Measurable residual disease recurrence as early warning of relapse in acute myeloid leukemia

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Abstract

The aim of this study was to investigate the clinical features and outcomes of measurable residual disease recurrence (MRD-R) by multiparameter flow cytometry in acute myeloid leukemia. We retrospectively analyzed clinical characteristics, residual disease status and outcomes of 767 patients with newly diagnosed acute myeloid leukemia who achieved complete remission within two cycles of induction at our center. Overall, 171 (22.3%) patients experienced MRD-R during follow-up. Patients with MRD-R had inferior outcomes compared to those without MRD-R, with 3-year cumulative incidence of morphological relapse (CIR), relapse-free survival (RFS) and overall survival (OS) being 63.6% *vs*. 30.6% (*P*<0.001), 13.9% *vs*. 67.2% (*P*<0.001) and 39.0% *vs*. 79.2% (*P*<0.001), respectively. The outcomes for patients in the groups with minute MRD-R (<0.1%) or overt MRD-R (≥0.1%) were comparable, with 3-year CIR, RFS and OS of 65.2% *vs*. 68.2% (*P*=0.76), 28.9% *vs*. 27.5% (*P*=0.85), and 44.8% *vs*. 38.9% (*P*=0.39), respectively. Early intervention at the time of MRD-R postponed morphological relapse, extending the median interval from MRD-R to morphological relapse to 4.2 months *vs*. 1.7 months without intervention (*P*=0.033). Considering these findings together, MRD-R indicated a higher incidence of relapse and poorer outcomes and could serve as an early warning event for relapse in clinical practice. Early intervention could delay relapse, thereby creating a time window for transplantation.

Introduction

Acute myeloid leukemia (AML) is a clonal hematologic malignancy characterized by heterogenous genetic and molecular profiles. Although 60% to 80% of patients achieve complete remission (CR) following induction chemotherapy, about 20% to 40% of patients in remission experience relapse, which accounts for shorter survival.[1] Measurable residual disease (MRD) generally refers to the persistence of trace leukemic cells in patients who have attained morphological CR. Increasing evidence indicates that persistent MRD during treatment is associated with inferior outcomes in AML.[2-4] Multiparametric flow cytometry (MFC) serves as a cornerstone for MRD assessment, being capable of detecting as few as one leukemic cell among 10,000-100,000 normal bone marrow cells, with near-universal applicability in AML. The European LeukemiaNet (ELN) recommends a threshold of less than 0.1% to define MRD negativity by MFC.[5] Notably, emerging studies indicate that even minute residual disease (<0.1%) after induction or consolidation therapy remains predictive of increased relapse rates and reduced survival.[6]

In addition to MRD-negative CR, MRD recurrence (MRD-R) has been increasingly recognized. A subset of patients who initially achieve MRD-negative CR later develop MRD-R during follow-up. While the conventional 0.1% threshold remains widely used to define MRD positivity, conclusive evidence supporting its prognostic validity in this context remains limited.[6-8] A study from MD Anderson Cancer Center (MDACC) showed that patients with MRD-R face a higher rate of relapse and poorer survival outcomes.[9] Recent studies have indicated that flow cytometry MRD surveillance can effectively pre-emptively detect relapse in high-risk AML.[10] However, the natural history and clinical significance of MRD-R detected by MFC requires further characterization. Furthermore, optimal management strat-

egies for this subset of patients remain undefined due to insufficient high-quality evidence. In this study, we aimed to characterize the clinical features and outcomes of MRD-R patients, as well as to evaluate the predictive value of MRD-R for impending relapse in remission-phase AML.

Methods

Patients

A total of 1,744 consecutive, newly diagnosed AML patients treated at our center from January 2014 to April 2021 were retrospectively assessed in this study. Inclusion criteria were as follows: (i) confirmed diagnosis of AML, excluding acute promyelocytic leukemia and core-binding factor AML; (ii) having achieved CR or CR with incomplete hematologic recovery within two courses of intensive chemotherapy; (iii) having at least one MRD-negative bone marrow assessment by MFC; and (iv) continuous MFC-based MRD monitoring during and following treatment. The study was approved by the Blood Diseases Hospital Ethics Committee in compliance with the Declaration of Helsinki. Written informed consent to participation was obtained from all patients.

Treatments

All patients received intensive induction therapy consisting of either cytarabine plus anthracycline with or without homoharringtonine or venetoclax, or cytarabine combined with aclarubicin with or without granulocyte colony-stimulating factor. Following CR, patients received three or four courses of consolidation therapy with high-, intermediate- or standard-dose cytarabine-based regimens. Hematopoietic stem cell transplantation (HSCT) was recommended for eligible patients.

Measurable residual disease detection by multiparametric flow cytometry

Fresh bone marrow samples were analyzed by eight-color MFC (BD FACSCanto). To determine AML MRD by MFC, a minimum of 500,000 events need to be acquired. A cell population was considered quantifiable for MRD when ≥50 phenotypically aberrant events demonstrating consistent leukemia-associated immunophenotypes and/or 'different-from-normal' patterns were identified. The lower limit of quantification and lower limit of detection for MFC-based MRD detection in our hospital were established at 0.01% and 0.004%, respectively. MRD results were routinely reported to clinicians within 4 days of sample receipt. All patients received post-treatment MRD assessments, with those in CR undergoing quarterly monitoring for 3 years, then biannually for 2 additional years if no disease progression. However, 89 cohort members did not complete regular MRD surveillance.

Study endpoints

The primary endpoint was cumulative incidence of relapse (CIR), measured from first MRD-negative CR to relapse with non-relapse deaths as competing risks (Fine-Gray test), censoring non-relapsing patients at their last follow-up. Secondary endpoints included overall survival (OS; MRD-negative CR to any death) and relapse-free survival (RFS; MRD-negative CR to first relapse/death), with surviving/event-free patients censored at their last follow-up. When analyzing MRD-R as a time-dependent variable, we employed the Mantel-Byar test with Simon-Makuch plots or time-dependent Cox models, with all survival analyses starting at MRD-negative CR unless specified. MRD-negative CR was defined as no detectable leukemic cells in the bone marrow sample (under the lower limit of detection), and MRD-R was defined as any newly detectable MRD level in a patient who previously had at least one MRD-negative bone marrow assessment without morphological relapse (MOR-R). MOR-R was defined as either ≥5% blasts in bone marrow/peripheral blood or the development of extramedullary disease.

Statistical analysis

Categorical variables are presented as frequencies and percentages, with between-group comparisons performed using χ^2 tests or the Fisher exact test (N<5). Continuous variables are expressed as medians with interquartile ranges (IQR), analyzed using the Mann-Whitney U test. Statistical significance was set at $P<0.05$. All analyses were conducted using GraphPad Prism 8 and R version 4.4.0.

