Multicenter prospective phase II study of decitabine priming with low-dose idarubicin, cytarabine, and G-CSF in children with refractory or relapsed acute myeloid leukemia

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Received: September 10, 2025.
Accepted: January 26, 2026.
Early view: February 5, 2026

https://doi.org/10.3324/haematol.2025.289170

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Supplementary Appendix

1. Methods

    a. Measurement of residual disease was performed with the use of multiparameter flow cytometry(MP-FCM)

    b. Bulk RNA sequencing was extracted from fresh peripheral blood or bone marrow cells of AML samples. For RNA sequencing library preparation, 1 μg of total RNA per sample was processed with the KAPA RNA HyperPrep Kit (Kapa Biosystems, cat. KK8540) following rRNA depletion with the KAPA RiboErase (HMR) Kit (Kapa Biosystems, cat. KK8482). The process included mRNA enrichment, fragmentation, cDNA synthesis, and purification. The library preparation involved end repair, A-tailing, adapter ligation, size selection, and PCR amplification. Library quality was assessed by measuring RNA concentration using a Qubit® RNA Assay Kit (Thermofisher), assessing insert size with the Qseq400 system (BIOPTIC) with an R1 cartridge, and quantifying the effective library concentration using the KAPA Library Quantification Kit (KAPA Biosystems). Qualified libraries were pooled and sequenced on the Illumina NovaSeq 6000 platform using a PE150 strategy to generate 150-bp paired-end reads, ensuring comprehensive transcriptome analysis.

2. Statistical Analysis

    a. The primary objective was to estimate the CR/CRi rate (probability) after the DP-IAG reinduction. Let π represent the probability of achieving CR/CRi. The sample size was determined by benchmarking on testing the null hypothesis H_0: $\pi \leqslant 0.50$ (Based on the results of the currently published clinical trials, the remission rate of salvage chemotherapy

regimens for refractory and relapsed acute myeloid leukemia ranges from 18% to 80%, with the majority having a remission rate of around 50% [1, 2]) against the alternative hypothesis H_1: $\pi > 0.50$. Assuming a target response rate of $\pi = 0.85$, a sample size of n = 100 patients provides >99% power to reject the null hypothesis at a two-sided significance level of 0.05. In terms of a confidence interval (CI), the sample size of 100 ensures that the half-length of the 95% confidence interval is not larger than 0.098, providing reasonable accuracy for estimation.

b. Descriptive statistics were summarized using tables and figures. Patient characteristics and treatment responses were reported as frequencies and percentages for categorical variables. Differences in continuous variables between two groups were assessed using the Student's t-test or the Wilcoxon rank-sum test, depending on distributional assumptions. Categorical variables were compared using the chi-square test or Fisher's exact test, as appropriate.

c. Overall survival (OS) was defined as the time from study enrollment to death from any cause. Event-free survival (EFS) was defined as the time from enrollment to the first occurrence of relapse, secondary malignancy, or death. Patients with no response (NR) to DP+IAG were considered treatment failures, with the event time set to 0 for EFS analysis. OS and EFS were estimated using the Kaplan–Meier method, and three-year estimates were reported along with log–log 95% confidence intervals (CIs).

d. All statistical analyses were conducted using SPSS version 27.0 and R version 4.4.1 (R Foundation for Statistical Computing, Vienna, Austria). The *survival* (v3.8-3) and *survminer* (v0.5.0) R packages were used to generate Kaplan–Meier estimates and survival curves. The genomic landscape was visualized using the *ComplexHeatmap* package

(v2.20.0). All statistical tests were two-sided, and a p-value < 0.05 was considered statistically significant. Data cutoff for analysis was December 31, 2024.

