

Minimally myelosuppressive regimen for remission induction in pediatric AML: long-term results of an observational study

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Key Points

- Most children with de novo AML experience complete hematologic remission when treated with minimally myelosuppressive therapy plus G-CSF.
- Reduced-intensity induction decreases toxicity without adversely affecting the long-term outcomes in children with newly diagnosed AML.

Treatment refusal and death as a result of toxicity account for most treatment failures among children with acute myeloid leukemia (AML) in resource-constrained settings. We recently reported the results of treating children with AML with a combination of low-dose cytarabine and mitoxantrone or omacetaxine mepesuccinate with concurrent granulocyte colony-stimulating factor (G-CSF) (low-dose chemotherapy [LDC]) for remission induction followed by standard postremission strategies. We have now expanded the initial cohort and have provided long-term follow-up. Eighty-three patients with AML were treated with the LDC regimen. During the study period, another 100 children with AML received a standard-dose chemotherapy (SDC) regimen. Complete remission was attained in 88.8% and 86.4% of patients after induction in the LDC and SDC groups, respectively ($P = .436$). Twenty-two patients in the LDC group received SDC for the second induction course. Significantly more high-risk AML patients were treated with the SDC regimen ($P = .035$). There were no significant differences between the LDC and SDC groups in 5-year event-free survival ($61.4\% \pm 8.7\%$ vs $65.2\% \pm 7.4\%$, respectively; $P = .462$), overall survival ($72.7\% \pm 6.9\%$ vs $72.5\% \pm 6.2\%$, respectively; $P = .933$), and incidence of relapse ($20.5\% \pm 4.5\%$ vs $17.6\% \pm 3.9\%$, respectively; $P = .484$). Clearance of mutations based on the average variant allele frequency at complete remission in the LDC and SDC groups was 1.9% vs 0.6% ($P < .001$) after induction I and 0.17% vs 0.078% ($P = .052$) after induction II. In conclusion, our study corroborated the high remission rate reported for children with AML who received at least 1 course of LDC. The results, although preliminary, also suggest that long-term survival of these children is comparable to that of children who receive SDC regimens.

Introduction

Acute myeloid leukemia (AML) comprises a heterogeneous group of disorders accounting for 15% to 20% of childhood leukemia.¹ Genetic classification of AML has enabled risk-adapted therapy for adult and pediatric disease.²⁻⁴ Treatments for most pediatric AML consist of very intensive chemotherapy for

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The sequencing data have been deposited in the Genome Sequence Archive at the BIG Data Center, Beijing Institute of Genomics, Chinese Academy of Sciences, under accession numbers HRA000692 (<https://bigd.big.ac.cn/gsa-human/browse/HRA000692>), HRA000696 (<https://bigd.big.ac.cn/gsa-human/browse/HRA000696>),

HRA000697 (<https://bigd.big.ac.cn/gsa-human/browse/HRA000697>), and HRA000030 (<https://bigd.big.ac.cn/gsa-human/browse/HRA000030>). For other original data, please contact Qian-fei Wang (wangqf@big.ac.cn), CAS Key Laboratory of Genomic and Precision Medicine, Beijing Institute of Genomics, Chinese Academy of Sciences, Beijing, China.

The full-text version of this article contains a data supplement.

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Table 1. Children's Hospital of Soochow University pediatric AML chemotherapy regimens