Results

Clinical characteristics

Of the 1,744 patients, 767 (43.98%) cases met the inclusion criteria and were included in the final analysis. Figure 1 depicts the detailed screening process. Of the 767 patients who met the inclusion criteria, 383 (49.93%) were male and 384 (50.07%) were female, with a median age of onset of AML at 41 years (IQR, 31-51). According to the 2017 ELN risk classification, 331 (43.16%) patients were categorized into the favorable-risk group, 296 (38.59%) into the intermediate-risk group, and the remaining 140 (18.25%) into the adverse-risk group. The baseline clinical characteristics of the cohort are presented in Table 1.

Responses and outcomes

All patients were treated with intensive chemotherapy, with 624 (81.4%) achieving CR after one cycle of induction and 143 (18.6%) requiring two cycles. MRD negativity, assessed by flow cytometry, was achieved by 657 patients (85.7%) within two cycles of induction, while the remaining 110 (14.3%) attained MRD negativity during consolidation therapy. With the median follow-up of sur-

vivors at 39.4 months, the 3-year CIR was 38.6% (95% confidence interval [95% CI]: 34.8-42.3%) (Figure 2A). The estimated 3-year RFS and OS rates were 56.7% (95% CI: 53.0-60.7%) and 70.3% (95% CI: 66.7-74.1%), respectively (Figure 2B, C). The 3-year CIR, RFS and OS censored at HSCT were 43.8% (95% CI: 39.4-48.2%), 53.8% (95% CI: 49.5-58.5%) and 69.3% (95% CI: 65.1-73.8%), respectively (*Online Supplementary Figure S1)*. Of these patients, 171 patients (22.3%) experienced MRD-R, with 108 (63.2%) subsequently progressing to MOR-R. The median MRD level at recurrence was 0.24% (IQR, 0.06-0.74%), including 59 cases (34.5%) with levels below 0.1%. The median time intervals were 6.0 months (IQR, 3.6-11.4) from MRD-negative CR to MRD-R and 3.3 months (IQR, 1.7-7.2) from MRD-R to MOR-R. Notably, these intervals varied significantly by risk stratification. In the favorable-risk group, the median time from MRD-negative CR to MRD-R was 7.5 months (IQR, 3.7-11.4), compared to 5.9 months (IQR, 3.5-11.4) in intermediate-risk patients and 3.8 months (IQR, 3.5-11.4) in adverse-risk patients. Similarly, the median time from MRD-R to MOR-R was 2.6 months (IQR, 1.6-7.1) in the favorable-risk group, 4.9 months (IQR, 1.7-7.2) in the intermediate-risk group, and 2.9 months (IQR, 1.7-7.2) in the adverse-risk group.

Impact of measurable residual disease recurrence

We first compared the outcomes of patients with MRD-R to those without MRD-R (non-MRD-R). Patients with MRD-R had a higher MOR-R rate of 63.2% compared to 28.0% in the non-MRD-R group, with 49.1% of MRD-R patients progressing to MOR-R within 3 months (*Online Supplementary Table S1*). The 3-year CIR, RFS and OS in the MRD-R and non-MRD-R groups were 63.6% (95% CI: 55.5-70.6%) *vs*. 30.6% (95% CI: 26.6-34.7%) (P<0.001), 13.9% (95% CI: 9.5-20.3%) *vs*. 67.2% (95% CI: 63.2-71.4%) (P<0.001) and 39.0% (95% CI: 31.5-48.4%) *vs*. 79.2% (95% CI: 75.6-83.0%) (P<0.001), respectively (Figure 3).

In our cohort, 89 non-MRD-R patients (14.9%) lacked consecutive 3-month serial MRD monitoring, including four who experienced MOR-R. To mitigate potential selection bias, we performed a sensitivity analysis after excluding these patients. The results consistently demonstrated significantly inferior outcomes in MRD-R patients compared with non-MRD-R patients, with 3-year CIR, RFS, and OS rates of 63.6% (95% CI: 55.5-70.6%) *vs*. 35.4% (95% CI: 30.8-40.0%) (P<0.001), 13.9% (95% CI: 9.5-20.3%) *vs*. 65.3% (95% CI: 60.9-69.9%) (P<0.001), and 39.0% (95% CI: 31.5-48.4%) *vs*. 78.5% (95% CI: 74.5-82.7%) (P<0.001), respectively (*Online Supplementary Figure S2*).

