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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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Running heads：A phase II study of DP-IAG in pediatric R/R AML

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Data Availability Statement: The deidentified datasets used and/or analyzed during the current study will be available from the corresponding author upon reasonable request. Access will be provided for two years after the publication date. Requests for data should be sent to hushaoyan@suda.edu.cn.

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Abstract

No standard salvage regimen exists for relapsed/refractory (R/R) pediatric AML. In this prospective, multicenter Phase II trial, 101 evaluable patients (<18 years) received one course of decitabine priming followed by low-dose idarubicin, cytarabine, and G-CSF (DP-IAG) as remission reinduction therapy. The primary objective was the rate of complete remission, including incomplete hematologic recovery (CR/CRi). Seventy-four patients (73.3%; 95% CI: 64.6–81.9%) achieved CR/CRi.Ultimately, 70 patients(69.3%) underwent allogeneic hematopoietic stem cell transplantation(allo-HSCT).At a median follow-up of 36.4 months (IQR: 10.3–51.3), the estimated 3-year overall survival for the entire cohort was 60.8% (95% CI: 55.9–65.7%). Infections were the most frequent non-hematologic adverse events; three patients died from toxicity after the first reinduction. DP-IAG demonstrated high remission rates and an acceptable safety profile, supporting its use as an effective salvage option and feasible bridge to HSCT. These findings provide a benchmark for future trials in pediatric R/R AML.

INTRODUCTION

Relapsed or refractory (R/R) acute myeloid leukemia (AML) occurs in approximately 30–40% of children treated for de novo AML [1]. Despite advances in risk stratification and supportive care, outcomes for children with relapsed or refractory AML (R/R AML) remain poor, and no standardized salvage regimen has been generally adopted. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is widely regarded as the only potentially curative approach for most patients in this setting [2, 3]. However, several factors, including a short duration of the first remission (CR1), high-risk molecular features, inadequate response to initial therapy, and prior treatment with HSCT, are associated with dismal outcomes following salvage treatment [3, 4].

Survival outcomes for children with relapsed AML have shown minimal improvement over the past two decades [5]. Most prospective trials in this setting have been Phase I/II studies with limited sample sizes and few reports of long-term survival. A notable exception is the randomized BFM study, which compared FLAG plus liposomal daunorubicin (DNX) versus FLAG alone in 394 children with first relapse or refractory AML. The overall survival did not differ significantly between the arms: 40% with FLAG-DNX and 36% with FLAG alone [6]. Since that time, the FLAG-based backbone has become the predominant approach to salvage therapy [6, 7].

In 2005, a collaboration initiative between institutions in China and St. Jude Children's Research Hospital was launched to reduce early mortality and treatment abandonment among children with leukemia. For pediatric patients with acute myeloid leukemia (AML) who were unable to tolerate standard intensive chemotherapy, due to medical frailty or a high risk of treatment abandonment, a low-dose chemotherapy (LDC) regimen was implemented. This regimen, consisting of low-dose cytarabine, low-dose mitoxantrone, and G-CSF, was adapted from protocols developed by Japanese investigators [8]. The unexpectedly favorable response rates observed in this population led to the broader adoption of the LDC regimen for newly diagnosed AML.

Recognizing that long-term survival of R/R AML typically requires allo-HSCT and that LDC regimens demonstrated efficacy in relapsed AML [9, 10], we hypothesized that a modest intensification of the LDC regimen with drugs that have alternative mechanisms, such as venetoclax and hypomethylating agents, could enhance salvage therapy without compromising tolerability. This modified LDC regimen replaced aclarubicin with idarubicin and incorporated decitabine priming based on a strong biological rationale. Preclinical data from our group and others have shown that chemotherapy-resistant pediatric AML is enriched for mutations in epigenetic regulators [11-13]. In a multicenter, randomized feasibility study, children receiving five days of decitabine before induction chemotherapy exhibited significantly broader DNA methylation changes than those receiving chemotherapy alone (2.518 vs. 539 genes affected), highlighting decitabine's robust epigenetic activity [14]. All 11

children who received decitabine achieved remission, and toxicity was limited primarily to expected hematologic adverse events. The combination of decitabine priming with standard chemotherapy has since demonstrated an acceptable safety profile in pediatric populations [15-17].

Here, we report the results of a prospective, multicenter, single-arm phase II trial evaluating the efficacy and safety of decitabine priming combined with low-dose chemotherapy in children with relapsed or refractory AML.

METHODS

Patients

This prospective, Phase II, multicenter clinical trial evaluated the combination of decitabine priming and low-dose chemotherapy (idarubicin, cytarabine, and G-CSF; DP-IAG) in pediatric patients with R/R AML. The study was conducted across 10 major medical centers in China and included patients aged <18 years with R/R AML (Figure 1). Diagnostic Criteria for Relapsed AML: After achieving complete remission (CR), the reappearance of leukemic cells in the peripheral blood, or a blast percentage ≥5% in the bone marrow (excluding other causes such as bone marrow regeneration following consolidation chemotherapy), or the presence of extramedullary leukemic cell infiltration. Diagnostic Criteria for Refractory AML:Newly diagnosed cases that do not respond to two courses of standard induction therapy.Cases that relapse within 12 months after consolidation and intensive

treatment following CR.Cases that relapse after 12 months and have no respond to conventional chemotherapy.Cases with two or more relapses.Cases with persistent extramedullary leukemia.Early relapse patients:Those who relapsed within 12 months after remission.Late relapse patients:Those who relapsed more than 12 months after remission.Immediate transplant:Children who directly proceed to the allogeneic hematopoietic stem cell transplantation process after the first DP-IAG treatment course.Delayed transplant:Children who continued to receive one or more courses of chemotherapy after the first DP-IAG treatment and then underwent allogeneic hematopoietic stem cell transplantation.

The study protocol was approved by the Medical Ethics Committee of the Children's Hospital of Soochow University (Approval No. 2017047-3) and registered with the Chinese Clinical Trial Registry (ChiCTR1800015872; www.chictr.org.cn). Informed consent was obtained from all patients or their legal guardians, in accordance with the Declaration of Helsinki.

