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

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Supplementary methods

Treatments

All patients received intensive induction therapy with cytarabine and anthracycline-based regimens, including 532 patients (69.36%) treated with DA (daunorubicin plus cytarabine), 50 (6.52%) with IA (idarubicin plus cytarabine), 163 (21.25%) with HAD (homoharringtonine plus cytarabine and daunorubicin), and 22 (2.87%) with AA (aclarubicin plus cytarabine). Among these, 3 DA-treated patients additionally received venetoclax, while 13 AA-treated patients received supplemental granulocyte colony-stimulating factor (G-CSF).

Following complete remission (CR), all patients received 3-4 cycles of consolidation therapy: 32 (17.21%) with standard-dose cytarabine, 170 (22.16%) with intermediate-dose, and 565 (73.66%) with high-dose cytarabine. Hematopoietic stem cell transplantation was recommended for eligible candidates.

MRD detection by MFC

Fresh bone marrow samples were analyzed by multiparameter flow cytometry (MFC) using a BD FACSCanto system with two optimized 8-color antibody panels: (1) CD38/CD117/CD34/CD33/CD13/HLA-DR/CD11b/CD45 and (2) CD15/CD34/CD56/CD33/CD7/CD14/CD19/CD45. To ensure sensitive detection of rare leukemic populations, we acquired $\geqslant$ 500,000 nucleated cells per sample, establishing analytical thresholds of 0.004% (LLOD, $\geqslant$20 aberrant events) and 0.01% (LLOQ, $\geqslant$50 events).

Measurable residual disease (MRD) identification incorporated both leukemia-associated immunophenotypes (LAIP) and different-from-normal (DfN) approaches. LAIP criteria included: (1) asynchronous antigen co-expression (e.g., CD34+CD117+CD33+), (2) $\geqslant$1 log marker over-expression (CD33/CD13/CD123), (3) aberrant lymphoid markers (CD7/CD56/CD19) on myeloid blasts, and (4) abnormal light scatter (SSC/CD45). The DfN approach utilized 50 age-matched normal controls to detect $\geqslant$2 consistent immunophenotypic deviations while excluding regenerating progenitors. MRD evaluation primarily followed the DfN approach, with complementary integration of LAIPs identified at initial diagnosis when available.

Clinical implementation featured: (1) standardized 4-day turnaround from sample receipt, (2) post-treatment MRD assessments for all patients, and (3) a risk-adapted monitoring schedule (quarterly for 3 years in CR patients, transitioning to biannually for 2 additional years if no disease progression). Notably, 89 patients (11.6%) were non-adherent to protocol-specified monitoring.

Study endpoints

Primary Endpoint: The cumulative incidence of relapse (CIR) was calculated from the date of first MRD-negative CR until relapse, with non-relapse deaths considered as competing events (analyzed using Fine-Gray's test). Patients without relapse were censored at last follow-up.

Secondary Endpoints: 1) Overall survival (OS): Time from MRD-negative CR to death from any cause (censored at last follow-up for surviving patients). 2) Relapse-free survival (RFS): Time from MRD-negative CR to first relapse or death (censored at

last follow-up for event-free patients). Both secondary endpoints were analyzed using Kaplan-Meier estimates with log-rank tests for comparisons. When analyzing MRD-R as a time-dependent variable, we employed the Mantel-Byar test with Simon-Makuch plots and time-dependent Cox models. Unless otherwise specified, the starting point for survival analysis was MRD-negative CR.

Multivariate analysis

Both MRD-R and transplant status were included as time-dependent covariates in the same multivariate model, which also adjusted for age, sex, WBC, consolidation therapy intensity, and ELN risk stratification. The time-dependent covariates (MRD-R and HSCT) were analyzed using an extended Cox proportional hazards model implemented through R's survival package. Patient follow-up periods were dynamically segmented based on MRD-R occurrence and transplant status transitions using the tmerge function. This created epoch-specific intervals with updated exposure status for both variables throughout follow-up.

Figure S1. Outcomes of the cohort under transplant censoring. A represents cumulative incidence of relapse (CIR), B denotes relapse-free survival (RFS), and C indicates overall survival (OS).