	Drug	Dose	Schedule	Days
Induction				
LDC	Cytarabine	10 mg/m ²	Every 12 h, subcutaneous	1-10
	Omacetaxine mepesuccinate* or mitoxantrone	1 mg/m ²	Once per day, IV	1-7
SDC		5 mg/m ²	Once per day, IV	1, 3, 5
	G-CSF	5 µg/kg	Once per day, subcutaneous	1-10
	Cytarabine	100 mg/m ²	Every 12 h, IV	1-10
	Etoposide	100 mg/m ²	Once per day, IV	1-5
	Omacetaxine mepesuccinate* or daunorubicin	3 mg/m ²	Once per day, IV	1-7
		50 mg/m ²	Once per day, IV	2, 4, 6
Postremission consolidation				
I	Cytarabine	2 g/m ²	Every 12 h, IV	1-3
	Mitoxantrone	10 mg/m ²	Once per day, IV	3-5
II	Cytarabine	3 g/m ²	Every 12 h, IV	1-3
	Etoposide	150 mg/m ²	Once per day, IV	1-3
III	Cytarabine	3 g/m ²	Every 12 h, IV	1, 2, 8, 9
	L-asparaginase	6000 U/m ²	Once per day, intramuscular	3, 10
IV	Cytarabine	2 g/m ²	Once per day, IV	1-5
	Fludarabine	30 mg/m ²	Once per day, IV	1-5
	G-CSF	300 µg/m ²	Once per day, subcutaneous	0-5

*Mitoxantrone (n = 59) and daunorubicin (n = 81) were substituted for omacetaxine mepesuccinate in the LDC group (n = 24) and in the SDC group (n = 19).

remission induction followed by intensive chemotherapy and/or hematopoietic stem cell transplantation (HSCT).⁵ Progress in supportive care has reduced early death rates and overall treatment-related mortality (TRM).^{6,7} However, concerns have been raised about the late effects of intensive chemotherapy in long-term survivors.⁸ Moreover, despite the increasing intensity of chemotherapy, optimal supportive care, and unlimited access to HSCT, overall survival (OS) has stagnated at approximately 75% in high-income countries.⁹⁻¹² In low- to middle-income countries (LMICs), intensified treatment has not been associated with improved survival rates in patients with pediatric AML.^{13,14} The dismal OS rates for AML in LMICs result from a combination of determinants, including AML biology, deficient supportive care, and therapy abandonment.¹⁵⁻¹⁹

Until recently, approximately half of Chinese families of children with AML did not initiate treatment or abandoned it. Although caregivers of Chinese children with AML are initially encouraged by the cure rates obtained with intensive therapy and HSCT, the financial burden and the potential for early and long-term toxicities are substantial obstacles that may discourage these families from initiating or completing treatment.¹⁹ Because treatment-related complications and treatment abandonment or refusal are more common in the early phases of AML treatment, we reasoned that decreasing the intensity of induction could reduce early treatment complications, increase treatment adherence, and allow families time to address the potential financial burden.²⁰

We recently analyzed the short-term outcomes of 46 children with de novo AML treated with low-dose chemotherapy (LDC).²⁰ The remission rate in children treated with LDC was comparable to that in children treated with standard-dose chemotherapy (SDC) at the same institution. However, the impact on long-term outcomes of

using LDC to induce remission remained uncertain. We have now accrued an additional 37 children with AML treated with LDC, and we focus our analysis on the long-term outcomes of the entire cohort.

Patients and methods

Patients

Children younger than 15 years with de novo AML, as defined by the World Health Organization (WHO) criteria, participated in this observational study. Patients were treated with either LDC or SDC for the first induction course. Allocation to LDC was based on the presence of comorbidities (active infection) or a high risk of treatment abandonment for socioeconomic reasons. Because of concerns that granulocyte colony-stimulating factor (G-CSF) might stimulate leukemia cells, patients who presented with white blood cell (WBC) counts of $\geq 50 \times 10^9/L$ did not receive LDC. Subsequently, because G-CSF proved to be well tolerated and did not increase WBC counts, patients who presented with WBC counts of $\geq 50 \times 10^9/L$ received LDC if their WBC counts decreased to $< 50 \times 10^9/L$ after cytoreductive therapy. Patients with acute promyelocytic leukemia, AML evolving from myelodysplastic syndrome, treatment-related AML, or myeloid neoplasms associated with Down syndrome were not included in the analysis. The study was approved by the ethics committee of the Children's Hospital of Soochow University. Informed consent was obtained from parents or legal guardians.