Response Criteria

Response definitions were based on the 2022 European LeukemiaNet (ELN) recommendations [18]. Complete remission (CR): <5% bone marrow blasts, no Auer rods, no extramedullary leukemia, and hematologic recovery defined as transfusion independence, an absolute neutrophil count (ANC) >1.0 × 10□/L, and platelet count >80 × 10□/L. Complete remission with incomplete hematologic recovery (CRi): same as CR, but without full recovery of ANC and/or platelets. Partial response (PR):

≥50% reduction in bone marrow blasts, with residual blasts between 5% and 19%. Non-response (NR): ≥20% bone marrow blasts. Bone marrow assessments were performed on day 26 ± 2 of reinduction I. Measurable residual disease (MRD) testing was also performed at this time point, but the results were not included in the response criteria.

Treatment

The DP-IAG regimen consisted of decitabine 20 mg/m²/day (intravenous, days -4 to 0; maximum dose 20 mg/day), followed by cytarabine 10 mg/m²/dose (subcutaneous, every 12 hours, days 1 to 10; total 20 doses), idarubicin 5 mg/m² (intravenous, days 1, 3, and 5), and G-CSF 5 μg/kg/day (subcutaneous, days 1 to 10). Targeted agents were incorporated based on molecular findings: sorafenib (200 mg/m²/day) was added for patients with *FLT3*-ITD–positive AML, and dasatinib (80 mg/m²/day) for those with *KIT* mutations or *BCR*::*ABL1* fusion (Supplementary Table 1).

All patients with R/R AML were advised to proceed to allogeneic hematopoietic stem cell transplantation (allo-HSCT). HSCT was recommended after completion of the first DP-IAG course, with a second DP-IAG course suggested, when necessary, as a bridge to transplantation. However, logistical considerations, including donor identification and selection, parental decision-making, and the degree of residual disease after the initial DP-IAG treatment, led to variability in subsequent therapies.Instead of receiving a second course of DP-IAG, 22 children were treated with alternative regimens, including: cytarabine 3 g/m² every 12 hours for 3 days

combined with etoposide 150 mg/m² once daily for 3 days (13 patients); homoharringtonine 3 mg/m² once daily for 3 days (7 patients); or mitoxantrone 5 mg/m² once daily for 3 days (2 patients) (Supplementary Table 2).

RESULTS

Patients

Between June 2018 and December 2022, 111 children with AML from ten medical centers in China were screened for eligibility. Ten patients were excluded: six did not meet the diagnostic criteria for R/R AML, three were transferred to other institutions during treatment, and one had a white blood cell count greater than 50 × 10□/L after five days of decitabine, rendering them ineligible for a G-CSF–containing regimen (Figure 1).

Clinical and Biological Characteristics

A total of 101 patients (60 males and 41 females), with a median age of 7.6 years (range, 4 months to 17.6 years), were treated with DP-IAG. Sixty-six patients (65.3%) were classified as having refractory AML, including 37 previously treated with the standard-dose arm and 29 with the low-dose arm of the CALSIII-AML18 protocol[19]. Thirty-five patients (34.7%) had relapsed AML, including 29 with early relapses and 6 with late relapses. The median white blood cell (WBC) count at enrollment was 9.5 × 10□/L (IQR, 4.1–35.2). The most frequent cytogenetic and molecular abnormalities were: *RUNX1::RUNX1T1* (20.8%), *KMT2A* rearrangements

(18.8%), *NUP98* rearrangements (7.9%), and *CBFβ::MYH11* (5.0%). Among patients with *KMT2A* rearrangements, the most common fusion was *KMT2A::MLLT3* (42.1%, 8/19). Mutation analysis showed *CEBPA* dmbZIP in 7.9% of cases, *KIT* mutations in 18.8%, and *FLT3-ITD* in 16.8%. Among patients with core-binding factor AML, *KIT* mutations co-occurred in 66.7% (14/21) of those with *RUNX1::RUNX1T1* and 40.0% (2/5) with *CBFβ::MYH11*. Eleven of them had a common mutation in exon 17 of the KIT gene and were treated with dasatinib. Dasatinib was initiated at the time the mutation was identified and continued until transplantation. 9 children got CR/CRi.The median duration of dasatinib exposure was 17 days (range, 12–32 days).Ten patients with *FLT3-ITD* received sorafenib, and one patient with *BCR::ABL1* fusion gene received dasatinib during the first course of reinduction chemotherapy. The distribution of genomic alterations is summarized by genetic pathway in Figure 2. Compared to patients with refractory AML, those in the relapsed group had significantly lower WBC counts at enrollment and higher frequencies of *RUNX1::RUNX1T1* fusions and *KIT* mutations ($P = 0.012$, $P = 0.003$, and $P < 0.001$, respectively; Table 1). No significant differences were observed between the two groups in terms of age, sex, French-American-British (FAB) subtype, or complex karyotype.

Treatment Responses

Following one course of DP-IAG, 74 of 101 patients (73.3%; 95% CI: 64.6–81.9%) achieved a morphologic complete remission (CR; n=16) or complete remission with

incomplete blood count recovery (CRi; n=58) (Figure 3A). MRD by flow cytometric assay was negative (<0.1%) in 36 patients (48.6%) (Figure 3B). The CR rate tended to be higher in the refractory AML group than in the relapsed group (78.8% vs. 62.9%; P = 0.085; Table 2). CR rates did not differ significantly by age, sex, white blood cell count at enrollment, FAB subtype, complex karyotype, or genetic risk category (Table 2). Response by genetic subgroup is shown in Figure 2B. Notably, all four patients with *ETV6* rearrangements achieved both morphologic and molecular remission (Supplementary Table 3). Patients with *KMT2A* rearrangements, excluding *KMT2A::MLLT3*, had a CR rate of 90.9% (Supplementary Table 4). Eighteen patients were non-responders after reinduction I. Three patients (3.0%) experienced treatment-related early deaths after reinduction I. An additional five patients refused further treatment.

Among the 101 patients, 70 ultimately underwent allogeneic hematopoietic stem cell transplantation (69.3%). Of these, 20 patients received transplantation after one cycle of DP-IAG chemotherapy, while 41 patients underwent transplantation after receiving two or more cycles of chemotherapy. Additionally, 9 children who did not respond to DP-IAG chemotherapy withdrew from the study ,after individualized treatment, they eventually received transplantation.