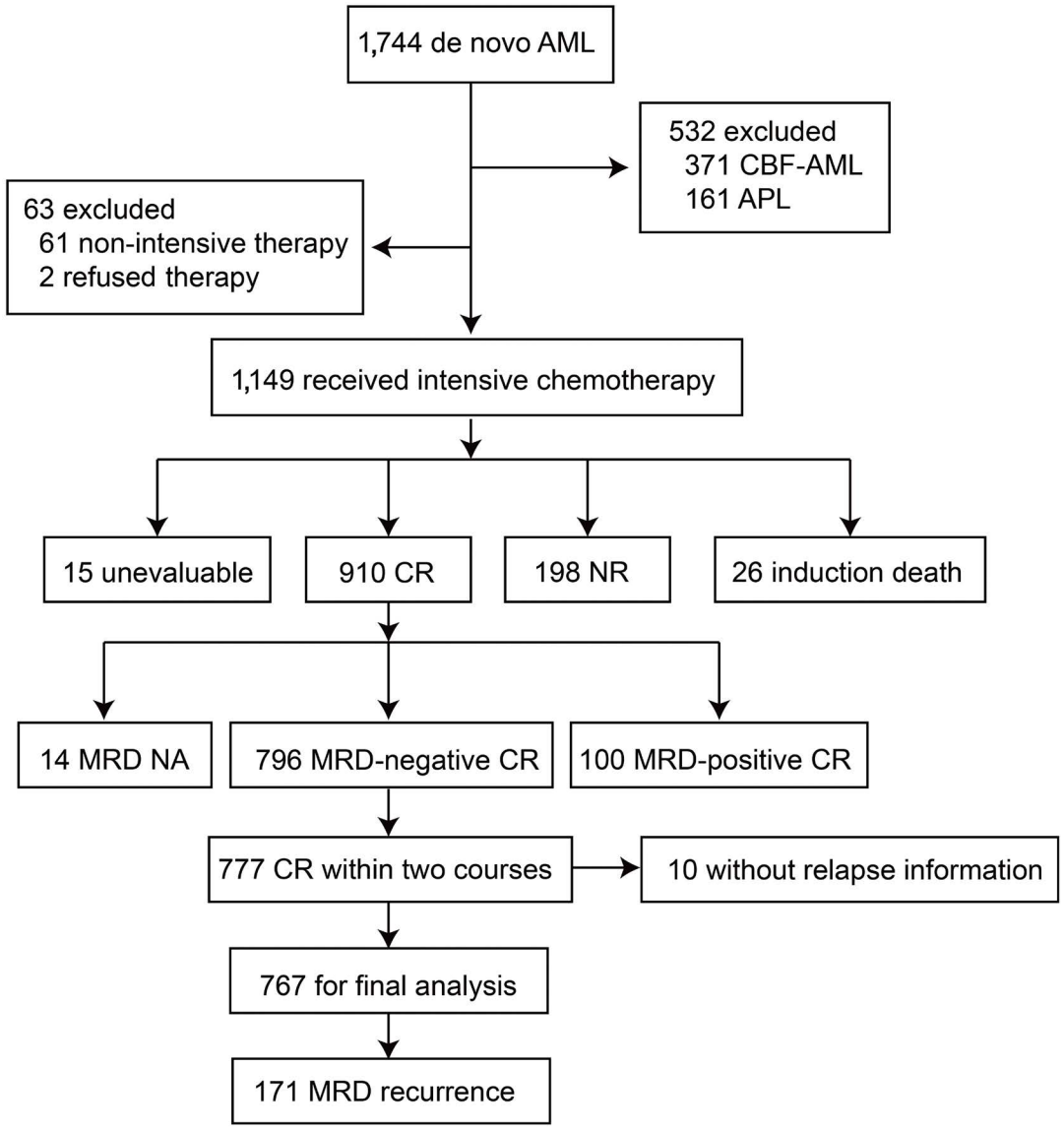

Figure 1. Flow diagram of patients' selection. AML: acute myeloid leukemia; CBF-AML: core-binding factor AML; APL: acute promyelocytic leukemia; CR: complete remission; NR: no response; MRD: measurable residual disease; NA: not available.

Current guidelines set a threshold of 0.1% for MRD detection by MFC. To investigate the clinical relevance of MRD levels below this cutoff, we stratified MRD-R cases into minute MRD-R (<0.1%) and overt MRD-R (≥0.1%) subgroups. Baseline characteristics including age, gender, white blood cell count and ELN risk stratification showed no significant differences between these subgroups (*Online Supplementary Table S2*). Importantly, clinical outcomes for patients in the minute and overt MRD-R groups were comparable (with MRD-R as time zero), with 3-year CIR, RFS and OS at 65.2% (95% CI: 50.5-76.5%) *vs*. 68.2% (95% CI: 57.7-76.6%) (*P*=0.76), 28.9% (95% CI: 18.9-44.2%) *vs*. 27.5% (95% CI: 19.8-38.2%) (*P*=0.85), and 44.8% (95% CI: 32.3-62.3%) *vs*. 38.9% (95% CI: 29.8-50.8%) (*P*=0.39), respectively (Figure 4).

Subgroup analysis

As shown in *Online Supplementary Table S3*, patients in the MRD-R group were significantly older than those in the non-MRD-R group (*P*=0.013) and showed a significant difference in ELN risk stratification (*P*=0.046). However, no significant differences were observed in white blood cell count (*P*=0.568) or gender distribution (*P*=0.573). To strengthen the robustness of our findings, we performed multivariate analyses incorporating the following covariates: age, sex, white blood cell count, consolidation therapy intensity, MRD-R status (time-dependent variable), transplantation (time-dependent variable), and ELN2017 risk stratification. As shown in *Online Supplementary Table S4*, the multivariate analysis revealed that age, white blood cell count, ELN2017 risk stratification, and MRD-R status were all independent risk factors for both OS and RFS, while transplantation served as a protective factor. Notably, MRD-R status demonstrated particularly strong prognostic significance, with MRD-R patients showing significantly worse outcomes compared to non-MRD-R patients (OS: HR=7.807, 95% CI: 2.503-24.352, *P*<0.001; RFS: HR=4.581, 95% CI: 1.882-11.151, *P*=0.001). To evaluate potential effect modification, we further analyzed interactions between MRD-R status and both ELN risk stratification and age. *Online Supplementary Table S4* demonstrates that neither ELN risk category nor age significantly modified the impact of MRD-R status on OS or RFS outcomes.

To address potential age-related bias, we performed a stratified analysis. Among patients under 40 years old, those with MRD-R had significantly worse outcomes compared to non-MRD-R patients, with 3-year CIR, RFS, and OS at 66.4% (95% CI: 53.5-76.5%) *vs*. 28.2% (95% CI: 22.7-34.0%) (*P*<0.001), 15.7% (95% CI: 9.2-26.7%) *vs*. 68.8% (95% CI: 63.2-74.9%) (*P*<0.001), and 40.4% (95% CI: 29.3-55.7%) *vs*. 79.4% (95% CI: 74.2-85.1%) (*P*<0.001), respectively (*Online Supplementary Figure S3A-C*). Similarly, in the 40- to 60-year-old age group, MRD-R remained strongly predictive of inferior outcomes across all endpoints, with 3-year CIR at 62.8% (95% CI: 51.1-72.5%) *vs*. 29.9% (95% CI: 24.2-35.8%) (*P*<0.001), the 3-year RFS rates at 12.0% (95% CI: 6.7-21.4%) *vs*. 68.0% (95% CI: 62.5-74.0%) (*P*<0.001), and the 3-year OS rates at 38.6% (95% CI: 28.4-52.5%) *vs*. 79.9% (95% CI: 74.9-85.1%) (*P*<0.001) (*Online Supplementary Figure S3D-F*). Among patients >60 years, while we observed no significant difference in CIR between groups at 49.2% (95% CI: 18.7-74.1%) *vs*. 57.7% (95% CI: 33.5-75.9%) (*P*=0.760), clinically significant differences emerged in sur-

Table 1. Clinical characteristics of the cohort.

Characteristics	All patients, N=767
Median follow-up, months	39.4
Sex, N (%)	
Male	383 (49.93)
Female	384 (50.07)
Age at diagnosis, years	
Median (IQR)	41 (31-51)
<60, N (%)	715 (93.22)
≥60, N (%)	52 (6.78)
Blood parameters, median (IQR)	
WBC, 10^9/L	14.7 (4.3-44.2)
Hemoglobin, g/L	87 (73-104)
Platelets, 10^9/L	49 (28-80)
2017 ELN risk classification, N (%)	
Favorable	331 (43.16)
Intermediate	296 (38.59)
Adverse	140 (18.25)
Induction regimens,* N (%)	
DA	532 (69.36)
IA	50 (6.52)
HAD	163 (21.25)
AA	22 (2.87)
Consolidation regimens, N (%)	
High-dose cytarabine	565 (73.66)
Intermediate-dose cytarabine	170 (22.16)
Standard-dose cytarabine	32 (17.21)
CR subcategory, N (%)	
CR	725 (94.52)
CRi	42 (5.48)
Timepoints of MRD- CR, N (%)	
After one cycle	534 (69.62)
After two cycles	123 (16.04)
After three or more cycles	110 (14.34)
HSCT in CR1, N (%)	
Yes	214 (27.90)
No	553 (72.10)
MRD recurrence, N (%)	171 (22.29)