Response evaluation and risk classification

Treatment response was evaluated on day 26 of each cycle of induction chemotherapy. Complete remission (CR) was defined as

WBC count $\geq 1.0 \times 10^9/L$, absolute neutrophil count $\geq 0.5 \times 10^9/L$, platelet count $\geq 50 \times 10^9/L$, and bone marrow showing $< 5\%$ leukemia cells by morphologic assessment. Partial remission (PR) was defined as $> 5\%$ but $< 20\%$ leukemia cells, and nonresponse (NR) was defined as $\geq 20\%$ leukemia cells in the bone marrow or the presence of extramedullary leukemia. Relapse was defined as $\geq 5\%$ leukemia cells in the bone marrow or evidence of extramedullary leukemia.

AML was provisionally classified into low-, intermediate-, and high-risk categories according to the criteria shown in supplemental Table 1. Definitive AML risk classification required morphologic evaluation of the bone marrow after 2 cycles of induction therapy. Patients who experienced CR retained the same initial risk classification, whereas patients who did not meet the CR criteria were reclassified as high-risk patients (supplemental Table 2).

Genomic analysis and clearance of clonal mutated variants

Bone marrow samples obtained at diagnosis and after induction I and induction II were analyzed by whole-exome sequencing. T cells isolated by flow cytometry from remission bone marrow samples served as a germline control. Genomic DNA was analyzed by standard techniques.²¹⁻²⁵

Treatment plan, supportive care, and toxicity monitoring

Details regarding treatment are provided in Table 1. Candidates for HSCT usually received it after the first consolidation course. LDC recipients who were classified as nonresponders to induction I received the SDC regimen for induction II but were included in the LDC group for analysis.

Patients who presented with WBC counts of $\geq 50 \times 10^9/L$ received cytoreductive therapy before starting remission induction. Options for cytoreductive therapy included cytarabine, hydroxyurea, and leukapheresis. Prophylactic antibiotics were not used, and fluconazole was used prophylactically when the absolute neutrophil count was $< 0.5 \times 10^9/L$. Measures for managing febrile neutropenia were based on the guidelines of the Infectious Diseases Society of America and the European Conference on Infections in Leukemia.^{26,27} Treatment-related toxicity and other adverse events were evaluated after each treatment course and were graded using Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

Definitions

Events were refractory disease, leukemia relapse, death as a result of any cause, abandonment of treatment, or secondary malignancy. Surviving patients who did not experience CR after induction II were considered to have refractory disease. Abandonment was defined as refusal to continue treatment before finishing at least 2 consolidation courses and was considered an event. Patients were followed until death or until the date of last contact. The database as of January 2020 was used for analysis.

Statistical analysis

OS and event-free survival (EFS) were calculated from the date of diagnosis. Cumulative incidence of relapse (CIR) was estimated by the Kalbfleisch-Prentice method, accounting for competing risk.