Survival Outcomes

Survival outcomes are summarized in Figure 4. As of December 31, 2024, with a median follow-up of 36.4 months (IQR, 10.3–51.3), the estimated 3-year overall

survival (OS) was 60.8% (95% CI, 51.9–71.2%), and the 3-year event-free survival (EFS) was 49.8% (95% CI, 40.9–60.7%) (Figure 4A). Patients who achieved CR/CRi after reinduction therapy (n = 74) had significantly superior 3-year OS and EFS compared to those with partial response or non-response (PR+NR) (both $P < 0.001$; Figure 4B-C). Similarly, patients who were MRD-negative after first reinduction (n=36) had significantly better 3-year OS and EFS than those (n= 35) who were MRD-positive ($P = 0.0021$ and $P = 0.0039$, respectively; Figure 4D-E). Twenty patients in CR/CRi or PR proceeded directly to allo-HSCT following reinduction I and achieved a 3-year OS of 95.0% (95% CI, 85.9–100.0%). Among the 55 patients who received a second course of salvage chemotherapy, 41 ultimately underwent allo-HSCT, with a 3-year OS of 70.7% (95% CI, 58.1–86.1%). This difference in OS between the immediate (n= 20; 95 CI 85.9-100%) and delayed transplant groups (n=41; 95 CI 58.1-86.1%) was statistically significant ($P = 0.025$; Figure 4F). Of the 22 patients who received reinduction II with standard-dose chemotherapy, 14 underwent subsequent allo-HSCT. Among the 33 patients who received DP-IAG chemotherapy, 27 proceeded to transplant. The 3-year OS in the standard-dose and low-dose groups was 66.7% (95% CI, 51.1–87.0%) and 78.6% (95% CI, 59.8–100.0%), respectively, with no significant difference between the groups ($P = 0.30$; Figure 4G). Among the 44 children who achieved CR/CRi before transplantation, they were divided into two groups based on the negative status of MRD. There was no significant difference in OS between the two groups($P = 0.08$; Figure 4H). For patients with refractory AML, regardless of whether they received

standard-dose chemotherapy or low-dose chemotherapy at the time of their initial diagnosis, there was no significant difference in 3-year OS (P = 0.72; Figure 4I).

Adverse Events

Adverse events observed during reinduction therapy are summarized in Table 3. All patients (100%) experienced grade 4 hematologic toxicity. The median time for granulocyte recovery (neutrophil count 0.5×10^9/L) in 78 patients with CR/CRi/PR was 23 days (range, 4-43 days), and the median time for platelet recovery (platelet count 20×10□/L) was 14.5 days (range, 3-46 days). The most common non-hematologic treatment-related complications were infections, including bacteremia and pneumonia. Gram-negative organisms accounted for most of the bloodstream infections. Among patients with pulmonary processes, 50% were classified as grade ≥3, and one patient died of severe pneumonia. Most other non-hematologic toxicities were grade <3 and resolved with supportive care. Three patients died during reinduction I due to treatment-related complications.

DISCUSSION

In this study, we demonstrated that salvage therapy for pediatric (R/R) AML using a low-intensity regimen preceded by a 5-day course of decitabine was associated with CR and OS rates that compare favorably to those reported with more intensive salvage approaches. For example, in the COG trial AAML1031, patients with high-risk or refractory AML who did not respond to initial induction with standard-dose daunorubicin, cytarabine, and etoposide (DAE) received a second,

induction regimen consisting of high-dose cytarabine (1,000 mg/m² × 8 doses) and mitoxantrone (12 mg/m² × 4 doses), followed by consolidation with cytarabine and etoposide and subsequent allogeneic HSCT. Despite this intensified approach, OS remained poor (34.6% ± 10.1%). Similar outcomes were observed in the earlier AAML0531 trial, with an OS of 37.9% ± 18.1% [20]. These findings highlight the lack of high-level evidence that intensifying chemotherapy improves outcomes in R/R AML. Nonetheless, intensive salvage regimens remain the standard approach (Supplementary Table 5).

Recently, the combination of venetoclax with hypomethylating agents has been extensively used in adult and elderly *de novo* or relapsed AML[21-23]. In a retrospective study from MD Anderson, 43 children with R/R AML were treated with venetoclax combined with hypomethylating agents or conventional chemotherapy, resulting in a 40% CR/CRi rate. The median EFS was only 3.7 months, and OS was 8.7 months [24]. In the Phase I dose-escalation study by Karol et al., the safety and preliminary efficacy of venetoclax were evaluated in combination regimens. At dose levels including venetoclax (360 mg/m²), cytarabine (8,000 mg/m²), with or without a single dose of idarubicin (12 mg/m²), the CR/CRi rate among 20 patients was approximately 65%. Negative MRD was observed in 64% of the 11 patients who did not receive idarubicin**,** compared to 33% of the 9 patients who did [25]. Despite the small number of patients, the limited benefit of intensified idarubicin-based therapy, alongside the COG AAML1031 trial's failure to improve outcomes with early intensification, suggests the need to re-evaluate salvage strategies for R/R AML.

Another agent, CPX-351,was evaluated in a Phase I/II trial for children with first relapse of AML [26]. In this trial, the salvage regimen included one cycle of CPX-351, followed by the FLAG regimen and HSCT as final consolidation. Following the initial course of CPX-351 alone, 28 of 37 evaluable patients (75.6%) achieved CR, CRi, or CRp, and the 2-year overall survival was 52.7% ± 21.1%. The high response rate after a single course of CPX-351 supports the concept that effective salvage therapy for R/R AML may be achievable with regimens of relatively lower intensity.

The interest in incorporating hypomethylating agents into the treatment of pediatric AML is longstanding [14]. The rationale is supported by the observation that early genetic events in pediatric AML tumorigenesis frequently involve genes that regulate DNA methylation, leading to global DNA hypermethylation [27]. A recent retrospective analysis demonstrated that methylation profiling refined risk stratification in patients treated on the COG AAML0531 and AAML1031 protocols, as well as the St. Jude AML02 and AML08 studies [28]. In these cohorts, risk classification, based on molecular markers and MRD by flow cytometry, was further improved by incorporating methylation status. A signal of decitabine priming efficacy in our study is suggested by the observation that among 37 newly diagnosed patients who had not achieved remission with an initial low-intensity regimen, 28 attained complete remission after receiving decitabine priming followed by a comparable low-intensity chemotherapy regimen**.** These findings support the hypothesis that hypomethylating agent priming may overcome chemotherapy resistance in leukemic cells by enhancing their sensitivity to cytotoxic agents. This therapeutic effect may

facilitate a higher proportion of patients proceeding to hematopoietic stem cell transplantation, often in better clinical condition and with a reduced leukemia burden.

