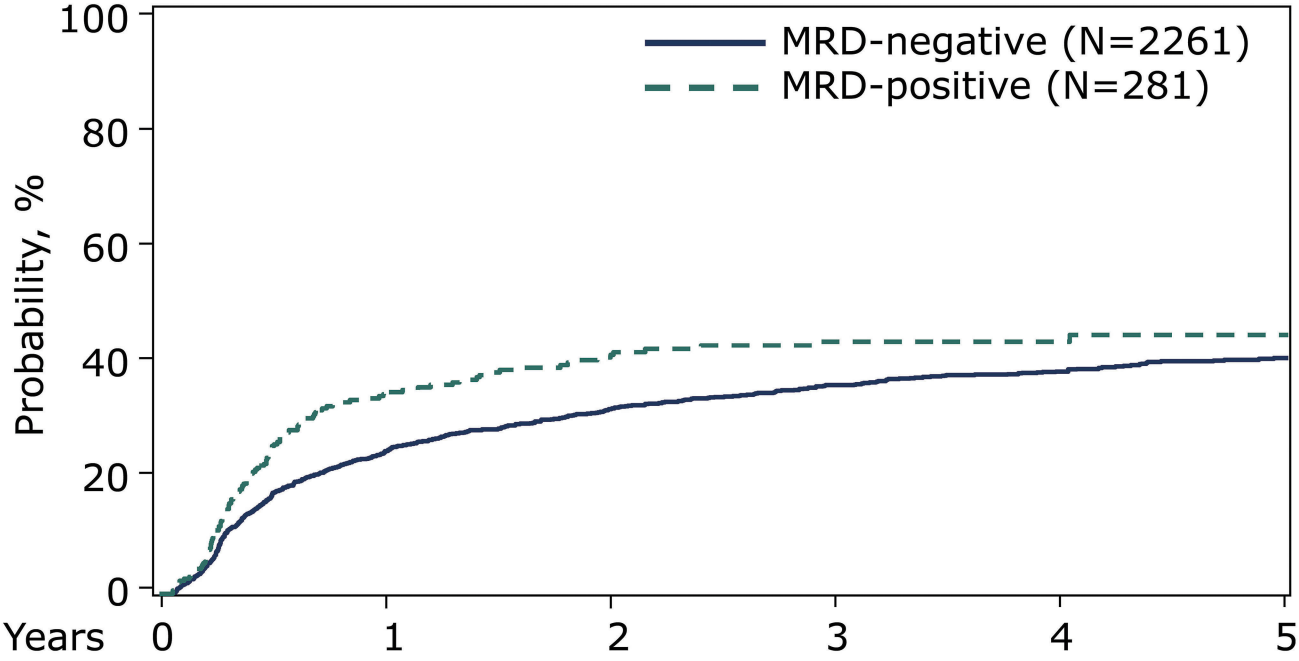
Figure 2A
Adjusted cumulative incidence of non-relapse mortality



No. at risk						
MRD-negative	2261	1367	1019	777	542	342
MRD-positive	281	133	96	63	41	23

Abbreviation: MRD, measurable residual disease

Figure 2B
Adjusted cumulative incidence of relapse



No. at risk						
MRD-negative	2261	1367	1019	777	542	342
MRD-positive	281	133	96	63	41	23

Figure 3 Relapse, disease-free survival, and overall survival, at largest centers

