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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Abstract

There is no standard salvage regimen for relapsed/refractory (R/R) pediatric acute myeloid leukemia (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 (alloHSCT). At a median follow-up of 36.4 months (Interquartile Range: 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; 3 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 R/R AML remain poor, and no standardized salvage regimen has been generally adopted. Allogeneic hematopoietic stem cell transplantation

(alloHSCT) 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 little 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 Berlin-Frankfurt-Münster (BFM) study, which compared FLAG plus liposomal daunorubicin (DNX) *versus* FLAG alone in 394 children with first relapse or refractory AML. The overall survival (OS) 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 (Memphis, TN, USA) was launched to reduce early mortality and treatment abandonment among children with leukemia. For pediatric patients with 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 granulocyte colony-stimulating factor (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 alloHSCT, 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 R/R 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).

The diagnostic criteria for refractory AML after achieving complete remission (CR) are 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. The diagnostic criteria for relapsed AML are: 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 response to conventional chemotherapy; cases with two or more relapses; cases with persistent extramedullary leukemia.

Early relapse patients are considered to be those who relapsed within 12 months after remission. Late relapse patients are considered to be those who relapsed more than 12 months after remission.

Children undergoing immediate transplant are those who directly proceed to the alloHSCT process after the first DP-IAG treatment course. Children undergoing delayed transplant are those who continue to receive one or more courses of chemotherapy after the first DP-IAG treatment and then undergo alloHSCT.

The study protocol was approved by the Medical Ethics Committee of the Children's Hospital of Soochow University (Approval N. 2017047-3) and was 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 principles of the Declaration of Helsinki.

Response criteria

Response definitions were based on the 2022 European LeukemiaNet (ELN) recommendations.[18] Complete remission (CR) is considered to be <5% bone marrow blasts, no Auer rods, no extramedullary leukemia, and hematologic recovery defined as transfusion independence, an absolute neutrophil count (ANC) >1.0×10^9/L, and platelet count >80×10^9/L. Criteria for CR with incomplete hematologic recovery (CRi) was the same as for CR, but without full recovery of ANC and/or platelets. Partial response (PR) is considered to be

≥50% reduction in bone marrow blasts, with residual blasts between 5% and 19%. No-response (NR) was ≥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 (*Online Supplementary Table S1*).

All patients with R/R AML were advised to proceed to alloHSCT. 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

