

Monitoring relapse in post-transplant acute myeloid leukemia requires integrated assessment, with flow cytometry showing the highest efficacy

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<https://doi.org/10.3324/haematol.2025.289192>

Received: November 20, 2025.

Accepted: March 17, 2026.

Early view: March 26, 2026.

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Supplementary Table 1. The summary of diagnostic assays used in this study

Assays		Criteria for relapse	Sensitivity
MFC		1) Detection of aberrant myeloblasts with an immunophenotype similar to that of the original blasts. 2) Detection of aberrant myeloblasts with an immunophenotype different from original blasts, with underlying clonal hematopoiesis, preleukemic cells, or dysplastic clones excluded.	0.1-0.01%
NGS		The detection of the same mutations in the pre-SCT myeloid neoplasms, or the emergence of any new mutations, excluding those typical of clonal hematopoiesis of indeterminate potential (CHIP).	2-5%
PCR	Semi-PCR	Detection of <i>FLT3</i> internal tandem duplication (ITD) mutation and mutations involving codons 835 and 836 in the tyrosine kinase domain (TKD). Positive results indicate relapse.	1%
	qRT-PCR	Detection of <i>RUNX1::RUNX1T1</i> and <i>CBFB::MYH11</i> fusion transcripts. Positive results indicate relapse.	0.01-0.001%
	<i>NPM1</i> PCR-NGS	Detection of <i>NPM1</i> exon 12 hotspot insertion mutations. Positive results indicate relapse.	0.005%
Cytogenetics		1) Detection of cytogenetic abnormalities that were present at initial diagnosis. 2) Emergence of the recipient's sex chromosome in sex-mismatched SCT. 3) Emergence of new clonal karyotypic abnormalities after excluding donor-derived clonal cytogenetic abnormalities of unknown clinical significance.	5% (Karyotyping) 0.5% (FISH)
Chimerism		Detection of recipient DNA($\geq 1\%$) in addition to donor-derived DNA (mixed chimerism) using sorted T lymphocytes and myeloid cells from BM or PB aspirate samples.	5%

Note: MFC: multiparameter flow cytometry (MFC); NGS: next-generation sequencing; PCR: polymerase chain reaction; MRD: minimal residual disease; SCT: Stem cell transplant; FISH: fluorescence in situ hybridization; BM: bone marrow; PB: peripheral blood.

The approximate turnaround time for the assays used in this study were as follows: MFC, ~12-24 hours; PCR-based assays, ~48- 72 hours; NGS, ~72 hours; conventional karyotyping, ~5 days; FISH, ~24-72 hours; chimerism analysis, ~48-72 hours; and morphologic evaluation, ~24-72 hours.