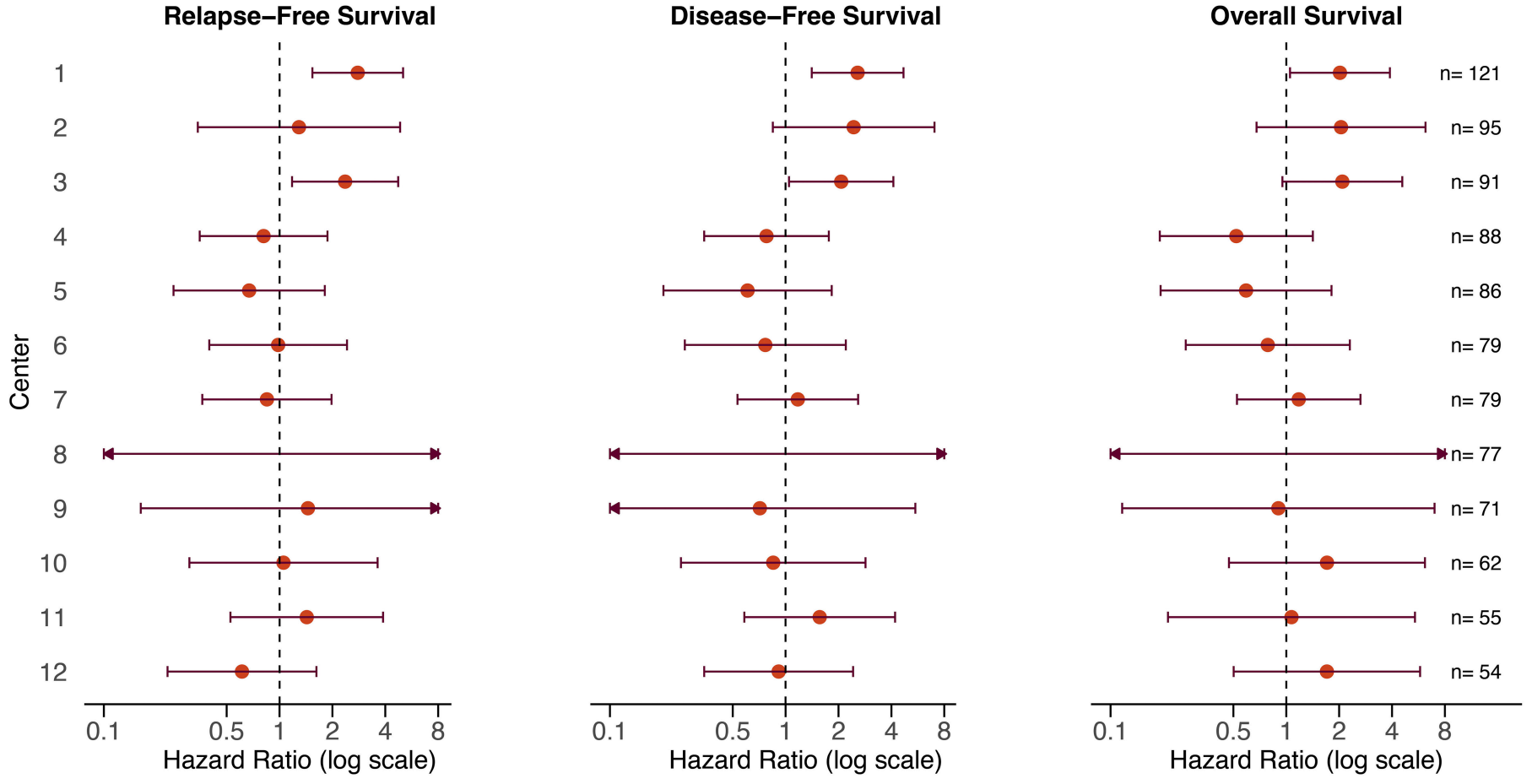
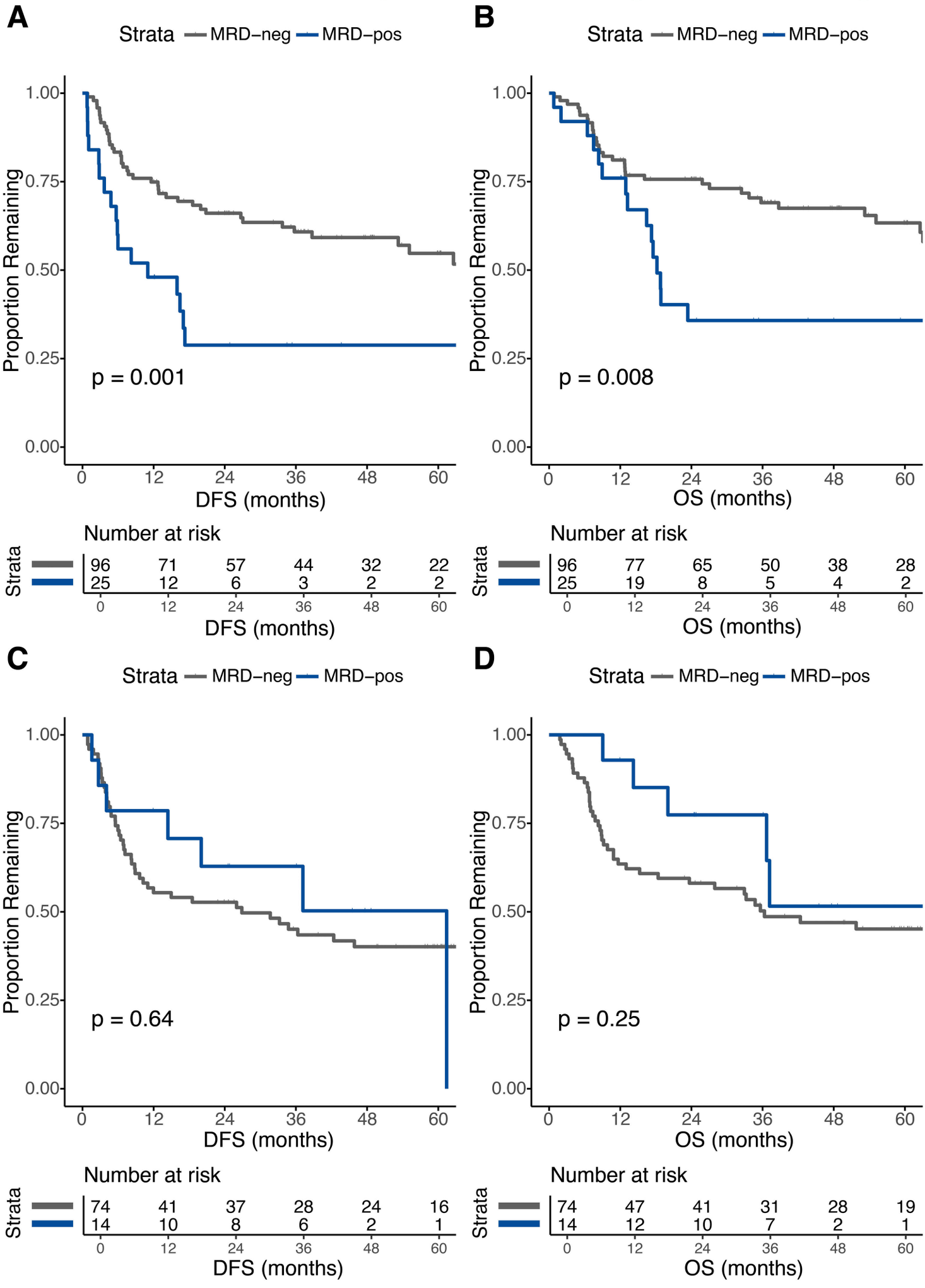


