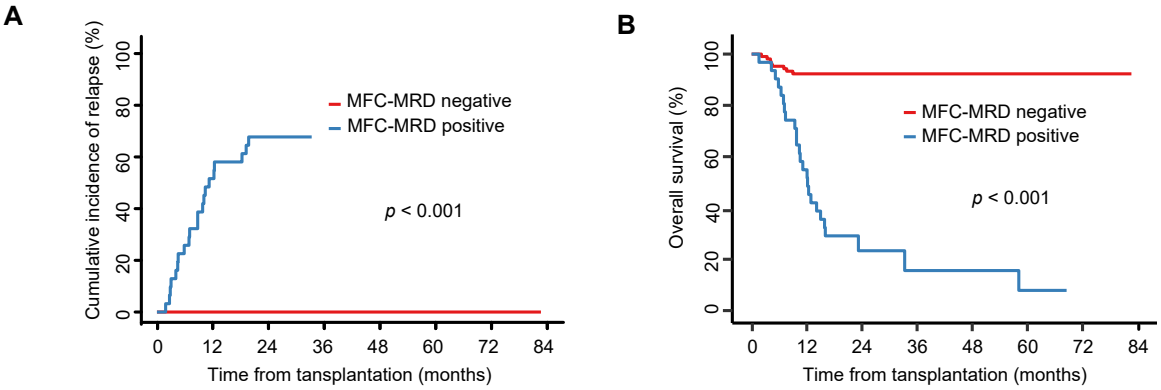
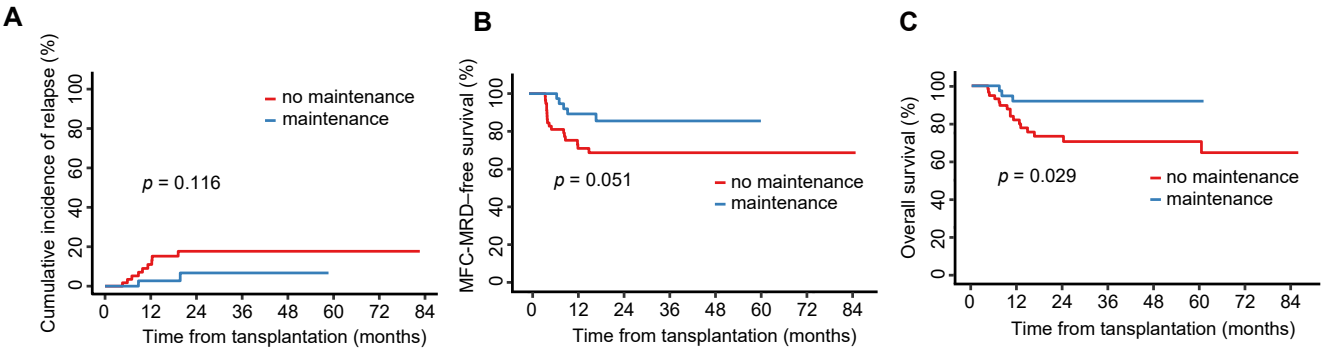
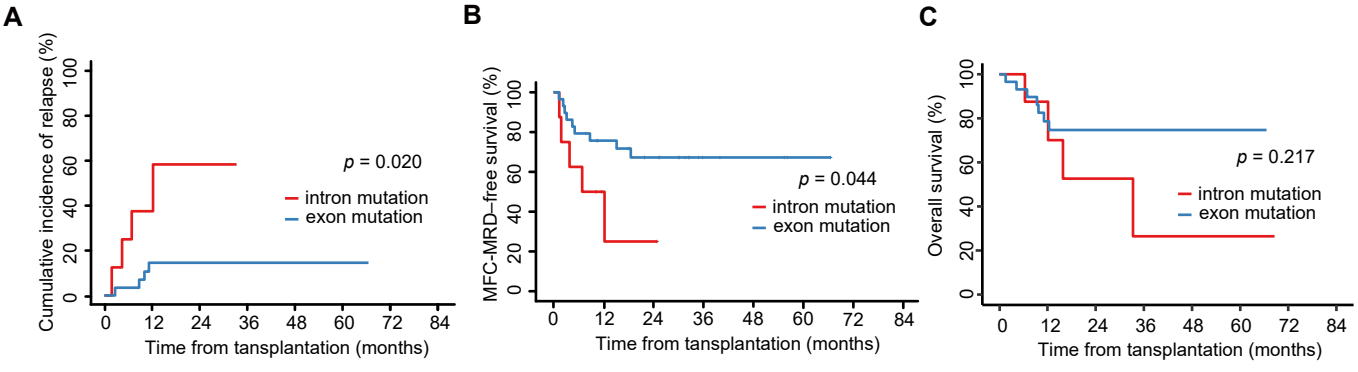
Supplemental Figure.1