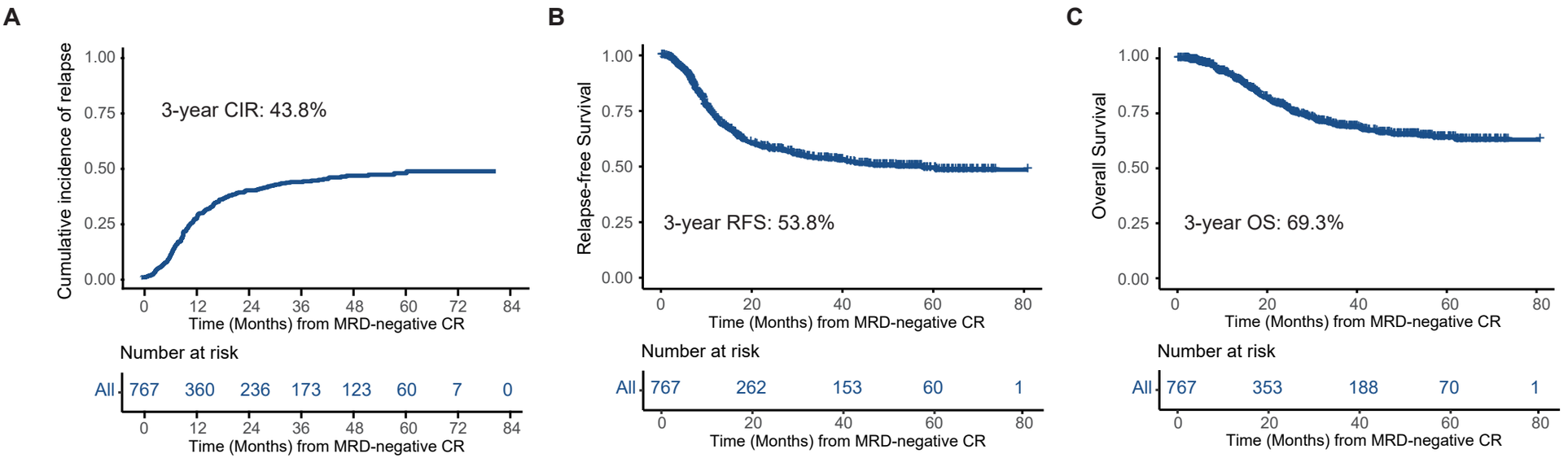

Figure S2. Sensitivity analysis assessing outcomes after exclusion of patients lacking serial measurable residual disease (MRD) monitoring. A represents cumulative incidence of relapse (CIR), B denotes relapse-free survival (RFS), and C indicates overall survival (OS). The blue line represents non MRD recurrence (Non MRD-R), and the red line indicates MRD-R