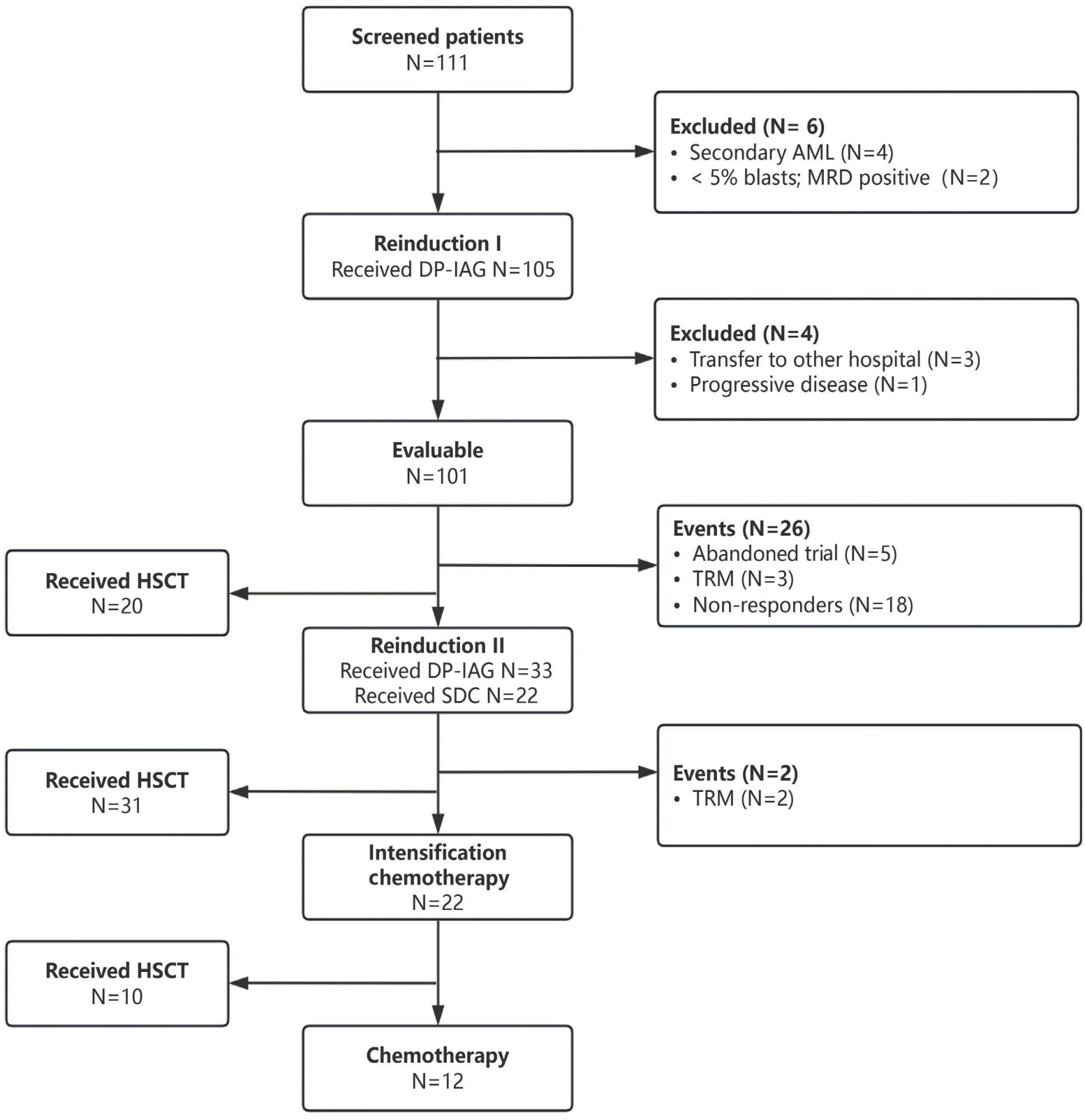


Figure 1. Flow diagram of participants with relapsed or refractory acute myeloid leukemia who received DP-IAG. alloHSCT: allogeneic hematopoietic stem cell transplantation; AML: acute myeloid leukemia; DP-IAG: one course of decitabine priming followed by low-dose idarubicin, cytarabine, and granulocyte colony-stimulating factor; MRD: measurable residual disease; N: number; SDC: standard-dose cytarabine; TRM: treatment-related mortality.

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 three days combined with etoposide 150 mg/m² once daily for three days (13 patients); homoharringtonine 3 mg/m² once daily for three days (7 patients); or mitoxantrone 5 mg/m² once daily for three days (2 patients) (*Online Supplementary Table S2*).

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: 6 did not meet the diagnostic criteria for R/R AML, 3 were transferred to other institutions during treatment, and one had a white blood cell (WBC) count >50×10⁹/L after five days of decitabine, making 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 WBC count at enrollment was 9.5×10⁹/L (Interquartile range [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. Nine children achieved 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/hematologic 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, WBC count at enrollment, FAB subtype, complex karyotype, or genetic risk category (Table 2). Response by genetic subgroup is shown in Figure 2B. Notably, all 4 patients with *ETV6* rearrangements achieved both morphologic and molecular remission (*Online Supplementary Table S3*). Patients with KMT2A rearrangements, excluding *KMT2A*::*MLLT3*, had a CR rate of 90.9% (*Online Supplementary Table S4*). Eighteen patients were non-responders after reinduction I. Three patients (3.0%) experienced treatment-related early deaths after reinduction I. An additional 5 patients refused further treatment.

Among the 101 patients, 70 ultimately underwent alloHSCT (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 a transplant.

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 no 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 alloHSCT 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 alloHSCT, 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 (N=41; 95%CI: 58.1-86.1%) transplant groups was statistically significant (*P*=0.025) (Figure 4F). Of the 22 patients who received rein-

duction II with standard-dose chemotherapy, 14 underwent subsequent alloHSCT. 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 two groups (P=0.30) (Figure 4G). The 44 children who achieved CR/CRi before transplantation were divided into two groups based on the negative MRD status; 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