Figure 4 Disease-free survival by MRD status at largest vs fourth-largest center



SUPPLEMENTARY MATERIAL

This supplementary material accompanies “Real-World Heterogeneity in the Prognostic Value of Pre-Transplant Flow Cytometry MRD in AML in CR1: CIBMTR Analysis”

Jesse M. Tettero[1,2], Mei-Jei Zhang[3,4], Wentong Liu[4], Mariam T. Nawas[5], Filippo Milano, MD, PhD[6], Nelli Bejanyan[7], Veronika Bachanova[8], Larisa Broglie[4,9], Wael Saber[4,10], Gege Gui[1], Laura W. Dillon[1], Roland B. Walter, MD, PhD, MS[6], Kristin M. Page[4,9], Christopher S. Hourigan[1], Firas El Chaer[11,12] on behalf of the MRD Working Group Consortium

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Contents

Figure S1 MRD-positive rates by center over time

SUPPLEMENTARY METHODS

Patient-, disease-, and transplant-related covariates that were included in the analysis are noted in **Table S1**. The main variables included in this analysis were age, sex, race, Karnofsky Performance Status score (KPS), Hematopoietic Cell Transplantation Comorbidity Index (HCT-CI), and disease-related characteristics (AML type, ELN 2017 risk classification, number of induction cycles, time from diagnosis to transplant, time from diagnosis to CR1), and transplant-related characteristics (conditioning regimen type, donor type, graft type, graft-versus-host-disease [GVHD] prophylaxis, and post-alloHCT anti-leukemic treatment modalities).

The covariates considered in the Cox models included age at transplant, KPS, HCT-CI, MRD at the time of transplant, cytogenetic risk group, time to achieve CR1, AML subtype [de novo vs therapy-related vs transformed from myelodysplastic syndrome (MDS)], number of cycles of induction and consolidation prior to transplant, conditioning intensity (using standard CIBMTR operational definitions), type of graft, type of donor, post-alloHCT maintenance, and year of transplant.

Data quality

CIBMTR is a research collaboration between the Medical College of Wisconsin and NMDP. More than 310 medical centers worldwide submit clinical data to CIBMTR and includes approximately 700,000 patients who have received HCT and cellular therapies (1). CIBMTR subjects data to automated and manual quality checks and on-site audits. These validations and verifications produce high-quality data. If a center fails to meet data-quality standards, its data are removed from research studies.

Ethics

CIBMTR protects the privacy of participants and obeys international laws and ethical guidelines (2). The NMDP Institutional Review Board reviews CIBMTR's research. Patients or guardians give informed consent for research.

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Table S1 Characteristics of patients, with MRD status by multiparameter flow cytometry

Characteristic	MRD-negative	MRD-positive	P value
No. of patients	**2261**	**281**	
No. of centers	129	74	
Patient characteristics			
Age, no. (%)			.42[a]
Median (range), years	58.3 (18.0-80.8)	57.5 (18.2-77.1)	
Age < 50	697 (30.8)	80 (28.4)	
Age ≥ 50	1564 (69.2)	201 (71.6)	
Age, by decade, no. (%)			.13[a]
18-29	194 (8.6)	23 (8.2)	
30-39	211 (9.3)	24 (8.5)	
40-49	292 (12.9)	33 (11.7)	
50-59	549 (24.3)	85 (30.2)	
60-69	807 (35.7)	83 (29.5)	
≥ 70	208 (9.2)	33 (11.7)	
Recipient sex, no. (%)			.07[a]
Male	1200 (53.1)	165 (58.9)	
Female	1061 (46.9)	116 (41.1)	
Race, no. (%)			.88[a]
White	1728 (76.4)	214 (76.2)	
Black or African American	259 (11.5)	28 (9.9)	
Asian	175 (7.7)	22 (7.8)	
Other[b]	32 (1.4)	5 (1.8)	
Not reported	67 (3.0)	12 (4.3)	
Ethnicity, no. (%)			.38[a]
Hispanic or Latino	169 (7.5)	17 (6.0)	
Not Hispanic or Latino	2032 (89.9)	255 (90.7)	
Not a US resident	12 (0.5)	3 (1.1)	
Not reported	48 (2.1)	6 (2.1)	
Karnofsky score, no. (%)			.57[a]
< 90	988 (43.7)	115 (41.3)	
≥ 90	1247 (55.2)	164 (58.0)	
Not reported	26 (1.1)	2 (0.7)	
HCT-CI score, no. (%)			.27[a]
HCT-CI 0	382 (16.9)	52 (18.5)	
HCT-CI 1-2	707 (31.3)	92 (33.1)	
HCT-CI ≥ 3	1153 (51.0)	132 (46.6)	
Not reported	19 (0.8)	5 (1.8)	
Disease characteristics			
Clinical onset of AML, no. (%)			.06[a]
De novo	1767 (78.2)	218 (77.3)	
Transformed from MDS	305 (13.5)	48 (17.4)	
Therapy-related	189 (8.4)	15 (5.3)	
ELN17 risk group, no. (%)			.02[a]
Favorable	327 (14.5)	30 (11.0)	
Intermediate	1032 (45.6)	116 (41.1)	
Adverse	832 (36.8)	130 (46.1)	
Not Reported	70 (3.1)	5 (1.8)	
Time to achieve CR1, no. (%)			<.01[a]
0-4 weeks	634 (28.0)	96 (34.2)	