*Three patients treated with daunorubicin + cytarabine received venetoclax add-on therapy, while 13 patients treated with aclarubicin + cytarabine received supplemental granulocyte colony-stimulating factor. IQR: interquartile range; WBC: white blood cells; ELN: European LeukemiaNet; DA: daunorubicin + cytarabine; IA: idarubicin + cytarabine; HAD: DA + homoharringtonine; AA: aclarubicin + cytarabine; CR: complete remission; CRi, complete remission with incomplete hematologic recovery; MRD-, measurable residual disease-negative; HSCT: hematopoietic stem cell transplantation; CR1: first complete remission.

vival outcomes. The MRD-R group had significantly inferior 3-year RFS at 20.0% (95% CI: 5.2-76.3%) *vs*. 45.2% (95% CI: 28.4-71.9%) (*P*=0.011). Similarly, 3-year OS was markedly lower in the MRD-R group at 30.0% (95% CI: 10.8-83.4%) *vs*. 70.9% (95% CI: 54.3-92.5%) (*P*=0.036), respectively (*Online Supplementary Figure S3G-I*).

We next compared the impact of MRD-R in each ELN risk group. Compared to non-MRD-R patients, those with MRD-R exhibited 3-year CIR of 50.2% (95% CI: 37.0-62.0%) *vs*. 27.1% (95% CI: 21.2-33.3%) (*P*<0.001) in the favorable-risk group, 67.0% (95% CI: 54.7-76.7%) *vs*. 32.0% (95% CI: 25.5-38.6%) (*P*<0.001) in the intermediate-risk group, and 79.8% (95% CI: 56.6-91.4%) *vs*. 37.1% (95% CI: 27.3-46.9%) (*P*<0.001) in the adverse-risk group (Figure 5A, D and G). The 3-year RFS rates were 21.7% (95% CI: 12.6-37.3%) *vs*. 72.9% (95% CI: 67.2-79.0%) (*P*<0.001), 14.0% (95% CI: 8.1-24.3%) *vs*. 66.9% (95% CI: 60.8-73.7%) (*P*<0.001), and 4.0% (95% CI: 0.9-17.5%) *vs*. 53.3% (95% CI: 43.9-64.8%) (*P*<0.001) (Figure 5B, E and H), respectively, while the 3-year OS rates were 53.6% (95% CI: 40.8-70.4%) *vs*. 90.1% (95% CI: 86.1-94.2%) (*P*<0.001), 38.2% (95% CI: 27.8-52.5%) *vs*. 74.6% (95% CI: 68.4-81.2%) (*P*<0.001), and 19.5% (95% CI: 8.7-43.5%) *vs*. 62.5% (95% CI: 52.9-74.0%) (*P*<0.001) (Figure 5C, F and I).

Early intervention postponed relapse of acute myeloid leukemia

Among the 171 patients who experienced MRD-R, 29 (17.0%) did not receive any early intervention, 131 (76.6%) received drug treatments, and seven (4.1%) proceeded directly to HSCT. The remaining four patients (2.3%) were excluded because of incomplete treatment information. *Online Supplementary Table S5* presents the clinical features of these patients. Early intervention, compared to no intervention, can increase the proportion of patients achieving MRD negativity within 3 months (*P*=0.007). Among 29 patients without intervention, outcomes were suboptimal with only four patients (13.8%) achieving MRD negativity within 3 months, seven (24.1%) maintaining persistent MRD without MOR-R, and 18 (62.1%) progressing to relapse. All four

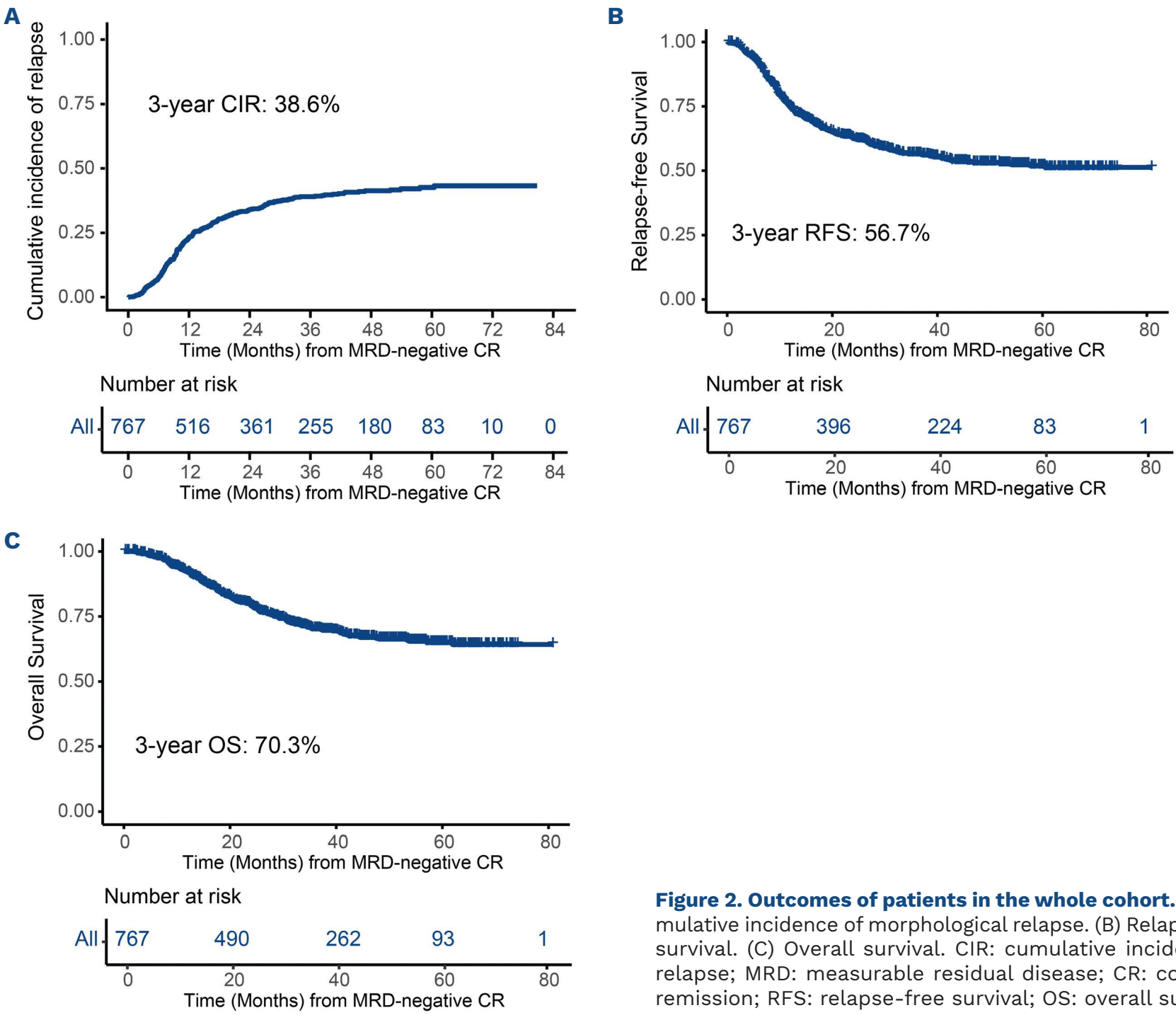

Figure 2. Outcomes of patients in the whole cohort. (A) Cumulative incidence of morphological relapse. (B) Relapse-free survival. (C) Overall survival. CIR: cumulative incidence of relapse; MRD: measurable residual disease; CR: complete remission; RFS: relapse-free survival; OS: overall survival.