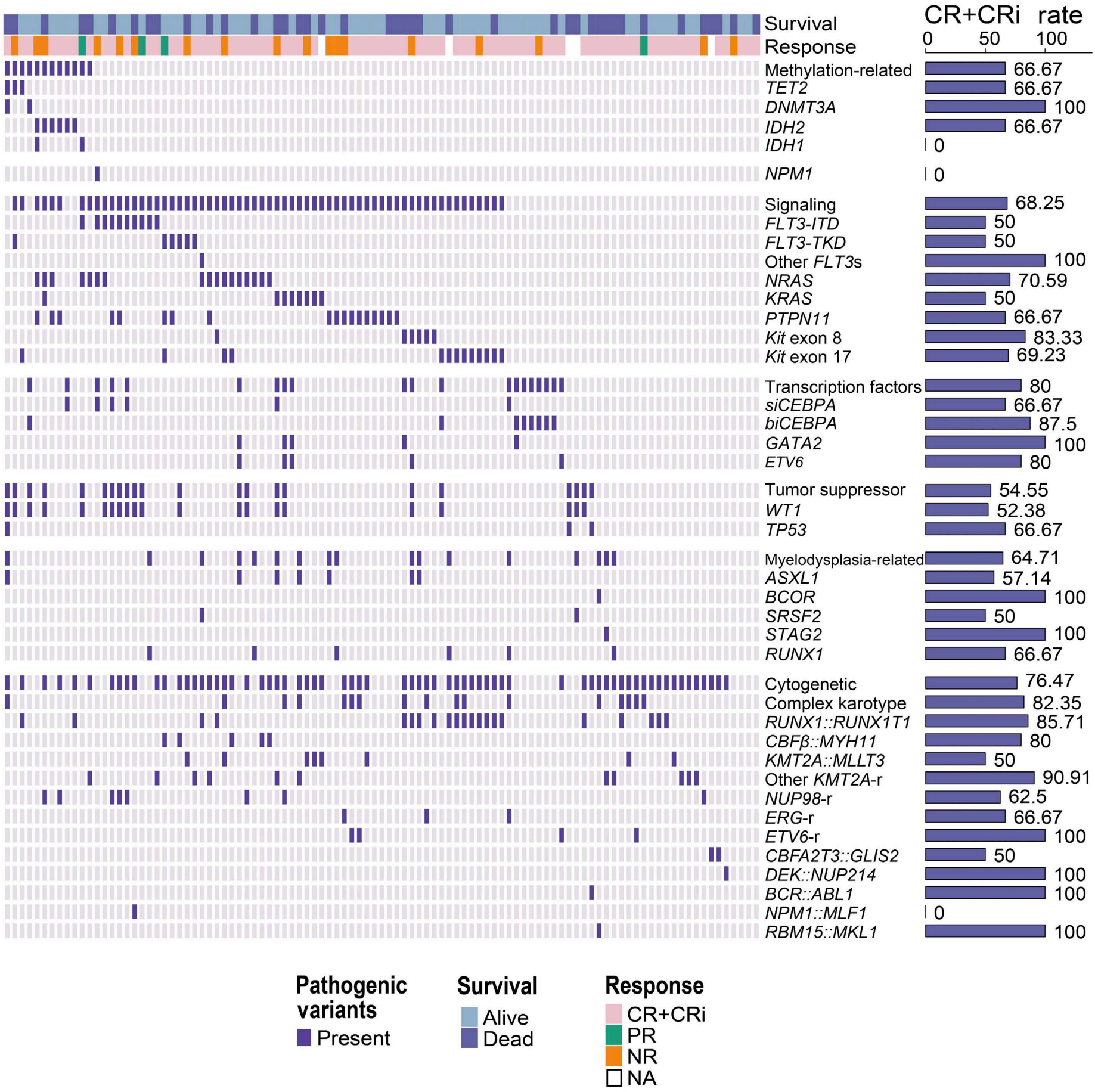


Figure 2. Genetic characteristics and treatment responses of participants with relapsed or refractory acute myeloid leukemia. (A) Genetic characteristics and treatment responses of participants with relapsed or refractory (R/R) acute myeloid leukemia (AML). (B) The genomic landscape highlights representative mutations and fusion genes, while bar charts depict the corresponding complete remission (CR)/CR with incomplete hematologic recovery (CRi) rates across molecular subgroups. NA: not available; NR: no-remission; PR: partial remission.

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

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 (ANC 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 20×10^9/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 demonstrate 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

Table 1. Clinical characteristics.