A subgroup analysis showed that patients who achieved CR, CRi, or PR after the first reinduction course (n = 20) and proceeded directly to HSCT experienced excellent outcomes. Among patients who were refractory to initial therapy (n = 66), overall survival was comparable regardless of whether their primary treatment consisted of low-intensity (n = 37) or standard-intensity (n = 29) regimens (Figure 4I). Although the study was not explicitly designed to address this question, our findings do not support the notion that intensifying chemotherapy to deepen remission is essential for HSCT success in relapsed/refractory AML. Instead, achieving a robust cytoreduction may be sufficient to proceed to transplant with favorable outcomes.

The responses observed in our patients with *ETV6* alterations were particularly intriguing. In our cohort, *ETV6* rearrangements were identified in four patients, including two with the well-characterized *ETV6::MNX1* fusion, and one each with *ETV6::MAGI2* and *ETV6::FRMPD1* fusions. The *ETV6::MNX1* fusion defines a high-risk AML subtype that occurs exclusively in children under two years of age and represents the second most common cytogenetic abnormality in infant AML, with a reported prevalence of 18% to 30% in this age group[29]. Clinical studies have shown poor outcomes in this subgroup, with 3-year event-free survival (EFS) and overall survival (OS) rates of 24% and 42%, respectively[30]. In contrast, the *ETV6::MAGI2* and *ETV6::FRMPD1* fusions identified in the other two patients have, to our

knowledge, not been previously reported in AML. Remarkably, all four patients in this subgroup achieved complete remission (CR) after a single course of reinduction therapy, with MRD-negative status at that time point (Supplementary Table 3). All four subsequently underwent allogeneic HSCT and remained alive and disease-free for over two years(Supplementary Table 3). Additionally, structural *ETV6* alterations were identified in four other patients. Among them, three achieved CR following one course of reinduction chemotherapy. Two of these patients proceeded to allogeneic HSCT, one immediately and the other after receiving a second course of low-dose reinduction, and both remained disease-free for more than three years. The fourth patient received a standard-dose consolidation regimen but died due to treatment-related complications. Taken together, these findings suggest that the combination of decitabine priming and low-dose chemotherapy, followed by timely HSCT, may be particularly effective in children with *ETV6* rearrangements or structural alterations. These encouraging results warrant further investigation.

*KMT2A*r occurs in approximately 20% of de novo pediatric AML cases and represents the most common recurrent cytogenetic abnormality in this population. While clinical outcomes vary depending on the specific fusion partner, the overall prognosis remains poor across most *KMT2A*r subtypes[31]. Preclinical studies have shown that decitabine, a DNA hypomethylating agent, can effectively eradicate *KMT2A*r acute lymphoblastic leukemia (ALL) cells in vitro and enhance the cytotoxicity of conventional chemotherapeutic agents [32]. In our trial, reinduction therapy, incorporating decitabine priming, achieved an overall complete remission (CR) rate of 73.7%. This

regimen showed high efficacy in patients with *KMT2A*r AML, except those harboring the *KMT2A::MLLT3* subtype (Supplementary Table 4). Despite these encouraging early responses, patients with *KMT2Ar* AML experienced inferior overall survival (OS) and EFS. All patients in this subgroup who did not undergo HSCT ultimately died from progressive disease, and relapse remained the primary cause of death among those who received HSCT. These findings highlight the urgent need for effective post-transplant relapse-prevention strategies in relapsed/refractory (R/R) AML with *KMT2A*r rearrangements. Recently, the FDA approved revumenib, a selective menin inhibitor, for the treatment of AML with *KMT2A*r, offering a promising targeted therapy, particularly in the post-HSCT setting[33].

Pediatric studies have shown that *KIT* mutations, particularly those involving exon 17, are associated with inferior outcomes in patients with *RUNX1::RUNX1T1* AML, even among those who achieve MRD negativity after induction chemotherapy [34]. In our cohort, *KIT* co-mutations were identified in 16 patients with CBF-AML, of whom 11 (68.8%) harbored mutations in exon 17**.** Notably, all but one of these eleven patients experienced disease relapses**,** suggesting that *KIT* exon 17 mutations may contribute significantly to relapse risk in this genetically defined AML subtype. A retrospective study from MD Anderson supports this observation, reporting that maintenance therapy with hypomethylating agents (azacitidine or decitabine) may help prolong remission in patients with CBF-AML [35]. In our study, patients with relapsed or refractory CBF-AML—including those with *KIT* exon 17 mutations—demonstrated high response rates and favorable survival outcomes when treated with decitabine

priming followed by a low-intensity chemotherapy regimen. Among the eleven patients with *KIT* exon 17 mutations, two died from disease relapse after declining allo-HSCT**.** In comparison, the remaining nine patients remain alive and disease-free following HSCT**.**These findings highlight the adverse prognostic impact of *KIT* exon 17 mutations in CBF-AML and the need for early molecular detection to guide risk-adapted therapy. Treatment intensification, such as decitabine priming before induction and postinduction chemotherapy, may lower relapse rates and limit HSCT to patients with suboptimal responses.

Outcomes of children with relapsed acute megakaryoblastic leukemia (AMKL) are dismal[36, 37]. In our cohort of six patients with R/R AMKL, only one patient is alive. These outcomes reinforce the urgent need for more effective and alternative treatment strategies for children with AMKL.

In conclusion, decitabine priming combined with low-dose chemotherapy showed promising efficacy and an acceptable safety profile in children with R/R AML. This regimen represents a potential bridging strategy to allogeneic HSCT. Early consideration of transplantation after remission may help improve long-term disease control, particularly for patients at high risk of relapse.

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Tables:

Table 1. Clinical characteristics.