cases of spontaneous MRD clearance occurred in patients without a history of transplantation, each presenting with minute MRD-R. Among 114 evaluable patients receiving drug intervention (excluding 17 with unevaluable MRD status), 42 (32.1%) achieved MRD negativity after the first cycle of pre-emptive treatment, 43 (32.8%) showed persistent MRD without MOR-R, and 29 (22.14%) relapsed, while all seven HSCT recipients (100%) achieved MRD negativity.

Although no significant differences were observed in outcomes between the intervention and non-intervention groups (*Online Supplementary Figure S4*), early intervention significantly postponed MOR-R in AML patients, with the median time from MRD-R to MOR-R being 4.2 months (IQR, 1.8-7.5) in the intervention group compared to just 1.7 months (IQR, 0.9-2.9) in the non-intervention group (P=0.033). Notably, achieving MRD negativity was associated with significantly lower rates of subsequent MOR-R (37.7% *vs*. 66.0%, P<0.001) and longer median time to relapse (7.3 months [IQR, 4.9-15.4] *vs*. 5.3 months [IQR, 4.2, 11.0], P=0.184) compared to those with persistent MRD. Final relapse rates demonstrated a treatment gradient, with the highest incidence in the group that received no intervention (69.0%), intermediate in drug-treated patients (63.4%), and lowest in HSCT recipients (42.9%). Patients achieving MRD negativity within 3 months after MRD-R had better survival outcomes than those with persistent MRD (with MRD-R as time zero and MRD clearance as a time-dependent covariate). Overall, MRD-negative patients had 3-year CIR, RFS, and OS rates of 40.2% (95% CI: 25.8-54.1%), 53.0% (95% CI: 40.0-70.3%), and 58.9% (95% CI: 44.9-77.2%) *vs*. 76.5% (95% CI: 59.5-87.1%) (P<0.001), 21.7% (95% CI: 12.0-39.1%) (P=0.005), and 43.5% (95% CI: 29.8-63.7%) (P=0.134) in MRD-persistent patients

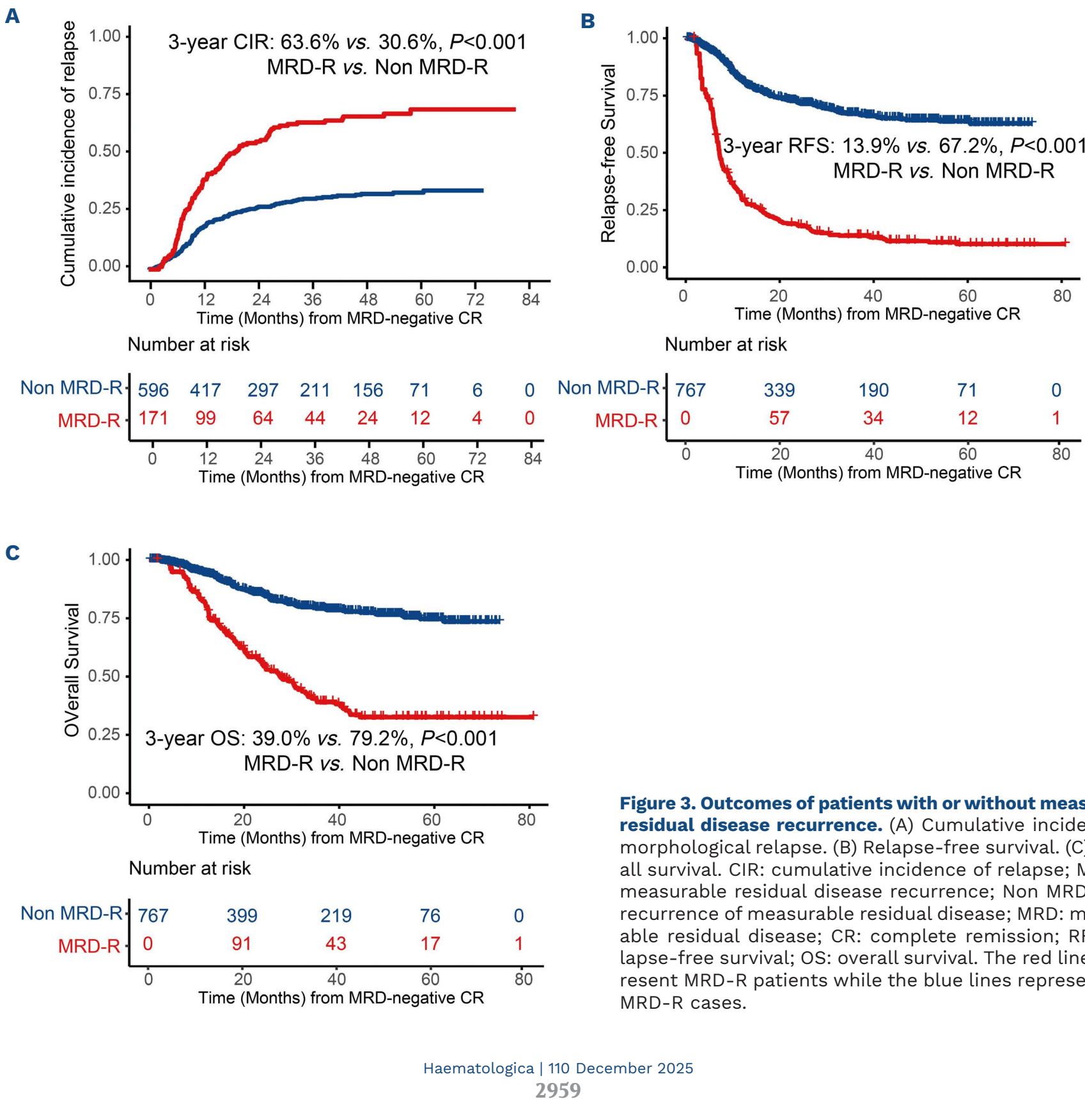

Figure 3. Outcomes of patients with or without measurable residual disease recurrence. (A) Cumulative incidence of morphological relapse. (B) Relapse-free survival. (C) Overall survival. CIR: cumulative incidence of relapse; MRD-R: measurable residual disease recurrence; Non MRD-R: no recurrence of measurable residual disease; MRD: measurable residual disease; CR: complete remission; RFS: relapse-free survival; OS: overall survival. The red lines represent MRD-R patients while the blue lines represent non MRD-R cases.