Supplemental Figure.2



Supplemental Figure.3



Supplementary Tables

Supplemental Table 1A. Categorized reasons for unavailability of Day +30 BM samples for PCR-NGS analysis

Category	Description	No. of patients, n (%)
No available sample	No residual BM sample was available for DNA extraction after completion of routine post-transplant clinical testing and prior research-related analyses	21 (42.0)
Insufficient DNA quantity	Residual BM sample was available after routine clinical testing and prior research-related analyses, but the amount of extractable DNA did not meet the minimum input requirement for PCR-NGS library preparation	29 (58.0)
Hypocellular bone marrow (subset)	Patients with hypocellular BM identified among the excluded samples	4 (8.0)
BM dry tap (subset)	Failure to obtain BM aspirate	0 (0.0)

Abbreviations: BM, bone marrow; PCR, polymerase chain reaction; NGS, next-generation sequencing; DNA, deoxyribonucleic acid.

Supplemental Table 1B. Baseline characteristics of included patients and those without available samples

Characteristic	Included patients	Unavailable samples	P value
Number of patients, n (%)	136 (73.1)	50 (26.9)	
Age at HSCT, median (IQR)	39.0 (29.0–49.0)	37.5 (25.8–46.8)	0.109
< 50 years, n (%)	107 (78.7)	10 (20.0)	0.844
≥ 50 years, n (%)	29 (21.3)	40 (80.0)	
Gender			0.828
Male, n (%)	65 (47.8)	23 (46.0)	
Female, n (%)	71 (52.2)	27 (54.0)	
WBC at diagnosis, median (IQR)	32.0 (11.9–105.5)	50.7 (24.4–111.4)	0.069
			0.756
<100*10^9/L, n (%)	101 (74.3)	36 (72.0)	
≥100*10^9/L, n (%)	35 (25.7)	14 (28.0)	
2022 ELN risk group			0.367
Favorable, n (%)	14 (10.3)	3 (6.0)	
Intermediate, n (%)	93 (68.4)	32 (64.0)	
Adverse, n (%)	29 (21.3)	15 (30.0)	
***FLT3-ITD* allele ratio**			0.651
<0.5, n (%)	31 (22.8)	13 (26.0)	
≥0.5, n (%)	42 (30.9)	12 (24.0)	
Miss, n (%)	63 (46.3)	25 (50.0)	
Combined with *NPM1* mutation			0.414
Yes, n (%)	58 (42.6)	18 (36.0)	
No, n (%)	78 (57.4)	32 (64.0)	

R/R AML			0.826
Yes, n (%)	32 (23.5)	11 (22.0)	
No, n (%)	104 (76.5)	39 (88.0)	
***FLT3* inhibitors before HSCT**			0.847
Yes, n (%)	62 (45.6)	22 (44.0)	
No, n (%)	74 (54.4)	28 (56.0)	
Disease status at HSCT			0.227
CR1, n (%)	125 (91.9)	43 (86.0)	
≥CR2, n (%)	11 (8.1)	7 (14.0)	
Pre-HSCT MRD			0.284
Negative, n (%)	108 (79.4)	36 (72.0)	
Positive, n (%)	28 (20.6)	14 (28.0)	
Time from diagnosis to transplantation (month), median (IQR)	6.6 (5.7–8.1)	7.2 (5.7–9.4)	0.069
Donor type			0.478
MSD, n (%)	47 (34.6)	13 (26.0)	
HID, n (%)	82 (60.3)	35 (72.0)	
URD, n (%)	7 (5.1)	2 (4.0)	
Donor age, median (IQR)	33.0 (24.0–46.0)	30.5 (19.0–44.3)	0.789
ABO blood type			0.228
Matched, n (%)	85 (62.5)	27 (54.0)	
Major, n (%)	14 (10.3)	11(22.0)	
Minor, n (%)	27 (19.9)	9 (18.0)	
Bi-directional, n (%)	10 (7.4)	3 (6.0)	
Infused CD34+ ($\times10^6$/kg) cells, median (IQR)	3.6 (2.9–4.9)	3.4 (2.5–4.3)	0.408
Infused nucleated ($\times10^8$/kg) cells, median (IQR)	11.0 (10.0–12.7)	11.0 (9.1–13.8)	0.959