Patients	Total	Refractory	Relapsed	*P*
Number（%）	**101 (100.0)**	**66 (100.0)**	**35 (100.0)**	
Sex				0.929
Male	60 (59.4)	39 (59.1)	21 (60.0)	
Female	41 (40.6)	27 (40.9)	14 (40.0)	
Age (year)				0.715
<3	15 (14.8)	11 (16.7)	4 (11.4)	
≥3, <10	53 (52.5)	33 (50.0)	20 (57.1)	
≥10	33 (32.7)	22 (33.3)	11 (31.4)	
WBC count, at enrollment, × 10^9/L				0.012
≥100	10 (9.9)	9 (13.6)	1 (2.9)	
≥50, < 100	8 (7.9)	8 (12.1)	0 (0.0)	
< 50	83 (82.2)	49 (74.3)	34 (97.1)	
FAB subtype				0.874
M1	1 (1.0)	1 (1.5)	0 (0.0)	
M2	37 (36.6)	22 (33.3)	15 (42.9)	
M4	15 (14.9)	9 (13.6)	6 (17.1)	
M5	23 (22.8)	16 (24.2)	7 (20.0)	
M7	6 (5.9)	5 (7.6)	1 (2.9)	
AML-unclassified	19 (18.8)	13 (19.7)	6 (17.1)	

Patients	Total	Refractory	Relapsed	*P*
Number（%）	**101 (100.0)**	**66 (100.0)**	**35 (100.0)**	
Karyotype				0.692
Complex*	17 (17.7)	11 (16.7)	6 (20.0)	
Non-Complex	79 (82.3)	55 (83.3)	24 (80.0)	
Fusion gene				
RUNX1::RUNX1T1	21 (20.8)	8 (12.1)	13 (37.1)	0.003
CBFB::MYH11	5 (5.0)	2 (3.0)	3 (8.6)	0.46
KMT2A::MLLT3	8 (7.9)	5 (7.6)	3 (8.6)	1.0
Other KMT2A-r	11 (10.9)	8 (12.1)	3 (8.6)	0.834
NUP98-r	8 (7.9)	7 (10.6)	1 (2.9)	0.325
ETV6-r	4 (4.0)	2 (3.0)	2 (2.9)	0.903
Mutations				
KIT	19 (18.8)	6 (9.1)	13 (37.1)	< 0.001
FLT3	17 (16.8)	13 (19.7)	4 (11.4)	0.437
CEBPA double mutations	8 (7.9)	4 (6.1)	4 (11.4)	0.573

Abbreviations: WBC, white blood cell; *KIT*, KIT proto-oncogene, receptor tyrosine kinase; *FLT3*, FMS-like tyrosine kinase 3 gene; *RUNX1*, RUNX family transcription factor 1.*Complex karyotype: ≥ three unrelated chromosome abnormalities in the absence of other class□defining recurring genetic abnormalities; excludes hyperdiploid karyotypes with three or more trisomies (or polysomies) without structural abnormalities.

Table 2. Treatment responses after DP-IAG therapy.

Feature	n	%	*P*
Total number of CR/CRi patients	**74**	**73.3**	
Sex			**0.35**
Male	46	76.7	
Female	28	68.3	
Age (year)			**0.865**
<3	11	73.3	
≥3, <10	40	75.5	
≥10	23	69.7	
WBC (at enrollment, × 10^9/L)			**0.821**
≥100	7	70.0	
≥50, < 100	7	87.5	
< 50	60	72.3	
FAB subtype			**0.818**
M1	1	100	
M2	28	75.7	
M4	9	60.0	
M5	17	73.9	
M7	4	66.7	
AML-unclassified	15	78.9	

Classification			**0.085**
Relapsed AML	22	62.9	
Refractory AML	52	78.8	
Karyotype			**0.72**
Complex karyotype*	14	82.4	
Non-complex karyotype	59	74.7	
Risk category#			**0.202**
Adverse	36	67.9	
Favorable or Intermediate	38	79.2	

*Complex karyotype: ≥ three unrelated chromosome abnormalities in the absence of other class□defining recurring genetic abnormalities; excludes hyperdiploid karyotypes with three or more trisomies (or polysomies) without structural abnormalities. #The risk categories are based on the ELN recommendations.[18]

Table 3. Summary of adverse events in DP-IAG therapy.

All adverse events	All Grades	Grade 1/2	Grade 3/4	Grade5(death)
	Total patients n (%)	Patients	Patients	Patients
Hematologic				
Neutropenia	101 (100.0)	0	101	0
Thrombocytopenia	101 (100.0)	0	101	0
Infections				
Pulmonary	12 (11.9)	6	5	1
Enterocolitis	7 (6.9)	4	2	1
Sepsis	13 (12.9)	0	12	1
Nonhematologic				
Oral mucositis	9 (8.9)	8	1	0
Gastrointestinal hemorrhage	3 (3.0)	2	1	0
Rhinorrhagia	7 (6.9)	6	1	0
Elevated cardiac troponin	3 (3.0)	3	0	0
Allergic reaction	5 (5.0)	4	1	0
Hepatotoxicity	6 (5.9)	4	2	0
Headache	2 (2.0)	2	0	0

Figure legends:

Figure 1. Flow diagram of participants with relapsed or refractory acute myeloid leukemia who received DP-IAG

Abbreviations: allo-HSCT, allogeneic hematopoietic stem cell transplantation

Figure 2. Genetic characteristics and treatment responses of participants with relapsed or refractory AML (A). The genomic landscape highlights representative mutations and fusion genes, while bar charts depict the corresponding CR/CRi rates across molecular subgroups (B).

Figure 3. Treatment responses.

Response rates (CR/CRi, PR, and NR) by study cohort after Reinduction I (A), and MRD status among patients achieving CR/CRi in each cohort (B).

Abbreviations: CR, complete remission; CRi,CR with incomplete hematologic recovery; PR, partial remission; NR, no remission; NA, not available; MRD, measurable residual disease.

Figure 4. Survival outcomes of enrolled patients.

(A) Overall survival (OS) and event-free survival (EFS) of all patients.

(B, C) OS and EFS by response status (CR/CRi vs. non-CR/CRi; $P < 0.001$ for both).

(D, E) OS and EFS by MRD level (<0.1% vs. 0.1–5%; $P = 0.0021$ and $P = 0.0039$).

(F) OS in children undergoing transplantation immediately after first reinduction