Supplementary Table 2. Treatment after post-transplant disease relapse

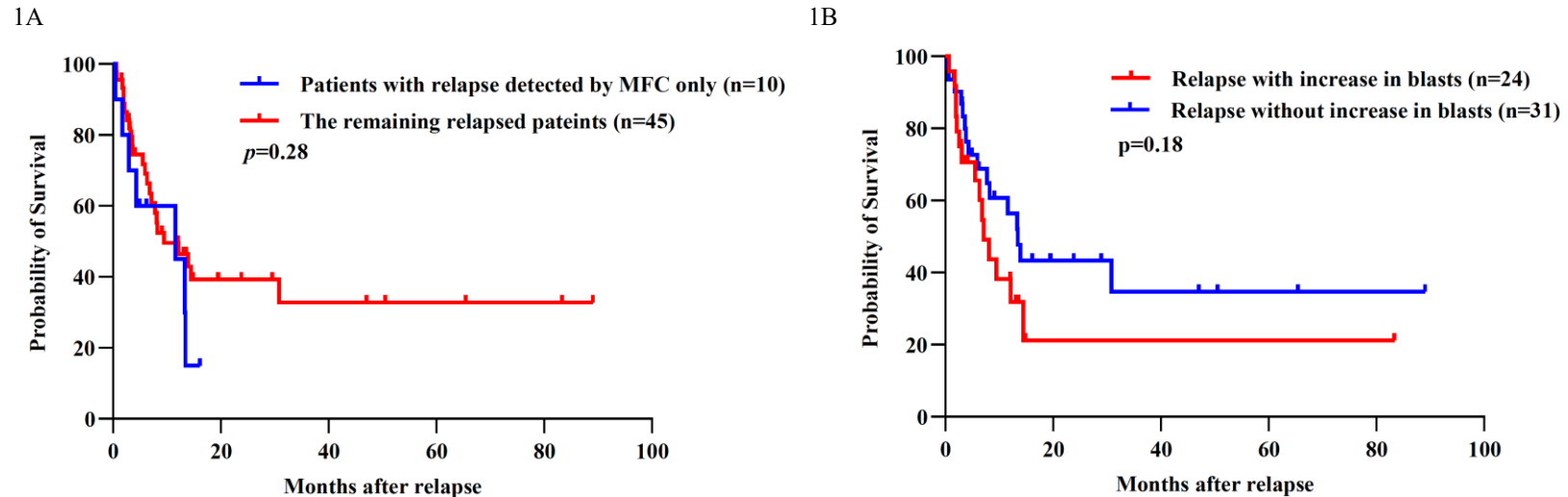
No.	HMA/VEN-based	Intensive chemotherapy	Targeted therapy	Immunotherapy	DLI	IST	Second allo-HSCT	No treatment	Therapy in detail
1	✓								1 cycle of Venetoclax + Azacitidine
2		✓	✓						Cytarabine; Revumenib; 1 cycle of Fludarabine + Cytarabine; Ziftomenib; Venetoclax; Camibirstat
3	✓		✓						1 cycle of Decitabine + Venetoclax + Quizartinib
4				✓					1 cycle of Nivolumab + Ipilimumab
5	✓								1 cycle of Azacitidine + Venetoclax
6				✓			✓		4 cycles of Vibecotamab; allo-HSCT
7	✓								1 cycle of Cladribine + low-dose cytarabine + Venetoclax
8								✓	No treatment
9	✓						✓		Decitabine; allo-HSCT
10	✓			✓	✓				7 cycles of low-dose Azacitidine + Venetoclax; DLI × 2; 1 cycle of Hu8F4; Azacitidine
11			✓						74 cycles of Venetoclax + Ivosidenib
12	✓								7 cycles of Azacitidine + Venetoclax + Ruxolitinib; 1 cycle of Cladribine + low-dose Cytarabine + Venetoclax
13		✓	✓						1 cycle of Venetoclax + Cladribine + Cytarabine + Idarubicin + Gemtuzumab ozogamicin
14				✓					2 cycles of Ipilimumab + Nivolumab
15			✓						2 cycles of decitabine + Ivosidenib
16	✓								1 cycle of Cladribine + low-dose Cytarabine alternating with Decitabine + Venetoclax
17							✓		Allo-HSCT
18	✓	✓	✓						1 cycle of Revumenib + Cobicistat; 1 cycle of Venetoclax + Azacitidine; 1 cycle of Vyxeos
19								✓	No treatment
20	✓	✓	✓		✓				5 cycles of Azacitidine; DLI × 2; 1 cycle of Emavusertib monotherapy; 1 cycle of Cladribine + Idarubicin + Cytarabine + Venetoclax; 1 cycle of IRAK4 inhibitor
21		✓	✓	✓					1 cycle of Fludarabine + Idarubicin + Gilteritinib + Blinatumomab