Patients	Total	Refractory	Relapsed	*P*
N (%)	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, years				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, x10^9/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)	
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

AML: acute myeloid leukemia; FAB: French-American-British classification system; *FLT3*: FMS-like tyrosine kinase 3 gene; *KIT*: KIT proto-oncogene, receptor tyrosine kinase; N: number; *RUNX1*: RUNX family transcription factor 1; WBC: white blood cell.*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.

mg/m² × 8 doses) and mitoxantrone (12 mg/m² × 4 doses), followed by consolidation with cytarabine and etoposide and subsequent alloHSCT. 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 (*Online Supplementary Table S5*).

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 CR with incomplete platelet recovery (CRp), and the 2-year OS 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 priming with a hypomethylating agent may overcome chemotherapy resistance in leukemic cells by enhancing their sensitivity to cytotoxic agents. This therapeutic effect may help a higher proportion of patients to proceed to HSCT, 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

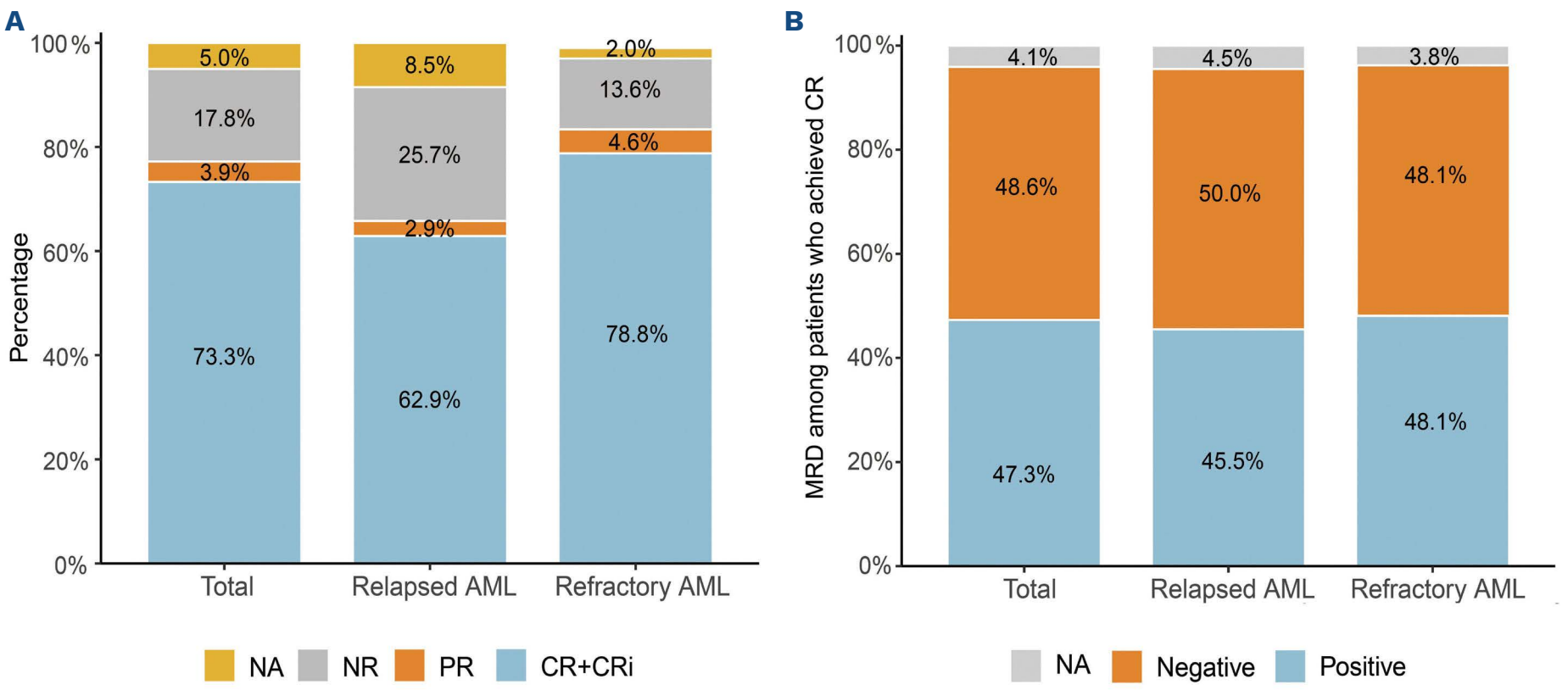


Figure 3. Treatment responses. Response rates (complete remission [CR]/CR with incomplete hematologic recovery [CRi], partial remission [PR], and no-remission [NR]) by study cohort after Reinduction I (A), and measurable residual disease (MRD) status among patients achieving CR/CRi in each cohort (B). NA: not available.