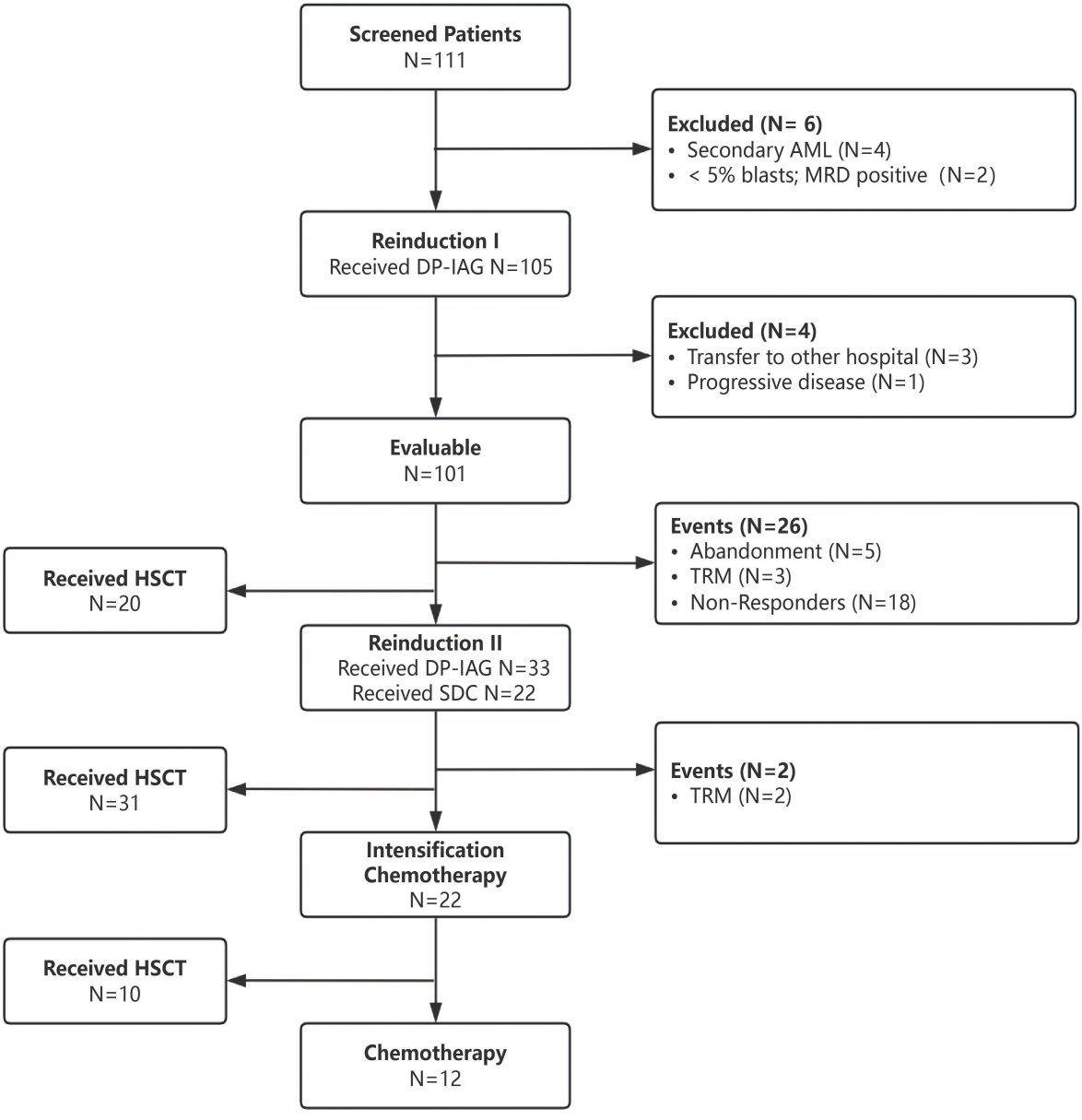
versus after additional chemotherapy ($P = 0.025$).

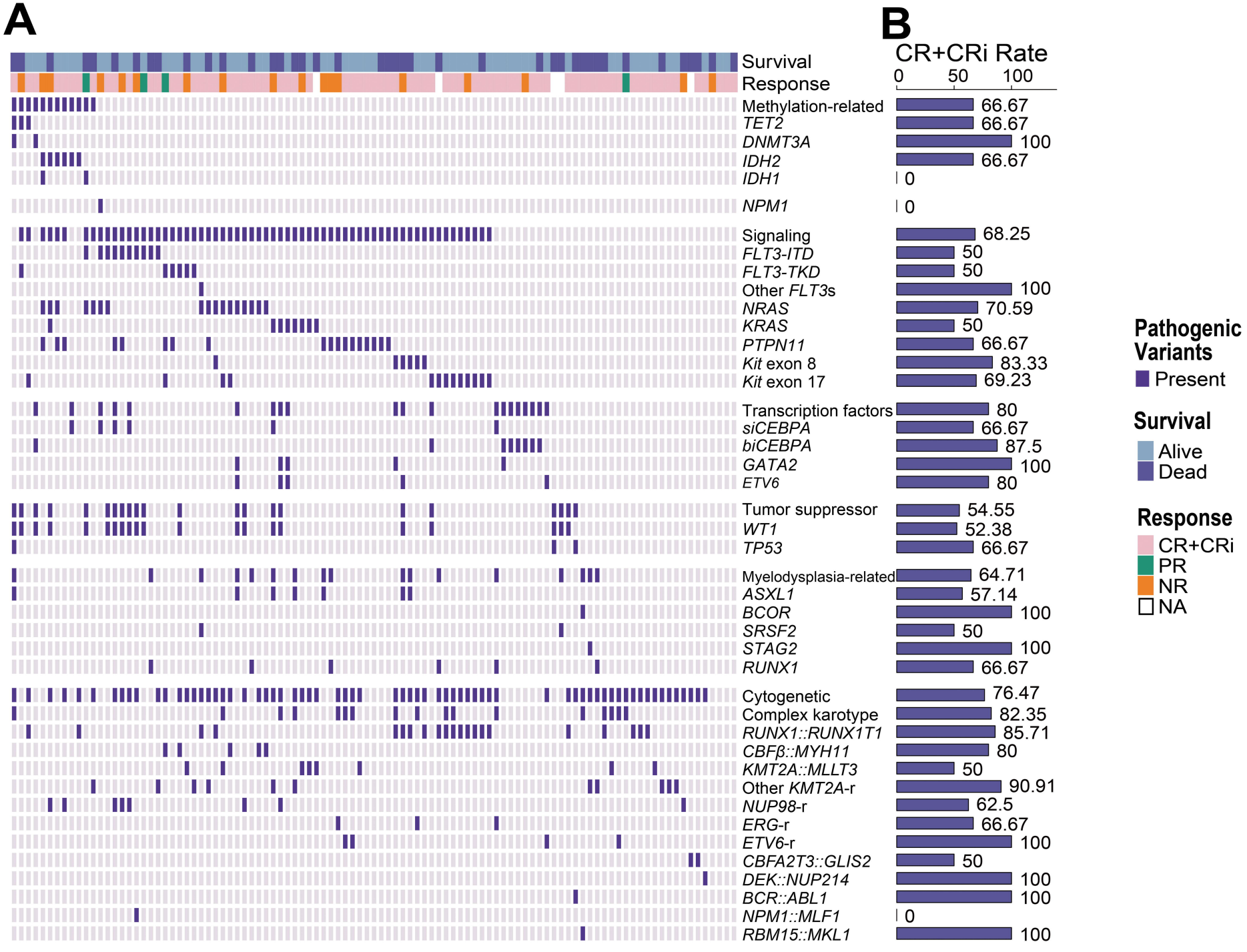
(G) OS in children treated with DP-IAG after Reinduction I versus those given standard-dose chemotherapy followed by transplantation ($P = 0.3$).