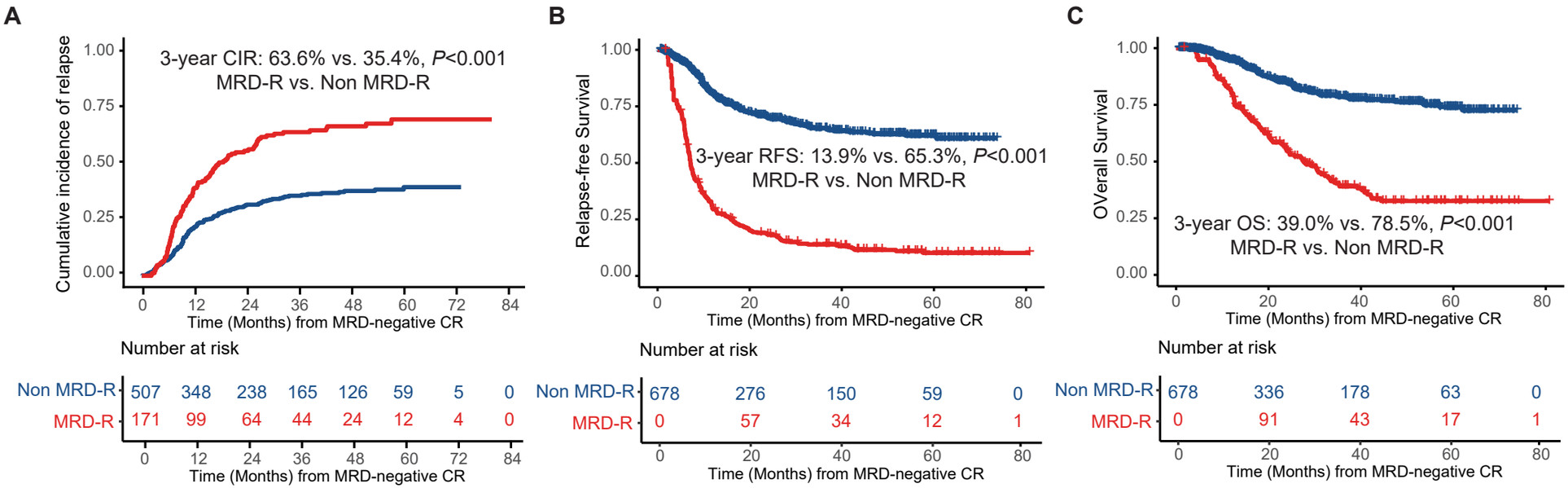

Figure S3. Outcomes stratified by age: <40y (A-C), 40-60y (D-F), >60y (G-I). A/D/G = cumulative incidence of relapse (CIR); B/E/H = relapse-free survival (RFS); C/F/I = overall survival (OS). Meaurable residual disease recurrence (MRD-R) (red) vs. non MRD-R (blue).