proceeded directly to HSCT experienced excellent outcomes. Among patients who were refractory to initial therapy (N=66), OS was comparable regardless of whether their primary treatment consisted of low-intensity (N=37) or standard-intensity (N=29) regimens (Figure 4 I). 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 a successful HSCT 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 4 patients, including 2 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 18-30% in this age group.[29] Clinical studies have shown poor outcomes in this subgroup, with 3-year EFS and OS rates of 24% and 42%, respectively.[30] In contrast, the *ETV6*::*MAGI2* and *ETV6*::*FRMPD1* fusions identified in the other 2 patients have, to our knowledge, not been previously reported in AML. Remarkably, all 4 patients in this subgroup achieved CR after a single course of reinduction therapy, with MRD-negative status at that time point (*Online Supplementary Table S3*). All 4 subsequently underwent alloHSCT and survived disease-free for over two years (*Online Supplementary Table S3*). Additionally, structural *ETV6* alterations were identified in 4 other patients. Among these, 3 achieved CR following one course of reinduction chemotherapy. Two of these patients proceeded to alloHSCT, 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.

KMT2Ar 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 *KMT2Ar* 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 CR rate of 73.7%. This regimen showed high efficacy in patients with *KMT2A*r AML, except those harboring the *KMT2A*::*MLLT3* subtype (*Online Supplementary Table S4*). Despite these encouraging early responses, patients with *KMT2Ar* AML experienced inferior 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 R/R AML with *KMT2A*r rearrangements. Recently, the US Food and Drug Administration 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

Table 2. Treatment responses after DP-IAG therapy.

Feature	N	%	*P*
Total N of CR/CRi patients	74	73.3	
Sex			0.35
Male	46	76.7	
Female	28	68.3	
Age, years			0.865
<3	11	73.3	
≥3 - <10	40	75.5	
≥10	23	69.7	
WBC at enrollment, x10^9/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	

AML: acute myeloid leukemia; CR: complete remission; CRi: CR with incomplete hematologic recovery; DP-IAG: one course of decitabine priming followed by low-dose idarubicin, cytarabine, and granulocyte colony-stimulating factor; FAB: French-American-British classification system; N: number; WBC: white blood cell. *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 European LeukemiaNet recommendations.[18]