Characteristic	MRD-negative	MRD-positive	P value
No. of patients	**2261**	**281**	
4-8 weeks	991 (43.8)	77 (27.4)	
> 8 weeks	636 (28.1)	108 (38.4)	
Number of induction, no. (%)			<.01[a]
1	1481 (65.5)	147 (52.1)	
2	513 (22.7)	91 (32.3)	
≥ 3	203 (9.0)	37 (13.5)	
Not reported	64 (2.8)	6 (2.1)	
Time from diagnosis to HCT, median (range), months	4.5 (1.5-31.2)	4.4 (1.9-17.2)	
Transplant characteristics			
Donor type, no. (%)			<.01[a]
Matched sibling or other related donor	486 (21.5)	67 (23.8)	
Matched unrelated adult donor	835 (36.9)	82 (29.4)	
Haploidentical related(or partially mismatched	481 (21.3)	54 (19.1)	
Mismatched unrelated donor	141 (6.2)	16 (5.7)	
UCB, single or double	318 (14.1)	62 (22.0)	
Donor-recipient sex match, no. (%)			<.01[a]
M-M	698 (30.9)	80 (28.5)	
M-F	542 (24.0)	48 (17.1)	
F-M	360 (15.9)	46 (16.4)	
F-F	338 (14.9)	45 (16.0)	
UCB-recipient M	140 (6.2)	39 (13.9)	
UCB-recipient F	178 (7.9)	23 (8.2)	
Not reported	5 (0.2)	0 (0.0)	
Donor-recipient CMV serostatus, no. (%)			<.01[a]
+/+	710 (31.4)	77 (27.4)	
+/-	179 (7.9)	26 (9.3)	
-/+	610 (27.0)	62 (22.1)	
-/-	432 (19.1)	51 (18.1)	
UCB-recipient +	242 (10.7)	40 (14.2)	
UCB-recipient -	75 (3.3)	21 (7.5)	
UCB-recipient CMV unknown	1 (0.0)	1 (0.4)	
Not reported	12 (0.5)	3 (1.1)	
Conditioning intensity, no. (%)			.45[a]
MAC	1089 (48.1)	147 (52.0)	
RIC	749 (33.1)	83 (29.9)	
NMA	423 (18.7)	51 (18.1)	
Conditioning intensity based on melphalan, no. (%)			.11[a]
MAC	1089 (48.2)	146 (52.0)	
Mel-containing RIC	380 (16.8)	54 (19.2)	
Other RIC/NMA	792 (35.0)	81 (28.8)	
Conditioning intensity and age, no. (%)			.20[a]
MAC, age < 50	540 (23.9)	69 (24.6)	
RIC/NMA, age < 50	158 (7.0)	11 (3.9)	
MAC, age ≥ 50	549 (24.3)	77 (27.4)	
RIC/NMA, age ≥ 50	1014 (44.8)	124 (44.1)	
TBI, no. (%)			.32[a]
No	1421 (62.8)	168 (59.8)	
Yes	840 (37.2)	113 (40.2)	
In vivo T-cell depletion, ATG/alemtuzumab, no. (%)			.41[a]
No	1804 (79.8)	230 (81.9)	
Yes	457 (20.2)	51 (18.1)	

Characteristic	MRD-negative	MRD-positive	P value
No. of patients	**2261**	**281**	
Donor graft, no. (%)			<.01[a]
Bone marrow	388 (17.2)	59 (21.0)	
Peripheral blood stem cells	1555 (68.8)	160 (56.9)	
Umbilical cord blood	318 (14.1)	62 (22.1)	
Planned GVHD prophylaxis, major, no. (%)			.09[a]
Ex vivo T-cell depletion	14 (0.6)	2 (0.7)	
CD34 selection	82 (3.6)	17 (6.0)	
PTCy	628 (27.8)	81 (28.8)	
Tac-based	1269 (56.1)	137 (48.8)	
CSA-based	253 (11.2)	42 (14.9)	
Other	15 (0.7)	2 (0.7)	
GVHD prophylaxis, broad, no. (%)			.03[a]
PTCy	628 (27.8)	81 (28.8)	
Tac-based	1269 (56.1)	137 (48.8)	
CSA-based	253 (11.2)	42 (14.9)	
Other	111 (4.9)	21 (7.5)	
Planned post-HCT maintenance, no. (%)			.88[a]
No	2074 (91.7)	257 (91.5)	
Yes	187 (8.3)	24 (8.5)	
Year of HCT, no. (%)			<.01[a]
2013	153 (6.8)	12 (4.3)	
2014	408 (18.0)	29 (10.3)	
2015	429 (19.0)	41 (14.6)	
2016	396 (17.5)	42 (14.9)	
2017	311 (13.8)	47 (16.7)	
2018	292 (12.9)	60 (21.4)	
2019	272 (12.0)	50 (17.8)	
Year of HCT, no. (%)			<.01[a]
2013-2016	1386 (61.3)	124 (44.1)	
2017-2019	875 (38.7)	157 (55.9)	
MRD detection method, no. (%)			.01[a]
Flow cytometry only	446 (19.7)	79 (28.1)	
Cytogenetic, flow cytometry	173 (7.7)	19 (6.8)	
Molecular, flow cytometry	924 (40.9)	99 (35.2)	
Cytogenetic, molecular, flow cytometry	718 (31.8)	84 (29.9)	
Cytogenetic testing results, no. (%)			<.01[a]
Cytogenetics negative	782 (34.6)	62 (22.1)	
Cytogenetics positive	109 (4.8)	41 (14.6)	
Not reported	1370 (60.6)	178 (63.3)	
Any molecular testing results, no. (%)			<.01[a]
Molecular negative	1019 (45.1)	102 (36.3)	
Molecular positive	623 (27.6)	81 (28.8)	
Not reported	619 (27.4)	98 (34.9)	
Follow-up of survivors, median (range), months	48.4 (3.3-95.0)	36.8 (6.4-81.3)	