3. Supplementary Tables

Supplementary Table 1. Experimental Regimen (Reinduction I)

Regimen	Drug	Dose (>10kg)	Dose (≤10kg)	Route	Days
DP-IAG	Decitabine	20 mg/m^2	0.67 mg/kg	IV, daily, 3h infusion	1-5*
	Idarubicin	5 mg/m^2	0.17 mg/kg	IV, daily, 1h infusion, at 10 AM	6-8
	Cytarabine	10 mg/m^2	0.33 mg/kg	Subcutaneous, daily, q12h at 8 AM	6-15
	G-CSF	5 μg/m^2	5 μg/kg	Subcutaneous, daily at 1PM	6-15
	#Sorafenib	200 mg/m^2	6.66 mg/kg	Oral, daily	16-variable#
	&Dasatinib	80 mg/m^2	2.66 mg/kg	Oral, daily	16-variable&

Abbreviations: IV, intravenous; G-CSF, granulocyte colony-stimulating factor.
*For patients with relapsed AML presenting with a white blood cell (WBC) count >50 × 10⁹/L at admission, cytoreductive therapy was initiated using decitabine (20 mg/m²/day, maximum dose 20 mg/day) in combination with cytarabine (100 mg/m² every 12 hours) for up to 5 days. Once the WBC count decreased to <50 × 10⁹/L, IDAG (idarubicin, cytarabine, and G-CSF) induction therapy was initiated. Cytarabine administered solely for cytoreduction was not considered part of the induction regimen.
#Sorafenib or &Dasatinib was started on the first day following completion of chemotherapy and was held before the next course or during fever episodes. Their use was not interrupted for neutropenia in the absence of fever.

Supplementary Table 2. Post-Reinduction I standard-dose chemotherapy regimens (Investigator-selected)

Drug combinations	Dose (>10kg)	Dose (≤10kg)	Route	Days
Regimen A				
Cytarabine	3 g/m^2	100 mg/kg	IV, q12h, 3h infusion	1-3
Homoharringtonine	3 mg/m^2	0.1 mg/kg	IV, daily, 3h infusion	1-5
Regimen B				
Cytarabine	3 g/m^2	100 mg/kg	IV, q12h, 3h infusion	1-3
Etoposide	150 mg/m^2	5mg/kg	IV, daily, 4h infusion	1-3
Regimen C				
Cytarabine	3 g/m^2	100 mg/kg	IV, q12h, 3h infusion	1-3
Mitoxantrone	5mg/m^2,	0.17mg/kg	IV, daily, 2h infusion	1,3,5

Supplementary Table 3. Selected clinical data and treatment outcomes of patients with *ETV6* rearranged acute myeloid leukemia

Sex	Age	FAB	Fusion gene	Genes	Response	MRD	Final treatment	EFS (months)
Female	1y2m	M5	*MNX1*::*ETV6*, *ETV6*::*CDK17*, *PSPC1*::*ZMYM2*	*PTPN11*/*IKZF1*	CRi	< 0.1%	HSCT	59
Female	1y3m	M5	*MNX1*::*ETV6*	*ETV6*	CRi	< 0.1%	HSCT	45
Female	2y3m	M5	*ETV6*::*MAGI2*	none	CR	< 0.1%	HSCT	28
Female	4m	M7	*ETV6*::*FRMPD1*、*LOH12CR1*::*DUSP16*	*PTPN11*	CRi	< 0.1%	HSCT	33

Supplementary Table 4. Selected clinical data and treatment outcomes of patients with *KMT2A* rearrangements

Sex	Age	FAB	Fusion gene	Genes	Response	MRD	Management	EFS (months)
Male	1y6m	M5	*KMT2A::MLLT3*	*PTPN11/SRCAP*	CR	<0.1%	HSCT	78
Male	8y10m	M5	*KMT2A::MLLT3*	none	CRi	<0.1%	HSCT	63
Female	7y4m	M5	*KMT2A::MLLT3*	*FLT3-TKD*	NR	68.14%	Palliative treatment	0
Male	7y7m	unclassified	*KMT2A::MLLT3*	*KRAS/EP300*	NR	57.67%	Palliative treatment	0