Abbreviations: HSCT, hematopoietic stem cell transplantation; IQR, interquartile range; WBC, white blood cell; ELN, European LeukemiaNet; *FLT3-ITD*, FMS-like tyrosine kinase 3 internal tandem duplication; R/R AML, relapsed/refractory acute myeloid leukemia; CR1, first complete remission; CR2, second complete remission; MRD, measurable residual disease; MSD, matched sibling donor; HID, haploidentical donor; URD, unrelated donor.

Supplemental Table 2. Primer sequences for *FLT3-ITD* detection

Primer name	Sequence (5′–3′)
PRIMER 1	GCAATTTAGGTATGAAAGCCAGCTAC

PRIMER 2	ATGGAAAAGAAATGCTGCAGAAAC
PRIMER 3	GCACGTACTCACCATTTGTCTTTG

Abbreviations: *FLT3-ITD*, FMS-like tyrosine kinase 3 internal tandem duplication.

Supplemental Table 3. Regression analyses of post-transplant outcomes in the *FLT3-ITD*–negative group

Outcomes	Variables in the model	Regression analyses		
		HR/sHR	95% CI	P value
OS	R/R AML (Yes vs. No)	3.90	1.66–9.13	0.002
	Pre-HSCT MRD positivity by MFC (pos vs. neg)	3.06	1.28–7.30	0.012
	Disease status at HSCT (≥ CR2 vs. CR1)	3.09	0.91–10.49	0.070
	2022 ELN risk group (adverse vs. other groups)	1.22	0.48–3.12	0.678
MFC-MRD–free survival	R/R AML (Yes vs. No)	3.92	1.76–8.77	0.001
	Pre-HSCT MRD positivity by MFC (pos vs. neg)	3.52	1.55–7.98	0.003
	Disease status at HSCT (≥ CR2 vs. CR1)	2.61	0.78–8.74	0.119
	2022 ELN risk group (adverse vs. other groups)	1.88	0.83–4.26	0.129
CIR	R/R AML (Yes vs. No)	4.41	1.53–12.70	0.006
	Pre-HSCT MRD positivity by MFC (pos vs. neg)	10.40	3.52–30.90	<0.001
	Disease status at HSCT (≥ CR2 vs. CR1)	1.38	0.16–11.90	0.771
	2022 ELN risk group (adverse vs. other groups)	4.01	1.40–11.50	0.010
Cumulative incidence of post-HSCT MFC-MRD relapse	R/R AML (Yes vs. No)	3.80	1.48–9.78	0.006
	Pre-HSCT MRD positivity by MFC (pos vs. neg)	5.70	2.24–14.50	<0.001
	Disease status at HSCT (≥ CR2 vs. CR1)	1.00	0.12–8.25	0.999
	2022 ELN risk group (adverse vs. other groups)	3.10	1.24–7.73	0.015

HRs for OS and MFC-MRD–free survival were estimated using Cox proportional hazards regression models; sHRs for CIR and cumulative incidence of post-HSCT MFC-MRD relapse were estimated using Fine–Gray models with death as a competing event. **Abbreviations**: HR, hazard ratios; sHR, subdistribution hazard ratio; CI, confidence interval; OS, overall survival; R/R AML, relapsed/refractory AML; MRD, measurable residual disease; MFC, multiparameter flow cytometry; HSCT, hematopoietic stem cell transplantation; CR1, first complete remission; CR2, second complete remission; ELN, European LeukemiaNet; CIR, cumulative incidence of relapse.

Supplemental Table 4. Molecular features of *FLT3-ITD* detected at day +30

Characteristic	*FLT3-ITD*–positive
Multiple *ITD* mutations, n (%)	9 (24.3)
Length median (bp, range)	33 (18–168)
VAF (%) (×10^{-4}, range)	19.5 (3.5–1029.0)
In-frame mutation (%)	48 (100.0)
insertion mutation (%)	23 (47.9)
Intron, n (%)	9 (18.7)
Exon, n (%)	39 (81.3)

Abbreviations: *FLT3-ITD*, FMS-like tyrosine kinase 3 internal tandem duplication; VAF, variant allele frequency.