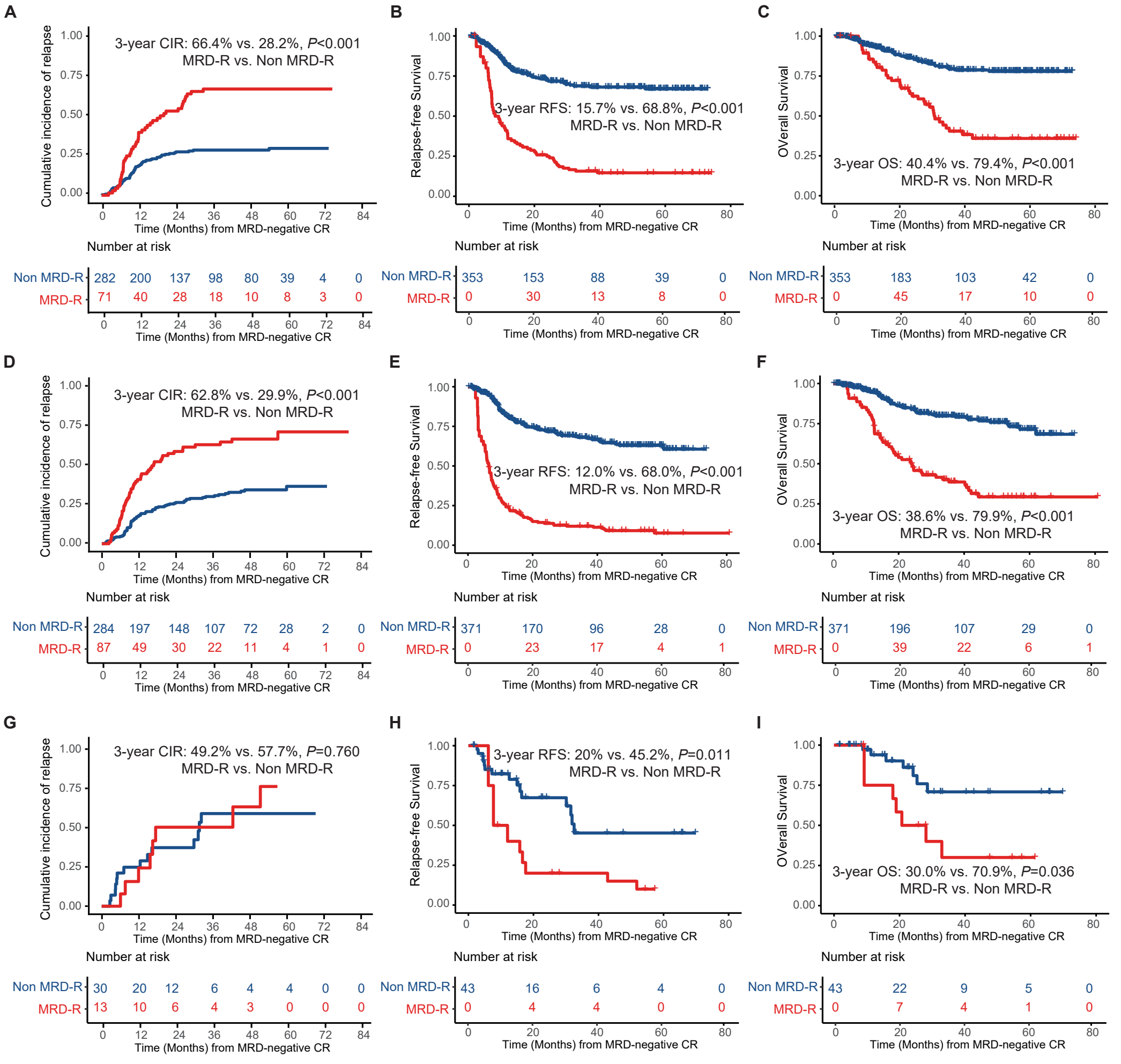

Figure S4. Outcomes of MRD-R group patients across different intervention groups. A represents cumulative incidence of relapse (CIR), B denotes relapse-free survival (RFS), and C indicates overall survival (OS). The blue line represents no intervention, the red line indicates direct transplantation, the green line corresponds to intensified drug therapy, and the light blue line signifies dose-reduced chemotherapy.

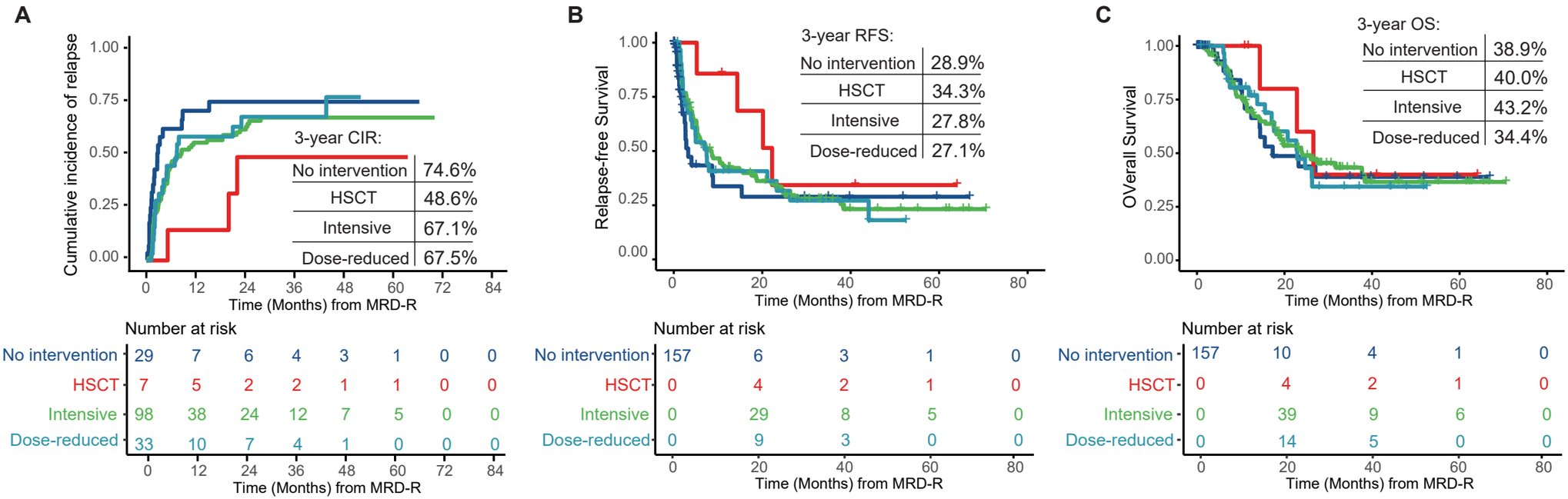

Table S1. Clinical characteristics of the relapsed patients in MRD-R and non MRD-R groups.