(Figure 6A-C). Specifically, in the drug intervention group, patients achieving MRD negativity showed superior outcomes compared to those with persistent MRD, with the 3-year CIR, RFS and OS being 43.0% (95% CI: 26.3-58.8%) *vs*. 79.6% (95% CI: 61.3-89.9%) (*P*=0.001), 50.9% (95% CI: 36.3-71.3%) *vs*. 18.4% (95% CI: 9.2-36.8%) (*P*=0.012), and 56.4% (95% CI: 40.5-78.6%) *vs*. 39.8% (95% CI: 25.7-61.8%) (*P*=0.134), respectively (Figure 6D-F). All the survival rates and time intervals are presented in *Online Supplementary Table S6*.

Discussion

This study revealed that 22.3% of AML patients experienced MRD-R after achieving MRD-negative CR, a population that exhibited significantly higher rates of MOR-R and worse clinical outcomes compared to those maintaining MRD negativity. Importantly, our findings demonstrate that early therapeutic intervention could effectively delay MOR-R, thereby creating a time window for HSCT.

Recently, Short *et al*. from MDACC reported a similar finding, but the rate of MRD-R was only 7.4% (55/704), significantly lower than that of our study.[9] Differences in treatment regimens, the frequency of MRD monitoring, and the cutoff value for MRD-R may account for this discrepancy. In our study, we defined any MRD level above the lower limit of detection as recurrence, with the lowest detectable level at 0.004%, compared to 0.05% in Short's report. Additionally, venetoclax was used in only three cases in our study, while it was used in 36% at MDACC, which may also contribute

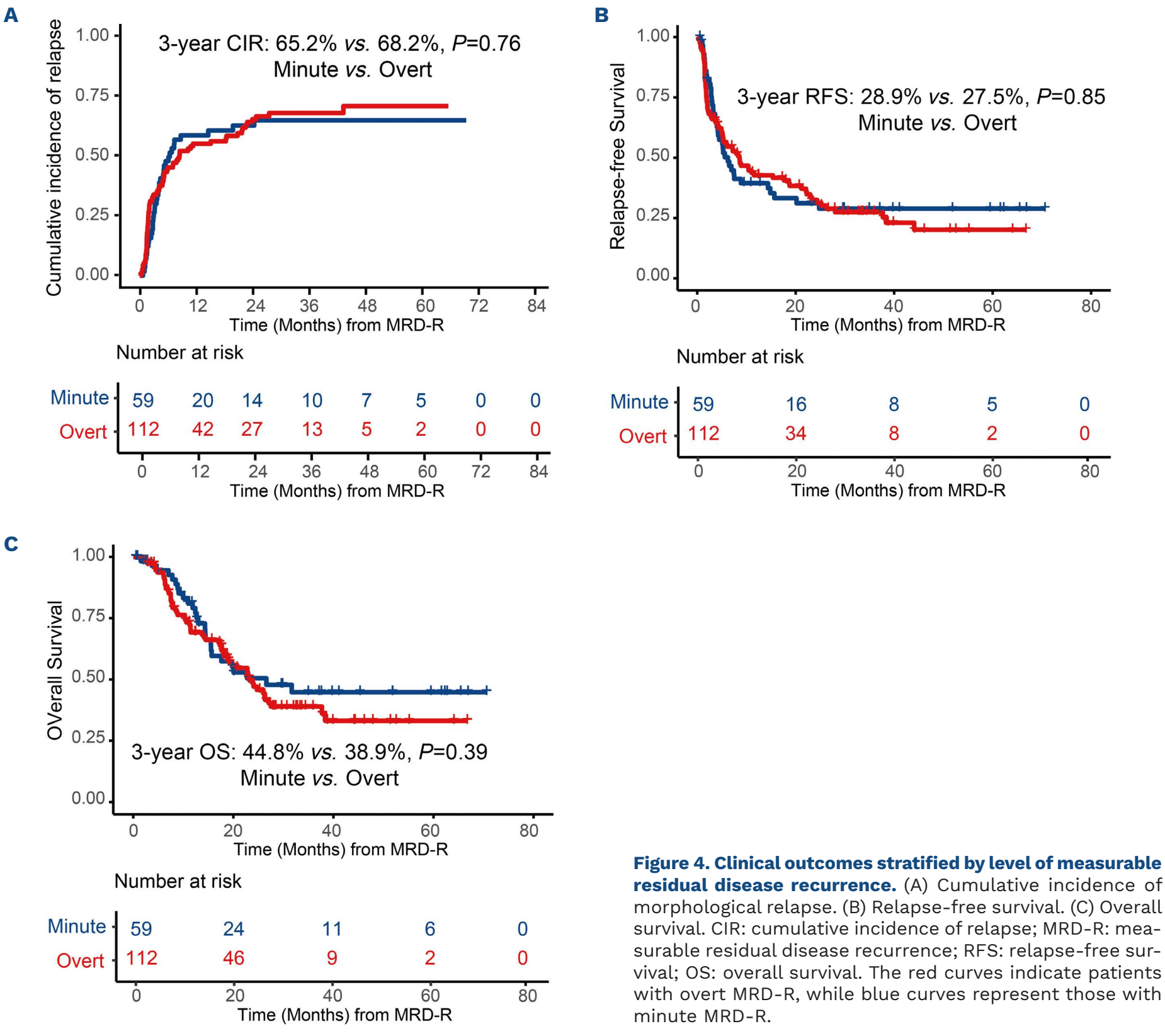

Figure 4. Clinical outcomes stratified by level of measurable residual disease recurrence. (A) Cumulative incidence of morphological relapse. (B) Relapse-free survival. (C) Overall survival. CIR: cumulative incidence of relapse; MRD-R: measurable residual disease recurrence; RFS: relapse-free survival; OS: overall survival. The red curves indicate patients with overt MRD-R, while blue curves represent those with minute MRD-R.