Table 2. Baseline characteristics of patients in the LDC and SDC cohorts

Characteristic	LDC*	SDC	Total	P†
Sex	83	100	183	.355
Male	45 (54.2)	61 (61.0)	106 (57.9)	
Female	38 (45.8)	39 (39.0)	77 (42.1)	
Age, y	83	100	183	.424
Median	6.1	7.5	6.8	
Range	0.8-14.2	0.3-13.8	0.3-14.2	
WBC count, $\times 10^9/L$	83	100	183	.001
Median	12.48	27.67	17.9	
Range	1.33-283.41	0.42-606	0.42-606	
FAB subtype	83	100	183	.058
M1/M2	38 (45.8)	43 (43.0)	81 (44.3)	
M4/M5	31 (37.4)	49 (49.0)	80 (43.7)	
M6	0	1 (1.0)	1 (0.5)	
M7	7 (8.4)	1 (1.0)	8 (4.4)	
Unclassified	7 (8.4)	6 (6.0)	13 (7.1)	
Gene rearrangements	83	100	183	.432
<i>RUNX1-RUNX1T1</i>	33 (39.8)	35 (35.0)	68 (37.1)	
<i>KMT2Ar</i>	11 (13.2)	14 (14.0)	25 (13.7)	
<i>CBF/MYH11</i>	6 (7.2)	15 (15.0)	21 (11.5)	
<i>BCR-ABL</i>	0	1 (1.0)	1 (0.5)	
Negative	33 (39.8)	35 (35.0)	68 (37.2)	
Gene mutations	76	92	168	.168
<i>C-KIT</i>	19 (25.0)	23 (25.0)	42 (25.0)	
<i>CEBPα</i> biallelic mutation	6(7.9)	3(3.3)	9(5.4)	
<i>FLT3-ITD</i>	4(5.3)	12(13.0)	16(9.5)	
<i>WT1</i>	4(5.3)	0	4(2.4)	
<i>NPM1</i>	2 (2.6)	1(1.1)	3 (1.8)	
<i>PTPN11</i>	2 (2.6)	2 (2.2)	4 (2.4)	
Other	6(7.9)	6(6.5)	12(7.1)	
Negative	33 (43.4)	45 (48.9)	78 (46.4)	
Provisional risk group	83	100	183	.035
Low	10 (12.0)	15 (15.0)	25 (13.7)	
Intermediate	56 (67.5)	49 (49.0)	105 (57.3)	
High	17 (20.5)	36 (36.0)	53 (29.0)	
Definitive risk group	77	95	172	.016
Low	10 (13.0)	15 (15.8)	25 (14.5)	
Intermediate	51 (66.2)	43 (45.3)	94 (54.7)	
High	16 (20.8)	37 (38.9)	53 (30.8)	

Data are presented as n (%), unless otherwise specified.

NR, no remission.

*Selected data on 46 study patients have been previously reported.²⁰

†The Mann-Whitney *U* test was used for continuous variables; Fisher's exact test was used for categorical variables with 2×2 charts; Monte Carlo analysis was used for categorical variables with $N \times 2$ charts.

Gray's test and the Fine-Gray regression model were applied to compare the CIRs in both groups. Fisher's exact test and Monte Carlo analysis were used to compare categorical variables between 2 or more groups. The Mann-Whitney *U* test and Kruskal-Wallis *H*