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; ATG, anti-thymocyte globulin; CD34, cluster of differentiation 34; CMV, cytomegalovirus; CR1, first complete remission; CSA, cyclosporine A; ELN17, European LeukemiaNet 2017; F-F, female donor to female recipient; F-M, female donor to male recipient; GVHD, graft-versus-host disease; HCT, hematopoietic cell transplantation; HCT-CI, Hematopoietic Cell Transplantation Comorbidity Index; MAC, myeloablative conditioning; MDS, myelodysplastic syndrome; M-F, male donor to female recipient; M-M, male donor to male recipient; MRD, measurable residual disease; Mel, melphalan; NMA, nonmyeloablative

conditioning; PTCy, post-transplant cyclophosphamide; RIC, reduced-intensity conditioning; Tac, tacrolimus; TBI, total body irradiation; UCB, umbilical cord blood.

[a] Pearson chi-square test hypothesis testing.

[b] Other races included Native Hawaiian or other Pacific Islander; American Indian or Alaska Native; or more than one race.

Table S2 Completeness of follow-up

	MRD-negative (N = 2261), %	MRD-positive (N = 281), %	MRD not reported (N = 549), %	Overall (N = 3091), %
1-year	99.6	99.5	99.6	99.6
2-year	94.8	93.3	96.8	95.0
3-year	91.2	88.6	94.3	91.5
4-year	88.4	85.2	92.5	88.9

Abbreviations: MRD, measurable residual disease.

Table S3 Selection criteria, patients with comprehensive report forms

Inclusion	Excluded	Remaining
Included adults (≥ 18 years old) who underwent first alloHCT for AML during 2013-2019		26,324
Included patients in first complete remission	9,068	17,256
Included all AML subtypes except for APML and myeloid sarcoma	APML (n = 68) Myeloid sarcoma (n = 147) Total (n = 215)	17,041
Excluded patients with syngeneic twin donors	22	17,019
Excluded patients at centers outside the US	4,443	12,576
Excluded patients without comprehensive report forms	748	3,257
Excluded patients who did not consented to research	40	3,217
Excluded patients at centers that did not meet CIBMTR data-quality standards (embargoed)	106	3,111
Excluded patients who did not have follow-up data	15	3,093

Abbreviations: alloHCT, allogeneic hematopoietic cell transplantation; AML, acute myeloid leukemia; APML, acute promyelocytic leukemia.

Table S4 Multivariate analysis of non-relapse mortality

Non-relapse mortality	N	HR (95% CI)	P
MRD-positive vs -negative	281 vs 2261	0.97 (0.71-1.32)	.831
Age ≥ 50 vs < 50	1765 vs 777	1.67 (1.33-2.09)	<.001
AML type			
De novo	1985	1	.033[a]
Transformed from MDS	353	1.38 (1.08-1.77)	.009
Therapy-related	204	1.09 (0.80-1.47)	.599
HCT-CI			
0	434	1	<.001[b]
1-2	799	1.31 (0.95-1.82)	.101
≥ 3	1285	1.89 (1.39-2.56)	<.001
Not reported	24	1.26 (0.45-3.51)	.656
Graft type			
BM	447	1	.017[a]
PBSC	1715	1.26 (0.96-1.65)	.098
UCB	380	1.62 (1.16-2.25)	.005

Abbreviations: AML, acute myeloid leukemia; BM, bone marrow; HCT-CI, Hematopoietic Cell Transplantation Comorbidity Index; HR, hazard ratio; MDS, myelodysplastic syndrome; MRD, measurable residual disease; PBSC, peripheral blood stem cells; UCB, umbilical cord blood.

[a] 2 degrees of freedom test

[b] 3 degrees of freedom test

Table S5 Multivariable analysis of relapse

Relapse	N	HR (95% CI)	P
MRD-positive vs -negative	281 vs 2261	1.42 (1.17-1.72)	<.001
AML type			
De novo	1985	1	<.001[a]
Transformed from MDS	353	1.55 (1.31-1.84)	<.001
Therapy-related	204	1.10 (0.87-1.39)	.441
Conditioning			
MAC	1236	1	<.001[a]
RIC	832	1.28 (1.10-1.49)	.002
NMA	474	2.02 (1.72-2.37)	<.001
ELN risk			
Favorable	375	1	<.001[b]
Intermediate	1148	1.15 (0.92-143)	.214
Adverse	962	1.75 (1.41-2.18)	<.001
Not reported	75	1.47 (0.98-2.21)	.063
Year of TX: 2017-2019 vs 2013-2016	1033 vs 1509	1.20 (1.05-1.38)	.008

Abbreviations: AML, acute myeloid leukemia; ELN, European LeukemiaNet 2017; HR, hazard ratio; MDS, myelodysplastic syndrome; MRD, measurable residual disease.