Supplemental Table 5. Regression analysis: Impact of *FLT3* inhibitor maintenance therapy on transplantation outcomes

Outcomes	Variables in the model	Regression analyses		
		HR/sHR	95% CI	P value
OS	R/R AML (Yes vs. No)	1.87	0.82–4.23	0.134
	Pre-HSCT MRD positivity by MFC (pos vs. neg)	2.24	1.02–4.90	0.044
	FLT3 inhibitor maintenance (Yes vs. No)	0.21	0.08–0.56	0.002
	+30d *FLT3-ITD* (pos vs. neg)	0.89	0.39–2.03	0.784
MFC-MRD–free survival	R/R AML (Yes vs. No)	2.06	0.95–4.43	0.066
	Pre-HSCT MRD positivity by MFC (pos vs. neg)	2.36	1.11–5.01	0.026
	FLT3 inhibitor maintenance (Yes vs. No)	0.28	0.12–0.68	0.005
	+30d *FLT3-ITD* (pos vs. neg)	1.04	0.49–2.23	0.915
CIR	R/R AML (Yes vs. No)	1.75	0.63–4.83	0.280
	Pre-HSCT MRD positivity by MFC (pos vs. neg)	7.63	2.16–27.02	0.002
	FLT3 inhibitor maintenance (Yes vs. No)	0.11	0.03–0.38	<0.001
	+30d *FLT3-ITD* (pos vs. neg)	0.82	0.62–2.78	0.750
Cumulative incidence of post-HSCT MFC-MRD relapse	R/R AML (Yes vs. No)	1.50	0.61–3.68	0.380
	Pre-HSCT MRD positivity by MFC (pos vs. neg)	3.38	1.29–8.86	0.013
	FLT3 inhibitor maintenance (Yes vs. No)	0.21	0.08–0.57	0.002
	+30d *FLT3-ITD* (pos vs. neg)	1.55	0.61–3.93	0.350
	2022 ELN risk group (adverse vs. other groups)	1.20	0.50–2.89	0.680
	Combined with *NPM1* mutation (Yes vs. No)	0.58	0.20–1.64	0.300

Maintenance therapy was modeled as a time-dependent variable. HRs for OS and MFC-MRD–free survival were estimated using Cox proportional hazards regression models; sHRs for CIR and cumulative incidence of post-HSCT MFC-MRD relapse were estimated using Fine–Gray models with death as a competing event. **Abbreviations**: HR, hazard ratios; sHR, subdistribution

hazard ratio; CI, confidence interval; OS, overall survival; R/R AML, relapsed/refractory AML; HSCT, hematopoietic stem cell transplantation; MRD, measurable residual disease; MFC, multiparameter flow cytometry; *FLT3-ITD*, FMS-like tyrosine kinase 3 internal tandem duplication; CIR, cumulative incidence of relapse; ELN, European LeukemiaNet.

Supplementary Methods

The capillary electrophoresis (CE)-based fragment analysis method

Deoxyribonucleic acid amplification was performed using AML bone marrow (BM) or peripheral blood samples. FMS-like tyrosine kinase 3 internal tandem duplication *(FLT3-ITD)* mutations were analyzed through fragment analysis using polymerase chain reaction (PCR) amplification with fluorescent-labeled primers (primers targeting exons 14-15), followed by CE-based fragment analysis. The *FLT3-ITD* allelic ratio was calculated as the ratio of the peak area of the mutant product to the peak area of the wild-type product. The detection limit of PCR-based capillary electrophoresis fragment analysis used in this study was an allele ratio of 0.02.

Minimal residual disease (MRD) detection by multiparameter

flow cytometry (MFC)

MFC-MRD was assessed in fresh BM samples using 8-color MFC (BD FACSCanto) as previously described [17]. Briefly, a minimum of 5×10^5 events were acquired, and MFC-MRD was considered quantifiable when ⩾50 aberrant events consistent with leukemia-associated immunophenotypes and/or different-from-normal patterns were identified. The lower limit of quantification and detection were 0.01% and 0.004%, respectively.

Assessment of Donor Chimerism

Donor chimerism was assessed by PCR analysis of short tandem repeats (STR) using total BM cells, with an analytical sensitivity of 1%–5%. The proportion of donor–recipient chimerism was calculated based on 24 STR loci. Donor chimerism ≥ 95% was considered as complete donor chimerism. Chimerism assessments were performed at 1, 2, 3, 6, 9, 12, 18, and 24 months post-transplant. After 2 years, the frequency of monitoring was determined based on the patient's specific condition.