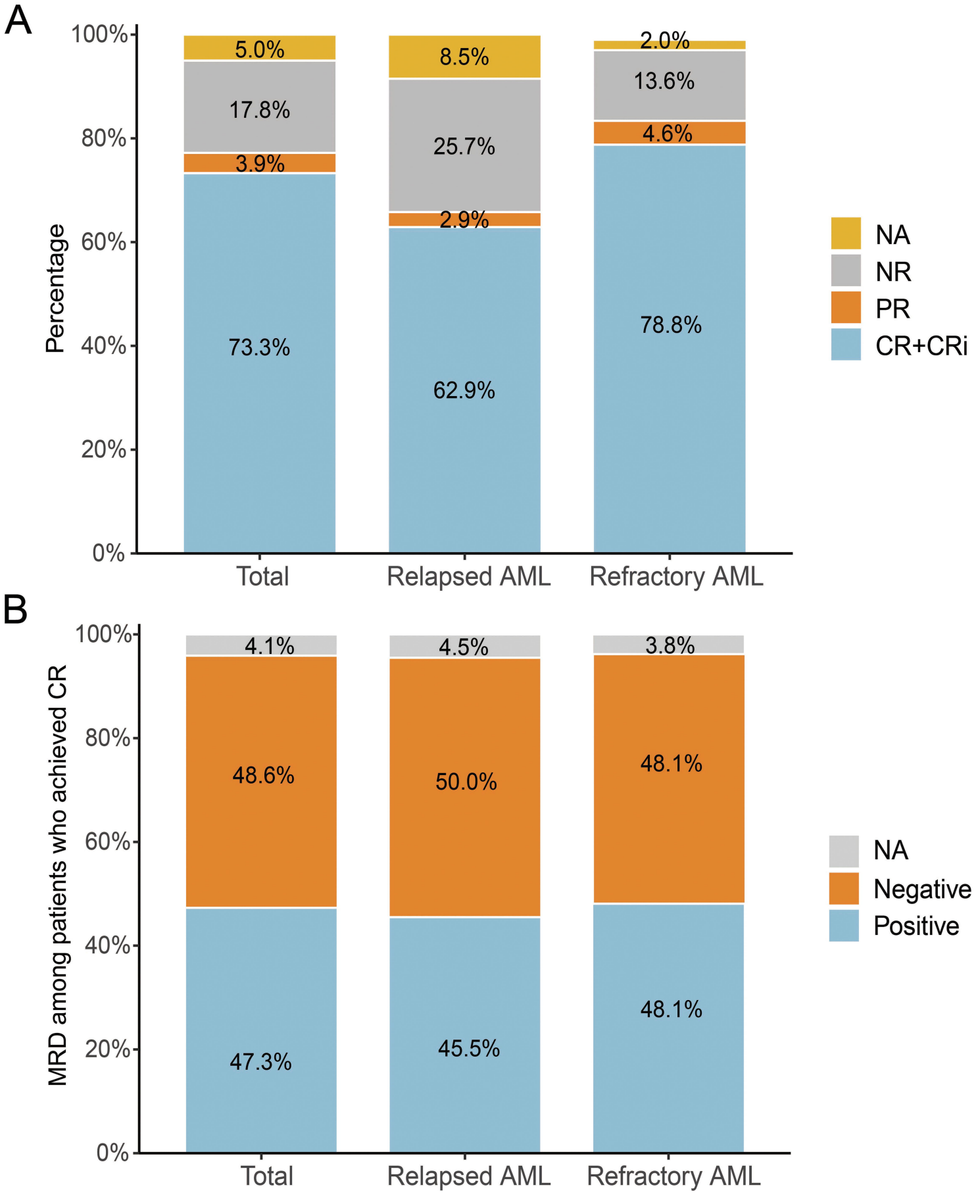
(H) OS by MRD status before transplantation (positive vs. negative; $P = 0.08$).

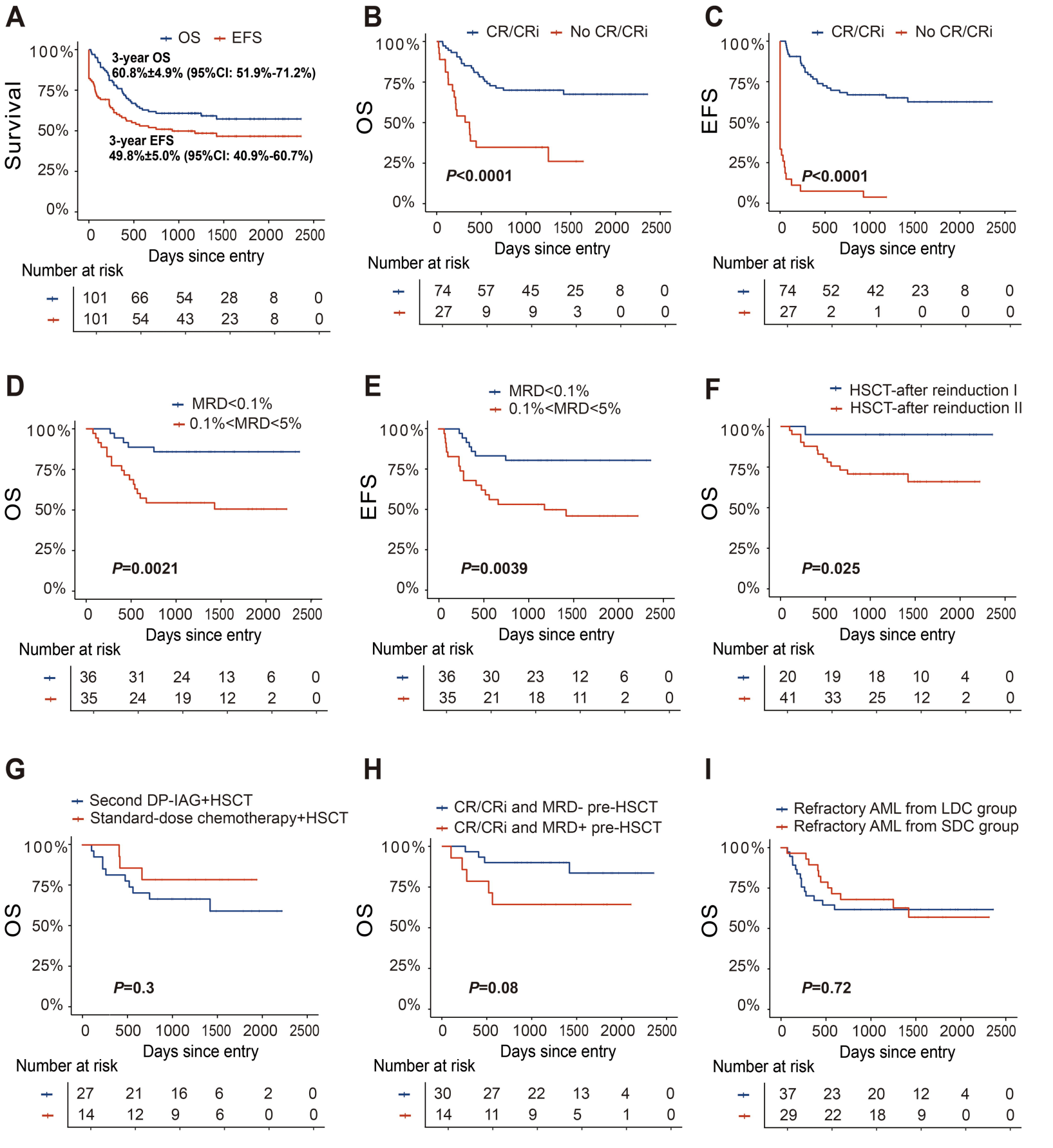
(I) OS in refractory AML patients initially treated with low-dose versus standard-dose chemotherapy ($P = 0.27$).