Male	8y10m	unclassified	*KMT2A::MLLT3*	*MECOM*	CRi	＜0.1%	HSCT	40
Female	4y9m	M2	*KMT2A::MLLT3*	*KRAS*	CRi	＜0.1%	chemotherapy	9
Female	9y	M5	*KMT2A::MLLT3*	*KRAS/FLI1/DNAH2/MEGF8*	abandonment	abandonment	abandonment	2
Male	6y	M4	*KMT2A::MLLT3*	*KIT/NRAS/NF1*	NR	51.09%	HSCT	0
Male	14y7m	M5	*KMT2A::ELL*	*EVI1*	CRi	3.78%	HSCT	7
Male	5y4m	M5	*KMT2A::MLLT10*	*EVI1/NRAS/PTPN11/SETD2*	CRi	0.43%	HSCT	17
Male	5y4m	unclassified	*KMT2A::MLLT10*	*KRAS/ASXL1/EVI1*	CRi	2%	chemotherapy	3
Male	2y4m	M4	*KMT2A::MYO1F*	*FLT3-TKD/KAT6A*	CRi	<0.1%	HSCT	36
Male	6y7m	M5	*KMT2A::LGALS1*	*RUNX1/DHX15/SLC6A2/TEK*	CRi	0.16%	HSCT	3
Male	14y10m	M5	*KMT2A::MLLT4*	*NRAS/TET1*	CRi	3.21%	HSCT	19
Male	8y	M2	*KMT2A-PTD*	none	CR	1.83%	HSCT	51
Female	10y10m	M2	*KMT2A-PTD*	*KRAS/CEBPA/WT1/RIT1/ASXL1*	NR	29.4%	HSCT	0
Female	9y10m	M5	*KMT2A::MLLT4*	none	CRi	1.79%	HSCT	4
Male	9y3m	M4	*KMT2A-PTD*	*KMT2A/FLT3-ITD*	CRi	3.94%	HSCT	22
Male	5y4m	M2	*KMT2A::ELL*	*PHF6/CCND3/STAG2*	CR	0.85%	chemotherapy	8

Supplementary Table 5. Completed Phase I/II/III Clinical Trials for Pediatric Refractory or Relapsed AML

Trials	Regimen	Study Design	Period	Number	CR rates	TRM	OS	Reference
CCG 2951	mitoxantrone/cytarabine	Phase II	1997-2000	101	73.5%	3%	2-year 24%	Wells RJ, *et al*.2003 [3]
BMF	FLAG/DNX vs FLAG	Phase III	2001-2009	394	69% vs 59 %	4.1% vs 4.6%	4-year 40% vs 36 %	Kaspers GJ , *et al*.2013 [4]
COG AAML 07P1	bortezomib/cytarabine/ idarubicin vs. bortezomi b/ cytarabine/etoposide	Phase II	2008-2011	37	57.1% vs 47.9%	14.3% v s 8.7%	2-year 39%	Horton TM , *et al*.2014 [5]
COG AAML 0523	clofarabine/cytarabine	Phase I/ II	2007-2012	49	48%	0	3-year 46%	Cooper TM , *et al*.2014 [6]
AML BFM-SG compassio nate use series	GO + cytarabine	Phase III	1995–2014	76	51%	3.9%	4-year 27%	Niktoreh N, *et al*.2019 [7]
COG AAML 1421	CPX-351followed by FLAG	Phase I/II	2016-2018	37	81.1%	0	2-year 52.7%	Cooper TM , *et al*.2020 [8]
ChiCTR18 00015872	DP + IAG	Phase Ⅱ	2018-2022	101	73.3%	3.0%	3-year 60.8%	

Abbreviations: BFM (Berlin, Frankfurt, Münster),COG (Childhood Oncology Group), CCG(Children's Cancer Group Study), SJCRH (St. Jude Children's Research Hospital). GO gemtuzumab ozogamicin; FLAG Fludarabine, Cytarabine, Granulocyte colony-stimulating factor; DNX Liposomal daunorubicin; CPX-351 Daunorubicin/Cytarabine Liposomal; DP + IAG decitabine priming +low-dose of idarubicin, cytarabine, and G-CSF chemotherapy.
NA, not available.

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