[a] 2 degrees of freedom test

[b] 3 degrees of freedom test

Table S6 Univariate analysis of outcomes by MFC-MRD status stratified according to ELN 2017 risk category

Outcome	Time	Favorable MRD− (N=327)	Favorable MRD+ (N=31)	P	Intermediate MRD− (N=1032)	Intermediate MRD+ (N=116)	P	Adverse MRD− (N=832)	Adverse MRD+ (N=129)	P
Overall survival	1-y	80% (75–84)	68% (51–83)	0.158	74% (71–76)	73% (65–81)	0.916	68% (65–71)	59% (50–67)	0.039
	3-y	70% (65–75)	NE	0.107	58% (55–61)	52% (43–62)	0.286	49% (46–53)	41% (33–51)	0.101
	5-y	59% (52–66)	NE	0.274	51% (48–55)	NE	0.894	40% (36–44)	NE	0.758
Relapse	1-y	20% (16–24)	16% (5–31)	0.618	21% (18–23)	40% (31–49)	<0.001	30% (27–33)	40% (31–48)	0.040
	3-y	27% (22–32)	NE	0.852	30% (27–33)	47% (38–57)	<0.001	44% (40–47)	49% (40–58)	0.265
	5-y	33% (27–39)	NE	0.420	33% (30–36)	NE	0.006	47% (44–51)	NE	0.469
Non-relapse mortality	1-y	11% (8–15)	19% (7–35)	0.283	15% (13–17)	11% (6–18)	0.246	13% (11–16)	15% (9–21)	0.735
	3-y	14% (11–19)	NE	0.536	20% (17–22)	14% (8–21)	0.127	17% (15–20)	15% (9–21)	0.435
	5-y	19% (14–24)	NE	0.486	22% (19–25)	NE	0.029	20% (17–24)	NE	0.100
Disease-free survival	1-y	69% (64–74)	65% (47–80)	0.619	64% (61–67)	49% (40–58)	0.002	56% (53–60)	46% (37–54)	0.024
	3-y	58% (53–64)	NE	0.766	50% (47–53)	39% (30–48)	0.018	39% (36–43)	36% (28–45)	0.554
	5-y	48% (41–55)	NE	0.964	45% (41–48)	NE	0.227	32% (29–36)	NE	0.691

NE = not estimable.

Table S7 Univariate analysis of survival outcomes excluding CBF and NPM1-mutated AML

	MRD-negative (N = 1987)		MRD-positive (N = 264)		
Outcomes	**N**	**Prob (95% CI)**	**N**	**Prob (95% CI)**	**P Value**
Overall survival	1987		264		0.006
1-year	1393	72 (70-74)%	166	65 (59-71)%	0.026
3-year	779	55 (53-58)%	68	45 (39-52)%	0.005
5-year	341	47 (44-50)%	25	43 (37-50)%	0.305
Relapse	1987		264		<0.001
1-year	1201	25 (23-27)%	125	38 (32-44)%	<0.001
3-year	678	36 (33-38)%	58	46 (40-52)%	0.002
5-year	305	39 (37-42)%	23	47 (41-54)%	0.025
Non-relapse mortality	1987		264		0.269
1-year	1201	14 (12-15)%	125	14 (10-19)%	0.933
3-year	678	18 (17-20)%	58	16 (12-21)%	0.366
5-year	305	21 (19-23)%	23	16 (12-21)%	0.037
Disease-free survival	1987		264		0.001
1-year	1194	61 (59-64)%	123	48 (42-54)%	<0.001

	MRD-negative (N = 1987)		MRD-positive (N = 264)		
Outcomes	**N**	**Prob (95% CI)**	**N**	**Prob (95% CI)**	**P Value**
3-year	676	46 (44-48)%	57	38 (32-44)%	0.013
5-year	305	39 (37-42)%	22	37 (31-43)%	0.443

Table S8 Distribution of MRD-negative and -positive patients at largest centers

Center	MRD-negative No. (%)	MRD-positive No. (%)	Total
Center 1	96 (79)	25 (21)	121
Center 2	90 (95)	5 (5)	95
Center 3	73 (80)	18 (20)	91
Center 4	74 (84)	14 (16)	88
Center 5	73 (85)	13 (15)	86
Center 6	65 (82)	14 (18)	79
Center 7	62 (78)	17 (22)	79
Center 8	76 (99)	1 (1)	77
Center 9	69 (97)	2 (3)	71
Center 10	55 (89)	7 (11)	62
Center 11	49 (89)	6 (11)	55
Center 12	39 (72)	15 (28)	54

Note: Analysis of 12 centers with more than 50 patients who underwent first transplant for acute myeloid leukemia.

Abbreviations: MRD, measurable residual disease.

Table S9 Relapse and MRD, multivariable analysis of largest centers

Center	MRD-positive No. (%)	HR (95% CI)	P value
Center 1	25 (21)	2.79 (1.54-5.05)	.001
Center 2	5 (5)	1.29 (0.34-4.86)	.71
Center 3	18 (20)	2.36 (1.18-4.74)	.02
Center 4	14 (16)	0.81 (0.35-1.88)	.63
Center 5	13 (15)	0.67 (0.25-1.81)	.43
Center 6	14 (18)	0.98 (0.40-2.42)	.97
Center 7	17 (22)	0.85 (0.36-1.98)	.70
Center 8	1 (1)	0.00 (0.0-inf)	1.00
Center 9	2 (3)	1.45 (0.16-13.0)	.74
Center 10	7 (11)	1.05 (0.31-3.62)	.93
Center 11	6 (11)	1.43 (0.53-3.89)	.48
Center 12	15 (28)	0.61 (0.23-1.62)	.32

Note: Analysis of 12 centers with more than 50 patients who underwent first transplant for acute myeloid leukemia included other covariables: AML type, conditioning regimen, ELN risk, and year of transplant.
Abbreviations: HR, hazard ratio; MRD, measurable residual disease.