Abbreviations: AML, acute myeloid leukemia; OS, overall survival; EFS, event-free survival; CR, complete remission; CRi, CR with incomplete hematologic recovery; MRD, measurable residual disease.


Screened Patients
N=111
Excluded (N= 6)
• Secondary AML (N=4)
• < 5% blasts; MRD positive (N=2)
Reinduction I
Received DP-IAG N=105
Excluded (N=4)
• Transfer to other hospital (N=3)
• Progressive disease (N=1)
Evaluable
N=101
Events (N=26)
• Abandonment (N=5)
• TRM (N=3)
• Non-Responders (N=18)
Received HSCT
N=20
Reinduction II
Received DP-IAG N=33
Received SDC N=22
Events (N=2)
• TRM (N=2)
Received HSCT
N=31
Intensification
Chemotherapy
N=22
Received HSCT
N=10
Chemotherapy
N=12


A
B
CR+CRi Rate
0
50
100
Survival
Response
Methylation-related
66.67
TET2
66.67
DNMT3A
100
IDH2
66.67
IDH1
0
NPM1
0
Signaling
68.25
FLT3-ITD
50
FLT3-TKD
50
Other FLT3s
100
NRAS
70.59
KRAS
50
PTPN11
66.67
Kit exon 8
83.33
Kit exon 17
69.23
Transcription factors
80
siCEBPA
66.67
biCEBPA
87.5
GATA2
100
ETV6
80
Tumor suppressor
54.55
WT1
52.38
TP53
66.67
Myelodysplasia-related
64.71
ASXL1
57.14
BCOR
100
SRSF2
50
STAG2
100
RUNX1
66.67
Cytogenetic
76.47
Complex karotype
82.35
RUNX1::RUNX1T1
85.71
CBFβ::MYH11
80
KMT2A::MLLT3
50
Other KMT2A-r
90.91
NUP98-r
62.5
ERG-r
66.67
ETV6-r
100
CBFA2T3::GLIS2
50
DEK::NUP214
100
BCR::ABL1
100
NPM1::MLF1
0
RBM15::MKL1
100
Pathogenic Variants
Present
Survival
Alive
Dead
Response
CR+CRi
PR
NR
NA


A
100%
80%
60%
40%
20%
0%
Percentage
5.0%
17.8%
3.9%
73.3%
8.5%
25.7%
2.9%
62.9%
2.0%
13.6%
4.6%
78.8%
Total
Relapsed AML
Refractory AML
NA
NR
PR
CR+CRi
B
100%
80%
60%
40%
20%
0%
MRD among patients who achieved CR
4.1%
48.6%
47.3%
4.5%
50.0%
45.5%
3.8%
48.1%
48.1%
Total
Relapsed AML
Refractory AML
NA
Negative
Positive


A
OS
EFS
Survival
100%
75%
50%
25%
0%
3-year OS
60.8%±4.9% (95%CI: 51.9%-71.2%)
3-year EFS
49.8%±5.0% (95%CI: 40.9%-60.7%)
0
500
1000
1500
2000
2500
Days since entry
Number at risk
101 66 54 28 8 0
101 54 43 23 8 0
B
CR/CRi
No CR/CRi
OS
P<0.0001
Days since entry
Number at risk
74 57 45 25 8 0
27 9 9 3 0 0
C
CR/CRi
No CR/CRi
EFS
P<0.0001
Days since entry
Number at risk
74 52 42 23 8 0
27 2 1 0 0 0
D
MRD<0.1%
0.1%<MRD<5%
OS
P=0.0021
Days since entry
Number at risk
36 31 24 13 6 0
35 24 19 12 2 0
E
MRD<0.1%
0.1%<MRD<5%
EFS
P=0.0039
Days since entry
Number at risk
36 30 23 12 6 0
35 21 18 11 2 0
F
HSCT-after reinduction I
HSCT-after reinduction II
OS
P=0.025
Days since entry
Number at risk
20 19 18 10 4 0
41 33 25 12 2 0
G
Second DP-IAG+HSCT
Standard-dose chemotherapy+HSCT
OS
P=0.3
Days since entry
Number at risk
27 21 16 6 2 0
14 12 9 6 0 0
H
CR/CRi and MRD- pre-HSCT
CR/CRi and MRD+ pre-HSCT
OS
P=0.08
Days since entry
Number at risk
30 27 22 13 4 0
14 11 9 5 1 0
I
Refractory AML from LDC group
Refractory AML from SDC group
OS
P=0.72
Days since entry
Number at risk
37 23 20 12 4 0
29 22 18 9 0 0

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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