22	✓		✓		1cycle of Cytarabine + Decitabine + Venetoclax + Revumenib
23				✓	Allo-HSCT
24	✓			✓	1 cycle of Cladribine + Cytarabine + Venetoclax; allo-HSCT
25	✓		✓		1 cycle of Cladribine + low-dose Cytarabine + Venetoclax + Enasidenib
26			✓		VIP943 (targeting CD123)
27		✓			1 cycle of Cladribine+ low-dose cytarabine
28	✓				1 cycle of Azacitidine + Venetoclax + Magrolimab; 1 cycle of Cladribine + low-dose Cytarabine alternating with Decitabine
29	✓		✓	✓	5 cycle of Decitabine + Venetoclax + Revumenib; allo-HSCT
30	✓				1 cycle of Azacitidine + Magrolimab + Venetoclax
31	✓	✓			1 cycle of Azacitidine + Venetoclax; 1 cycle of Cladribine + Idarubicin + Cytarabine + Venetoclax
32		✓	✓	✓	1 cycle of Cladribine + Idarubicin + Cytarabine +Dexrazoxane + Venetoclax; 2 cycles of Revumenib + Venetoclax; allo-HSCT
33	✓		✓	✓	1 cycle of Azacitidine + Venetoclax + Gilteritinib; DLI × 2; 1 cycle of Mometinib + Gilteritinib
34		✓		✓	1 cycle of Cladribine + Idarubicin + Cytarabine + Venetoclax; allo-HSCT
35	✓				1 cycle of Cladribine + low-dose Cytarabine + Venetoclax
36	✓		✓		4 cycles of Decitabine; 2 cycles of Azacitidine + Venetoclax+HC-7366 (CDK9 inhibitor)
37				✓	Allo-HSCT
38	✓				4 cycle of Venetoclax + Decitabine; 1 cycle of Gemtuzumab ozogamicin
39			✓		Sorafenib
40	✓		✓	✓	1 cycle of Venetoclax+ Decitabin-cedazuridine+ Enasidenib; allo-HSCT
41			✓	✓	1 cycle of IMGN632; allo-HSCT
42				✓	Allo-HSCT
43	✓		✓		1 cycle of Azacitidine + Venetoclax + Enasidenib
44	✓		✓	✓	1 cycle of Decitabine + Venetoclax + Enasidenib; allo-HSCT

45	✓	✓	✓	1 cycle of Omacetaxine + venetoclax; 1 cycle of Azacitidine + Venetoclax + ADI-PEG; 1 cycle of Fludarabine + high-dose Cytarabine + Filgrastim + Venetoclax; 1 cycle of Revumenib
46		✓		1 cycle of Cyclophosphamide + Cytarabine + Topotecan + Go
47		✓	✓	2 cycles of Cladribine + Idarubicin + Cytarabine + Quizartinib
48	✓			1 cycle of Decitabine-cedazuridine; 1 cycle of Venetoclax + Azacitidine
49	✓		✓	1 cycle of Decitabine + Venetoclax + Gilteritinib; allo-HSCT
50			✓	Decitabine; Bortezomib
51	✓			1 cycle of Venetoclax + Azacitidine,
52			✓	Immunosuppressor tapering
53	✓		✓	1 cycle of Revumenib; 1 cycle of Clad + low-dose Cytarabine + Venetoclax
54	✓		✓	1 cycle of Cladribine + low-dose Cytarabine + Venetoclax + Revumenib
55	✓			1 cycle of Venetoclax + Azacitidine

HMA/VEN: hypomethylating agent/Venetoclax; DLI: donor lymphocyte infusion; IST: immunosuppressor taper; allo-HSCT: allogeneic-hematopoietic stem cell transplantation.

Supplementary Figure 1: Overall survival following disease relapse after hematopoietic stem cell transplantation (HSCT).



Supplementary Figure 1 legend:

A. Comparison of overall survival (OS) between patients with relapse detected exclusively by MFC (n=10) and those with relapse identified by others, including MFC (n=45).

B. Comparison of OS between cases with morphologic relapse (blasts $\geq 5\%$, n=24) and patients with MRD relapse (blasts $< 5\%$, n=31).

For each patient, the date of disease relapse was designated as day 1, with all subsequent time points calculated from this reference date. OS was defined as the time from disease relapse to death or the last follow-up. For the seven patients who relapsed after both their first and second allo-HSCT, survival following the first transplant was censored at the time of the second transplant, and OS following the second transplant was calculated from the date of relapse after the second transplant to the time of death or last follow-up.