Table S10 Disease-free survival and MRD, multivariable analysis of largest centers

Center	MRD-positive No. (%)	HR (95% CI)	P value
Center 1	25 (21)	2.57 (1.41-4.69)	.002
Center 2	5 (5)	2.44 (0.85-7.04)	.099
Center 3	18 (20)	2.07 (1.05-4.11)	.037
Center 4	14 (16)	0.78 (0.34-1.76)	.55
Center 5	13 (15)	0.61 (0.20-1.83)	.38
Center 6	14 (18)	0.77 (0.27-2.20)	.62
Center 7	17 (22)	1.17 (0.53-2.59)	.69
Center 8	1 (1)	0.00 (0.0-inf)	1.00
Center 9	2 (3)	0.71 (0.09-5.47)	.75
Center 10	7 (11)	0.85 (0.25-2.85)	.79
Center 11	6 (11)	1.56 (0.58-4.20)	.38
Center 12	15 (28)	0.91 (0.34-2.42)	.86

Note: Analysis of 12 centers with more than 50 patients who underwent first transplant for acute myeloid leukemia included other covariables: AML type, conditioning regimen, ELN risk, and HCT-CI.
Abbreviations: HR, hazard ratio; MRD, measurable residual disease.

Table S11 Overall survival and MRD, multivariable analysis of largest centers

Center	MRD-positive No. (%)	HR (95% CI)	P value
Center 1	25 (21)	2.02 (1.05-3.89)	.035
Center 2	5 (5)	2.05 (0.68-6.20)	.20
Center 3	18 (20)	2.09 (0.95-4.57)	.067
Center 4	14 (16)	0.52 (0.19-1.42)	.20
Center 5	13 (15)	0.59 (0.19-1.81)	.36
Center 6	14 (18)	0.79 (0.27-2.30)	.66
Center 7	17 (22)	1.18 (0.52-2.65)	.69
Center 8	1 (1)	0.00 (0.0-inf)	1.00
Center 9	2 (3)	0.90 (0.12-6.98)	.92
Center 10	7 (11)	1.71 (0.47-6.16)	.42
Center 11	6 (11)	1.07 (0.21-5.40)	.93
Center 12	15 (28)	1.70 (0.50-5.78)	.39

Note: Analysis of 12 centers with more than 50 patients who underwent first transplant for acute myeloid leukemia included other covariables: age, AML type, conditioning regimen, ELN risk, HCT-CI, and Karnofsky Performance Status score.

Abbreviations: HR, hazard ratio; MRD, measurable residual disease.

Table S12 Outcomes by MRD status, adjusting age by conditioning intensity and regimen

		Model 1: Adjusting conditioning intensity		Model 2: Adjusting conditioning intensity + melphalan regimen	
	N (MRD+ vs MRD–)	HR (95% CI)	P	HR (95% CI)	P
Relapse	281 vs 2261	1.42 (1.17-172)	<.001	1.47 (1.21-1.77)	<.001
NRM	281 vs 2261	0.97 (0.71-1.32)	.831	0.94 (0.69-1.28)	.684
DFS	281 vs 2261	1.31 (1.11-1.53)	.001	1.32 (1.13-1.56)	.001
OS	281 vs 2261	1.27 (1.06-1.51)	.009	1.28 (1.07-1.52)	.006

Abbreviations: DFS, disease-free survival; MRD, measurable residual disease; NRM, non-relapse mortality; OS, overall survival.

Table S13 Cox regression analysis of the impact of age group and MRD status on outcomes

		Adjusting age by < 50 vs ≥ 50	
	N (MRD+ vs MRD–)	HR (95% CI)	P
Relapse	281 vs 2261	1.42 (1.17-172)	<.001
NRM	281 vs 2261	0.97 (0.71-1.32)	.831
DFS	281 vs 2261	1.31 (1.11-1.53)	.001
OS	281 vs 2261	1.27 (1.06-1.51)	.009