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 11 patients experienced

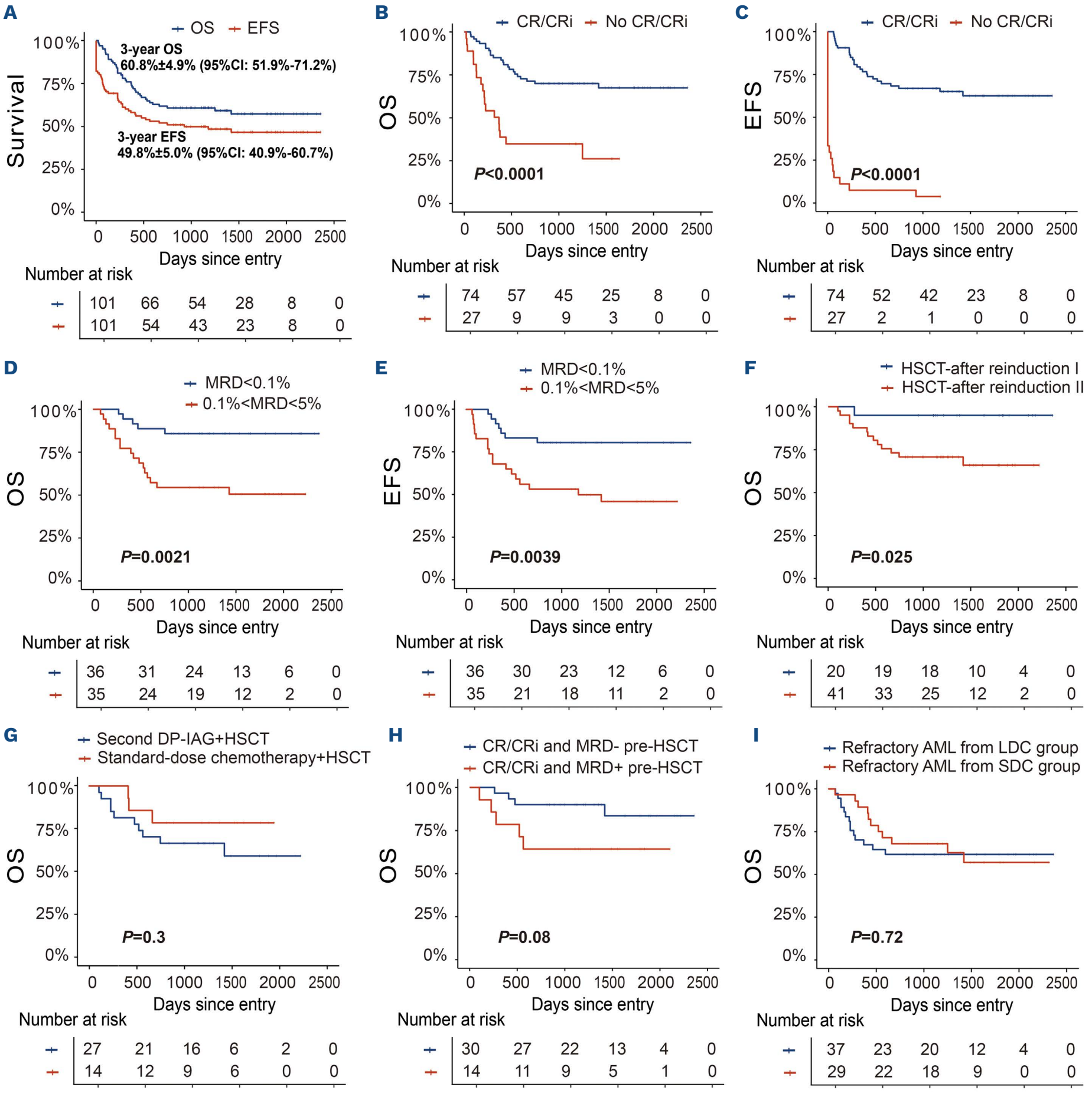


Figure 4. Survival outcomes of enrolled patients. (A) Overall survival (OS) and event-free survival (EFS) of all patients. (B and C) OS and EFS by response status (complete remission [CR]/CR with incomplete hematologic recovery [CRi] *vs*. non-CR/CRi; *P*<0.001 for both). (D and E) OS and EFS by measurable residual disease (MRD) level (<0.1% *vs*. 0.1-5%; *P*=0.0021 and *P*=0.0039). (F) OS in children with acute myeloid leukemia (AML) undergoing hematopoietic stem cell transplantation (HSCT) immediately after first reinduction *versus* after additional chemotherapy (*P*=0.025). (G) OS in children treated with one course of decitabine priming followed by low-dose idarubicin, cytarabine, and granulocyte colony-stimulating factor (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*=008). (I) OS in refractory AML patients initially treated with low-dose *versus* standard-dose chemotherapy (*P*=0.27). CI: Confidence Interval.

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

All adverse events	All Grades	Grade 1/2	Grade 3/4	Grade 5 (death)
	Total patients N (%)	Patients N (%)	Patients N (%)	Patients N (%)
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
Non-hematologic				
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

DP-IAG: one course of decitabine priming followed by low-dose idarubicin, cytarabine, and granulocyte colony-stimulating factor.

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 11 patients with *KIT* exon 17 mutations, 2 died from disease relapse after declining alloHSCT. In comparison, the remaining 9 patients are still alive and disease-free following HSCT. These findings highlight the adverse prognostic impact of *KIT* exon 17 mutations in core binding factor (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 reserve HSCT to patients with suboptimal responses. Outcomes of children with relapsed acute megakaryoblastic leukemia (AMKL) are dismal.[36,37] In our cohort of 6 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 alloHSCT. Early consideration of transplantation after remission may help improve long-term disease control, particularly for patients at high risk of relapse.

Disclosures

No conflicts of interest to disclose.

Contributions

RR and SH designed and directed the study; LF and LG drafted the manuscript and performed all the data analysis; WZ, LY, HL, HW, PX, NL, YZ, XX and JLuo helped interpret the results; YHe, YZ, XH, YHu, JLi, HH and YW helped integrate all the clinical data; XZ, XJ, NW, JLu, HJ, RR and SH helped revise the manuscript; CC assisted with statistical data analysis. All authors read and approved the final manuscript for publication.

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Data-sharing 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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