	MRD-R (N=108)	Non MRD-R (N=167)	*P*
Timepoint for relapse, n (%)			0.605
Post-chemotherapy	95 (87.96)	143 (85.63)	
Post-HSCT	13 (12.04)	24 (14.37)	
Genetics, n (%)			
KMT2A-r	12 (11.11)	15 (8.98)	0.554
FLT3-ITD	17 (15.74)	36 (21.56)	0.225
NPM1	21 (19.44)	44 (26.35)	0.191
CEBPA bZIP	24 (22.22)	33 (19.76)	0.623
DNMT3A	13 (12.04)	18 (10.78)	0.726
IDH1	1 (0.93)	12 (7.19)	0.035
IDH2	6 (5.56)	5 (2.99)	0.353
TP53	1 (0.93)	1 (0.60)	1.000

Abbreviation: MRD, measurable residual disease; MRD-R, MRD recurrence; *KMT2A*-r, *KMT2A* rearrangement; *CEBPA* bZIP, *CEBPA* basic leucine zipper domain; HSCT, hematopoietic stem cell transplantation.

Table S2. Clinical characteristic of patients from minute MRD-R and overt MRD-R groups.

Characteristics	Minute MRD-R (N=59)	Overt MRD-R (N=112)	*P*
Sex, n (%)			0.794
Male	30 (50.85)	59 (52.68)	
Female	29 (49.15)	53 (47.32)	
Age at diagnosis, yr, n (%)			
Median (IQR)	37 (32.5-52)	46 (34.5-54.25)	0.107
<40	30 (50.85)	41 (36.61)	
40-60	28 (47.46)	59 (52.68)	
⩾60	1 (1.69)	12 (10.71)	
WBC, ×109/L, median (IQR)	14 (6.17-42.54)	14 (2.455-38.85)	0.368
2017 ELN risk classification, n (%)			0.885
Favorable	22 (37.29)	41 (36.61)	
Intermediate	25 (42.37)	51 (45.54)	
Adverse	12 (20.34)	20 (17.86)	
Intervention after MRD-R, n (%)			<0.001
No intervention	19 (32.20)	10 (8.93)	
Drug intervention	33 (55.93)	98 (87.5)	
HSCT	5 (8.47)	2 (1.79)	
Unclear	2 (3.39)	2 (1.79)	
Intervention results*, n (%)			0.367
MRD negative	23 (41.82)	30 (31.58)	
MRD persistent without MOR-R	18 (32.73)	32 (33.68)	
MOR-R	14 (25.45)	33 (34.74)	

*Patients with unevaluable MRD status post-intervention (4 from the minute MRD-R group and 17 from the overt MRD-R group) were excluded from analyses and treated as missing data.

Abbreviation: MRD-R, measurable residual disease recurrence; IQR, interquartile range; WBC, White blood cell; ELN, European LeukemiaNet; HSCT, hematopoietic stem cell transplantation; MOR-R, morphologic relapse.

Table S3. Clinical characteristics of the patients in MRD-R and non MRD-R groups.

Characteristics	MRD-R (N=171)	Non MRD-R (N=596)	*P*
Sex, n (%)			0.573
Male	89 (52.05)	294 (49.33)	
Female	82 (47.95)	302 (50.67)	
Age at diagnosis, yr, n (%)			
Median (IQR)	43 (33, 53)	41 (30, 50)	0.013
< 40	71 (41.52)	282 (47.32)	
40-60	87 (50.88)	284 (47.65)	
≥ 60	13 (7.60)	30 (5.03)	
WBC, ×109/L, median (IQR)	13.74 (3.88, 39.24)	15.07 (4.43, 44.63)	0.568
2017 ELN risk classification, n (%)			0.046
Favorable	63 (36.84)	268 (44.97)	
Intermediate	76 (44.44)	220 (36.91)	
Adverse	32 (18.71)	108 (18.12)	
Genetics, n (%)			
KMT2A-r	15 (8.77)	57 (9.56)	0.783
FLT3-ITD	22 (12.87)	109 (18.29)	0.092
NPM1	38 (22.22)	196 (32.89)	0.006
CEBPA bZIP	39 (22.81)	120 (20.13)	0.443
DNMT3A	21 (12.28)	66 (11.07)	0.654
IDH1	8 (4.68)	36 (6.04)	0.493
IDH2	15 (8.77)	38 (6.38)	0.245
TP53	1 (0.59)	4 (0.67)	1.000
Timepoints of MRD- CR, n (%)			0.003
After one cycle	103 (60.23)	431 (72.32)	
After two cycles	37 (21.64)	86 (14.43)	
After three or more cycles	31 (18.13)	79 (13.26)	
HSCT in CR1, n (%)			0.256
YES	42 (24.56)	172 (28.86)	
NO	129 (75.44)	424 (71.14)	
MOR-R, n (%)	108 (63.16)	167 (28.02)	<0.001