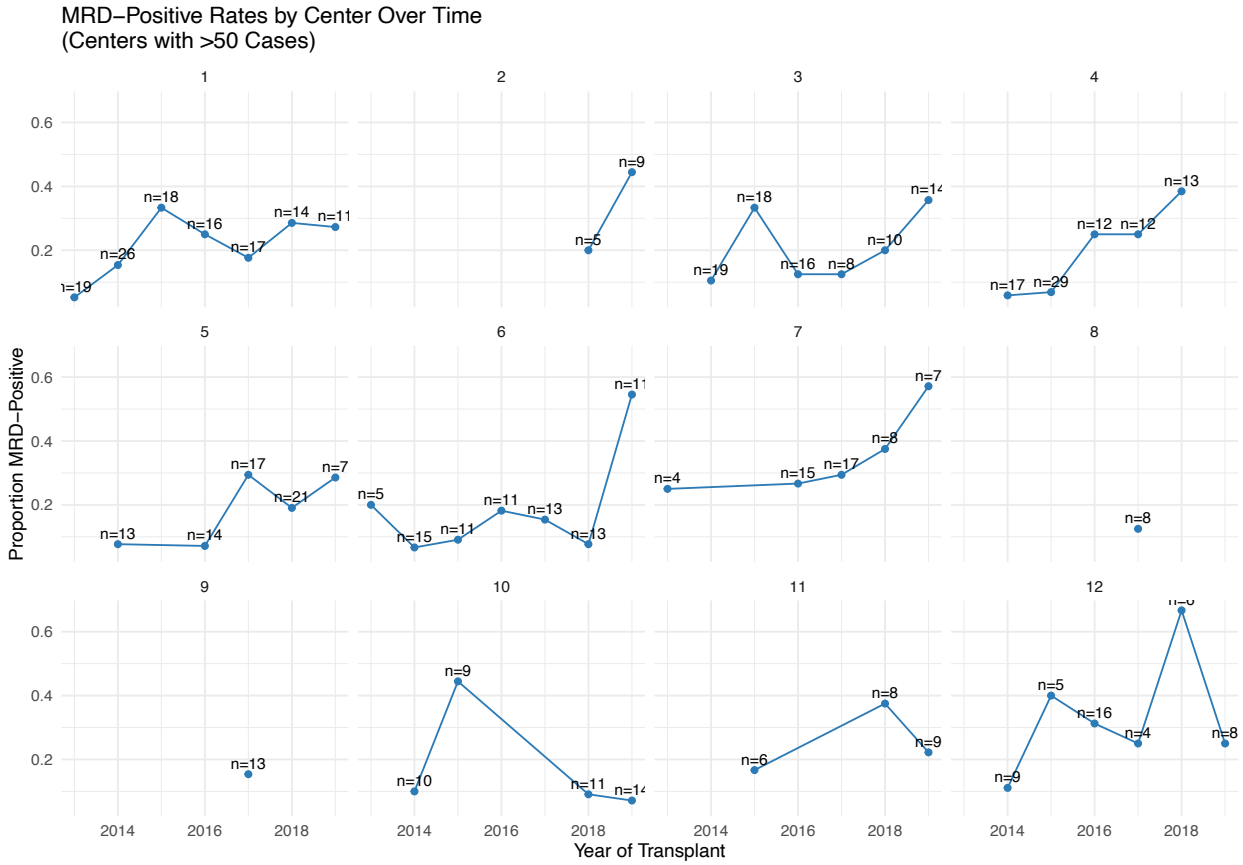
Abbreviations: DFS, disease-free survival; HR, hazard ratio; MRD–, negative measurable residual disease; MRD+,positive measurable residual disease; NRM, non-relapse mortality; OS, overall survival.

Table S14 Impact of age group, conditioning, and MRD status on survival

	RIC/NMA vs MAC	HR (95% CI)	P
Relapse	< 50 & MRD+ (n = 11 vs n = 69)	2.71 (1.23-5.99)	.014
	< 50 & MRD– (n = 157 vs n = 540)	1.84 (1.41-2.41)	<.001
	≥ 50 & MRD+ (n = 123 vs n = 78)	1.65 (1.06-2.57)	.026
	≥ 50 & MRD– (n = 1015 vs n = 549)	1.44 (1.20-1.73)	<.001
NRM	< 50 & MRD+ (n = 11 vs n = 69)	1.04 (0.13-8.45)	.973
	< 50 & MRD– (n = 157 vs n = 540)	0.58 (0.32-1.05)	.071
	≥ 50 & MRD+ (n = 123 vs n = 78)	1.15 (0.59-2.25)	.689
	≥ 50 & MRD– (n = 1015 vs n = 549)	1.08 (0.86-1.35)	.501
DFS	< 50 & MRD+ (n = 11 vs n = 69)	2.39 (1.14-4.99)	.020
	< 50 & MRD– (n = 157 vs n = 540)	1.42 (1.12-1.81)	.004
	≥ 50 & MRD+ (n = 123 vs n = 78)	1.50 (1.04-2.17)	.031
	≥ 50 & MRD– (n = 1015 vs n = 549)	1.30 (1.13-1.50)	<.001
OS	< 50 & MRD+ (n = 11 vs n = 69)	1.50 (0.57-3.96)	.416
	< 50 & MRD– (n = 157 vs n = 540)	1.04 (0.77-1.39)	.810
	≥ 50 & MRD+ (n = 123 vs n = 78)	1.25 (0.86-1.83)	.241
	≥ 50 & MRD– (n = 1015 vs n = 549)	1.23 (1.06-1.43)	.008

Abbreviations: DFS, disease-free survival; HR, hazard ratio; MAC, myeloablative conditioning; MRD, measurable residual disease; NMA, nonmyeloablative conditioning; NRM, non-relapse mortality; OS, overall survival, RIC, reduced-intensity conditioning.

Figure S1 MRD-positive rates by center over time



Note: The proportion of patients testing positive for measurable residual disease (MRD) over time for the 12 largest transplant centers, those with more than 50 patients who underwent first transplant for acute myeloid leukemia. The x-axis shows the year of transplant, while the y-axis represents the proportion of MRD-positive cases. Each panel (facet) corresponds to a different center, with data points connected by a line to highlight the trend. Additionally, the total number of patients for each year is annotated above the points, facilitating comparisons of sample sizes across time. The logistic regression analysis indicates no statistically significant change in MRD positivity (P = .13).