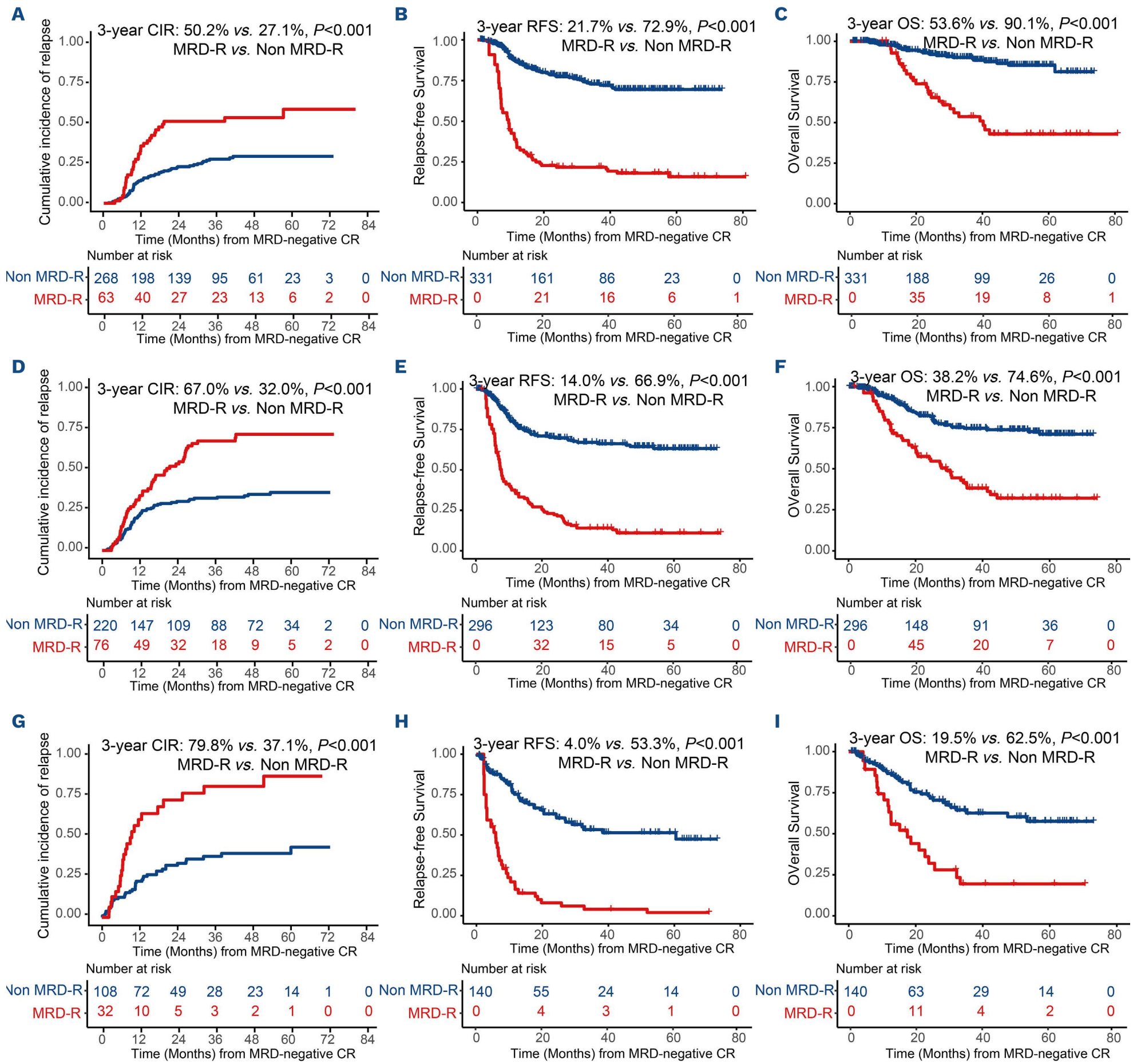

Figure 5. Clinical outcomes stratified by European LeukemiaNet 2017 risk classification and measurable residual disease recurrence status. (A, D, G) Cumulative incidence of relapse. (B, E, H) Relapse-free survival. (C, F, I) Overall survival. (A-C) Outcomes for favorable-risk patients; (D-F) for intermediate-risk; and (G-I) for adverse-risk patients according to the European LeukemiaNet classification. Patients with measurable residual disease recurrence are represented by red curves, while those without recurrence of measurable residual disease are shown in blue. CIR: cumulative incidence of relapse; MRD-R: measurable residual disease recurrence; Non MRD-R: no recurrence of measurable residual disease; MRD: measurable residual disease; CR: complete remission; RFS: relapse-free survival; OS: overall survival.

to the differences between the two studies.[9] These protocol differences likely account for the observed disparity in MRD-R detection rates between the two studies while still supporting the clinical significance of MRD recurrence in AML management.

The current ELN guidelines recommend a cutoff of 0.1% for MRD-R by MFC.[3,4] However, emerging evidence challenges this conventional cutoff. Recent computational analyses of flow cytometric MRD surveillance in high-risk AML demonstrated that lower thresholds (e.g., 0.040%) achieved superior sensitivity (74%) for relapse prediction while maintaining high specificity (87%), outperforming the traditional 0.1% threshold (sensitivity 51%, specificity 98%).[10] Maurillo *et al.* demonstrated that even at a much lower threshold of 3.5×10^{-4}, MRD positivity following consolidation therapy was significantly associated with worse RFS and OS.[11] Our findings corroborate and extend these observations, demonstrating that AML patients with any detectable MRD-R, whether meeting or falling below the ELN threshold, exhibited substantially poorer clinical outcomes compared to those maintaining MRD negativity. These consistent findings across studies strongly suggest that the current 0.1% cutoff may lack sufficient sensitivity for optimal risk stratification, highlighting the need for further multicenter studies to establish more precise, biologically relevant thresholds for MRD-R definition in AML management.

The optimal management strategy for AML patients with MRD-R remains undefined, as current guidelines lack consensus regarding pre-emptive treatment approaches. Our study demonstrated that while a small subset of patients (4/29, 13.8%) achieved spontaneous MRD clearance without intervention, comparable to the 19% rate observed in the QUAZAR AML-001 trial,[12] these cases may represent false-positive MRD results given their exceptionally favorable outcomes. Emerging evidence supports