Abbreviation: MRD, measurable residual disease; MRD-R, MRD recurrence; IQR, interquartile range; WBC, White blood cell; ELN, European LeukemiaNet; *KMT2A*-r, *KMT2A* rearrangement; *CEBPA* bZIP, *CEBPA* basic leucine zipper domain; CR1, the first complete remission; HSCT, hematopoietic stem cell transplantation; MOR-R, morphologic relapse.

Table S4. Multivariable Cox regression analysis of OS and RFS in the cohort.

	OS		RFS	
	HR*	*P*	HR*	*P*
Age	1.020 (1.005-1.036)	0.009	1.012 (1.001-1.024)	0.038
Female	1.053 (0.794-1.395)	0.722	1.072 (0.857-1.343)	0.542
WBC	1.004 (1.002-1.006)	<0.001	1.004 (1.002-1.006)	<0.001
Intermediate-risk	2.648 (1.660-4.223)	<0.001	1.588 (1.142-2.207)	0.006
Adverse-risk	5.238 (3.094-8.866)	<0.001	3.161 (2.141-4.668)	<0.001
CR1 HSCT	0.608 (0.423-0.873)	0.007	0.406 (0.293-0.561)	<0.001
HDAC consolidation	0.865 (0.436-1.717)	0.678	1.634 (0.866-3.084)	0.129
MDAC consolidation	0.761 (0.378-1.532)	0.445	1.384 (0.726-2.638)	0.324
MRD-R	7.807 (2.503-24.352)	<0.001	4.581 (1.882-11.151)	0.001
Intermediate-risk: MRD-R	0.541 (0.272-1.077)	0.08	0.870 (0.507-1.493)	0.613
Adverse-risk: MRD-R	0.512 (0.233-1.123)	0.095	0.901 (0.476-1.705)	0.749
Age: MRD-R	0.992 (0.970-1.015)	0.499	1.004 (0.985-1.022)	0.698

*All HRs presented are adjusted values from the multivariate Cox model containing both time-dependent variables (MRD-R and HSCT status) along with age, sex, WBC, consolidation therapy intensity, and ELN risk stratification.

Abbreviations: OS, overall survival; RFS, relapse-free survival; HR, hazard ratio; WBC, White blood cell count; CR1 HSCT, Hematopoietic stem cell transplantation in first complete remission; HDAC, High-dose cytarabine; MDAC, Medium-dose cytarabine; MRD-R: Measurable residual disease recurrence.

Table S5. Clinical characteristics of patients from various interventions groups after MRD recurrence.