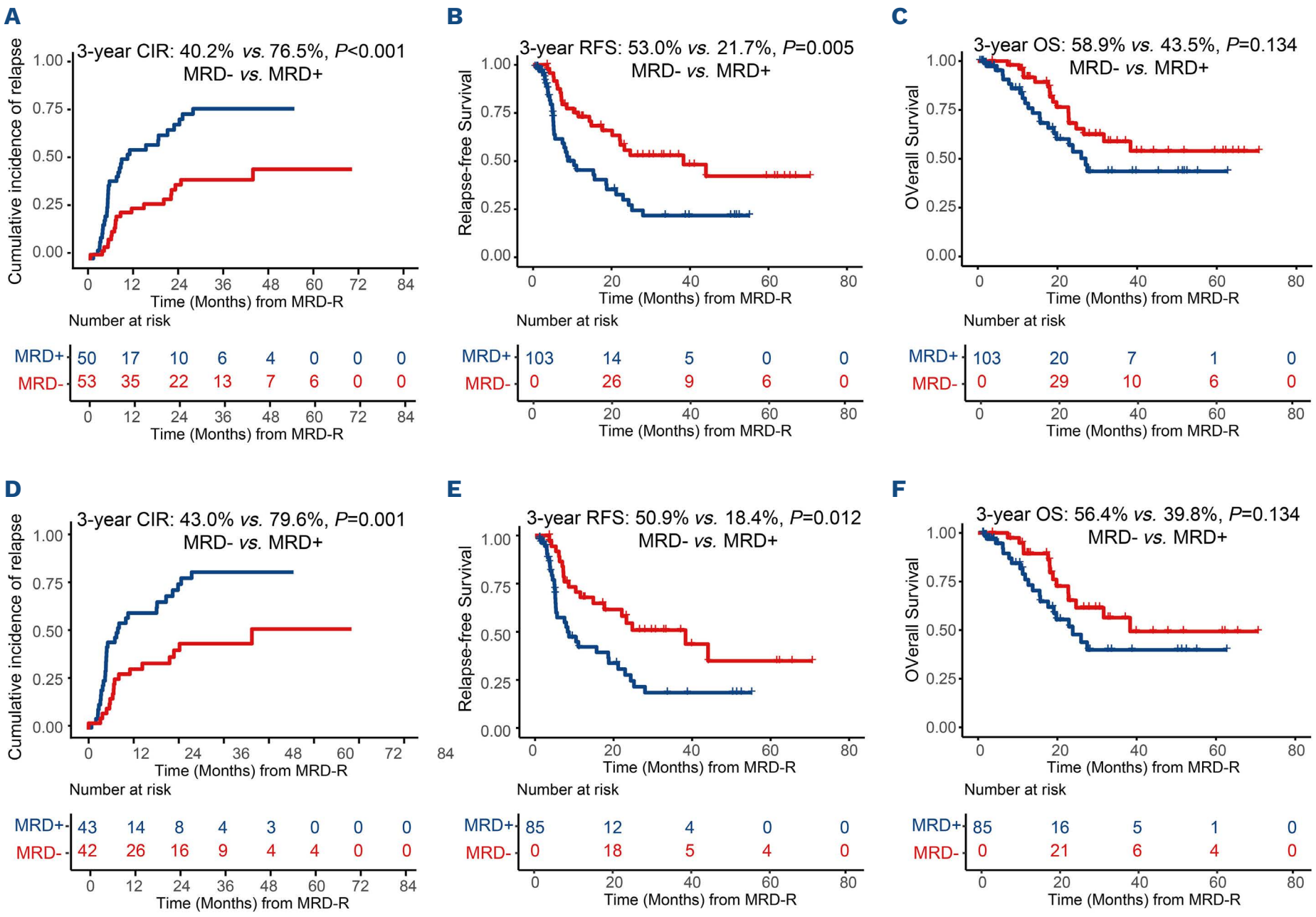

Figure 6. Clinical outcomes of patients with measurable residual disease recurrence stratified by response to early intervention. (A-C) Entire cohort. (D-F) Drug intervention group. (A, D) Cumulative incidence of relapse. (B, E) Relapse-free survival. (C, F) Overall survival. Red curves indicate patients who achieved clearance of measurable residual disease after intervention, while blue curves represent those with persistent measurable residual disease. CIR: cumulative incidence of relapse; MRD-: measurable residual disease-negative; MRD+: measurable residual disease-positive; MRD-R: measurable residual disease recurrence; RFS: relapse-free survival; OS: overall survival.

the efficacy of hypomethylating agents in preventing or delaying relapse for MRD-positive AML patients or patients with myelodysplastic syndrome,[12-14] and our findings further reinforce the clinical benefit of timely intervention, showing that treated patients experienced significantly prolonged progression intervals (median 4.2 months *vs*. 1.7 months in untreated cases). This dramatic difference underscores the aggressive proliferative potential of residual leukemic clones and emphasizes the critical importance of rapid therapeutic intervention. To address potential selection bias from rapidly progressive disease, we systematically compared the time intervals between MRD-R and clinical intervention in the treatment group against the time from MRD-R to relapse in the non-intervention group. This analysis revealed a striking >30-fold difference in median timeframes (1.5 days [IQR, 1-12] for intervention *versus* 52 days [IQR, 6-88.25] to relapse), demonstrating that pre-emptive therapy was consistently initiated during the biologically early phase of MRD-R. The profound temporal dissociation between these intervals strongly suggests that: (i) early intervention occurred before natural disease progression could manifest clinically, and (ii) the therapeutic benefit observed is unlikely to be attributable solely to selection of indolent cases, as interventions were consistently initiated within a standardized 4-day reporting window, preceding any clinical evidence of disease tempo. This rapid turnaround ensured early treatment during the biologically susceptible phase of MRD-R, before relapse manifestations could influence case selection. The relatively short median time of 1.5 days from MRD-R detection to intervention in our study may reflect our center's specific workflow and resource availability. While this rapid response likely contributed to preventing progression to MOR-R, we acknowledge that such timelines may be challenging to achieve in other settings due to variations in healthcare infrastructure, referral processes, and logistical constraints. Nevertheless, our findings suggest that minimizing delays between MRD-R and intervention could be beneficial. However, conventional chemotherapy alone proved insufficient for many patients, with substantial progression rates persisting despite treatment. While immediate HSCT represents the optimal curative approach, practical constraints often limit its feasibility due to the narrow therapeutic window available for transplantation preparation. Consequently, there is an urgent clinical need to develop optimized therapeutic strategies for this high-risk population, with particular emphasis on integrating novel targeted agents including FLT3 inhibitors (e.g., gilteritinib), BCL-2 inhibitors (e.g., venetoclax), and emerging immunotherapeutic approaches (e.g., antibody-drug conjugates or chimeric antigen receptor T-cell therapy) into current treatment paradigms to significantly improve long-term outcomes. This study has several limitations that should be acknowledged. First, this was a retrospective study. Second, the relatively small cohort size may reduce the statistical power to fully characterize the clinical features and outcomes of MRD-R patients. Additionally, non-randomized treatment allocation after MRD-R is an important limitation, which may introduce confounding by indication. These limitations highlight the need for well-designed, large-scale prospective studies to validate our findings and further elucidate the optimal management strategies for MRD-R in AML patients.

In conclusion, this study confirmed that MRD-R serves as a robust predictor of disease progression in AML patients, with even minimal residual disease levels (<0.1%) conferring a significant risk of subsequent relapse. The strong correlation between MRD-R and poor clinical outcomes establishes the former as a critical early warning indicator that should prompt immediate therapeutic intervention. These results have two key clinical implications: first, the implementation of standardized, frequent MRD monitoring protocols is essential for timely detection of disease recurrence; second, there is an urgent need to develop optimized treatment strategies specifically for MRD-R patients, potentially incorporating novel targeted therapies or early transplantation approaches to prevent overt relapse and improve long-term survival.

Disclosures

No conflicts of interest to disclose.

Contributions

JW and HW participated in the design of the study concept. BG collected and organized the clinical data. MY participated in data analysis and drafting and revising the manuscript. SQ, BL, YW and YM interpreted the results. All authors read and approved the final manuscript.

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Data-sharing statement

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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