Characteristics	No intervention (N=29)	Drug intervention (N=131)	*P*	HSCT (N=7)	*P*
Sex, n (%)			0.047		1.000
Male	11 (37.93)	75 (57.25)		2 (28.57)	
Female	18 (62.07)	56 (42.75)		5 (71.43)	
Age at diagnosis, yr, n (%)					
Median (IQR)	34 (29, 45)	46 (34.5, 55)	0.002	43 (34, 50)	0.350
< 40	19 (65.52)	48 (36.64)		3 (42.86)	
40-60	10 (34.48)	70 (53.44)		4 (57.14)	
≥ 60	0 (0)	13 (9.92)		0 (0)	
WBC, ×109/L, median (IQR)	15 (7.42, 25.88)	13 (2.76, 40.09)	0.345	52 (16.63, 82.09)	0.221
2017 ELN risk classification, n (%)			0.920		0.006
Favorable	11 (37.93)	49 (37.40)		1 (14.29)	
Intermediate	14 (48.28)	60 (45.80)		1 (14.29)	
Adverse	4 (13.79)	22 (16.79)		5 (71.43)	
Drug intervention regimen, n (%)					-
Intensive		98 (74.81)			
Non-intensive		33 (25.19)			
Intervention Results, n (%)			<0.001		<0.001
MRD negative	4 (13.79)	42 (32.06)		7 (100)	
MRD persistent without MOR-R	7 (24.14)	43 (32.82)		0 (0)	
MOR-R	18 (62.07)	29 (22.14)		0 (0)	
Unevaluable	0(0)	17(12.98)		0(0)	

Excluding 4 patients with unclear interventions. Abbreviation: MRD, measurable residual disease; IQR, interquartile range; WBC, White blood cell; ELN, European LeukemiaNet; HSCT, hematopoietic stem cell transplantation; MOR-R, morphologic relapse.

Table S6. Survival rates and time intervals reported in the manuscript.

Survivals	3-year CIR	3-year RFS	3-year OS
The whole cohort	38.6%	56.7%	70.3%
MRD-R status			
MRD-R	63.6%	13.9%	39.0%
Non MRD-R	30.6%	67.2%	79.2%
P	<0.001	<0.001	<0.001
MRD-R level			
Minute	65.2%	28.9%	44.8%
Overt	68.2%	27.5%	38.9%
P	0.76	0.85	0.39
ELN classification			
Favorable			
MRD-R	50.2%	21.7%	53.6%
Non MRD-R	27.1%	72.9%	90.1%
P	<0.001	<0.001	<0.001
ELN classification			
Intermediate			
MRD-R	67.0%	14.0%	38.2%
Non MRD-R	32.0%	66.9%	74.6%
P	<0.001	<0.001	<0.001
ELN classification			
Adverse			
MRD-R	79.8%	4.0%	19.5%
Non MRD-R	37.1%	53.3%	62.5%
P	<0.001	<0.001	<0.001
Age <40 years			
MRD-R	66.4%	15.7%	40.4%
Non MRD-R	28.2%	68.8%	79.4%
P	<0.001	<0.001	<0.001
Age 40-60 years			
MRD-R	62.8%	12.0%	38.6%
Non MRD-R	29.9%	68.0%	79.9%
P	<0.001	<0.001	<0.001
Age >60 years			
MRD-R	49.2%	20.0%	30.0%
Non MRD-R	57.7%	45.2%	70.9%
P	0.76	0.011	0.036
Intervention results			
The whole cohort			
MRD-	40.2%	53.0%	58.9%
MRD+	76.5%	21.7%	43.5%
P	<0.001	0.005	0.134

Intervention results			
Drug intervention			
MRD-	43.0%	50.9%	56.4%
MRD+	79.6%	18.4%	38.9%
P	0.001	0.012	0.134
Time interval	MRD-R to MOR-R		MRD-negative to MRD-R
The whole MRD-R cohort	3.3 months, IQR [1.7, 7.2]		6 months, IQR [3.6, 11.4]
ELN classification			
Favorable-risk	2.6 months, IQR [1.6, 7.1]		7.5 months, IQR [3.7, 11.4]
Intermediate-risk	4.9 months, IQR [1.7, 7.2]		5.9 months, IQR [3.5, 11.4]
Adverse-risk	2.9 months, IQR [1.7, 7.2]		3.8 months, IQR [3.5, 11.4]
Intervention group		*P*	
Intervention	4.2 months, IQR [1.8, 7.5]	0.033	
No intervention	1.7 months, IQR [0.9, 2.9]		
Intervention result			
MRD-	7.3 months, IQR [4.9, 15.4]	0.184	
MRD+	5.3 months, IQR [4.2, 11.0]		

Abbreviation: CIR, cumulative incidence of relapse; RFS, relapse-free survival; OS, overall survival; MRD, measurable residual disease; MRD-R, MRD recurrence; ELN, European LeukemiaNet; IQR, interquartile range.