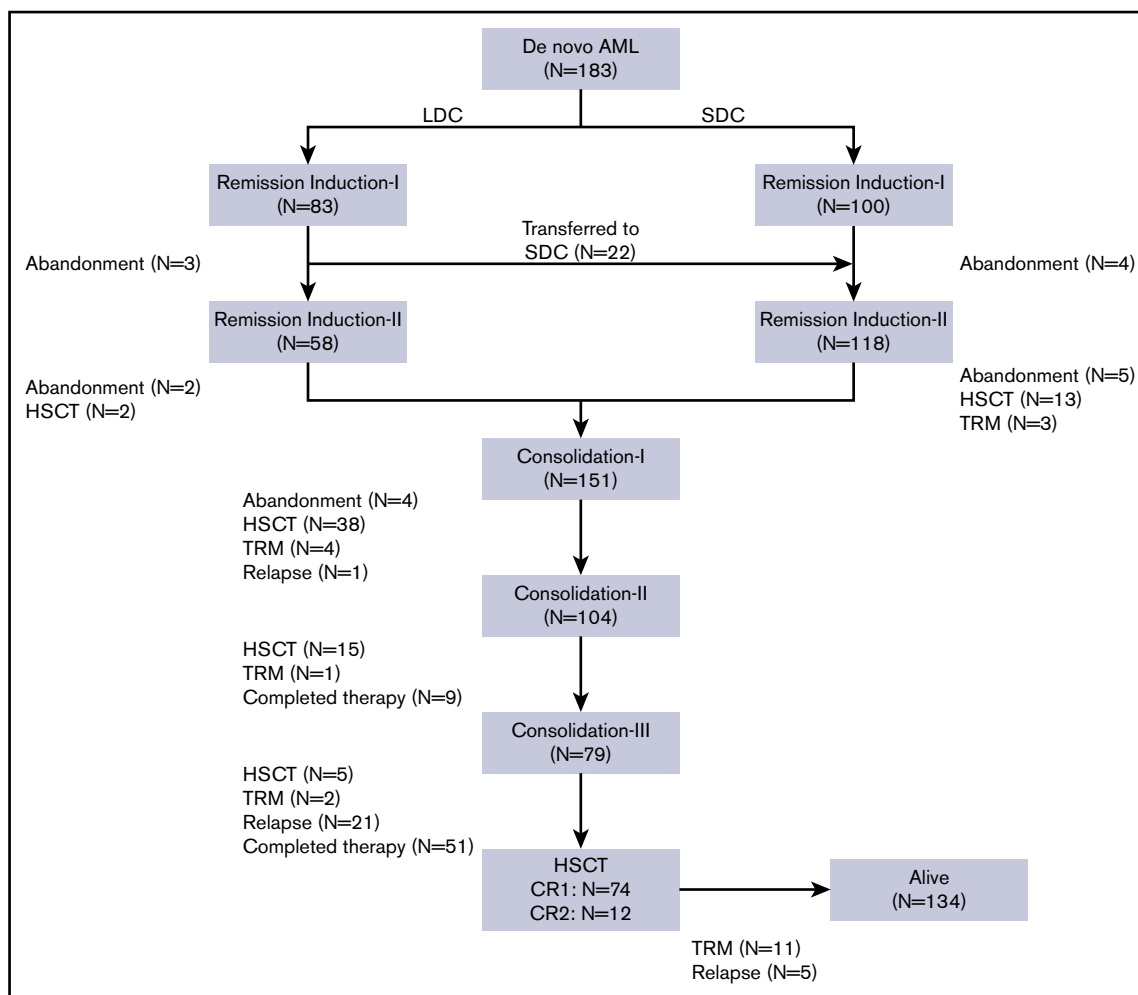


Figure 1. Flowchart of the study.

test were used to compare continuous variables between 2 or 3 groups. The risk group was used in survival analyses as a stratification factor or covariate. Kaplan-Meier analysis was used to estimate survival functions. The stratified log-rank test and the Cox regression model were applied to compare OS and EFS in the LDC and SDC groups. In light of the strong relation and overlap between the major outcomes (OS, EFS, and CIR), variables with $P < .1$ in univariable analysis were examined by the multivariable Cox regression method. All tests were two-sided. P values of $< .05$ were considered to indicate significance.

Results

Patient characteristics

From July 2012 to March 2018, 183 children (age younger than 15 years) with de novo AML were treated consecutively at the Children's Hospital of Soochow University. Of these, 83 patients (45 males, 38 females) received the LDC regimen. The median age of the 83 patients was 6.1 years (range, 0.8-14.2 years). Median WBC count at diagnosis was $12.48 \times 10^9/L$ (range, $1.33 \times 10^9/L$ to $283.41 \times 10^9/L$). Nine patients who presented with WBC counts of $>50 \times 10^9/L$ received cytarabine alone as cytoreductive therapy before starting an LDC plus G-CSF regimen.

According to the French-American-British (FAB) classification, the M1/M2 subtype was observed in 38 patients (45.8%), the M4/M5 subtype in 31 patients (37.4%), and the M7 subtype in 7 patients (8.4%). Seven patients (8.4%) were not classifiable. Gene rearrangement analysis showed *RUNX1-RUNX1T1* to be present in 33 (39.8%), *KMT2Ar* in 11 (13.2%), and *CBF/MYH11* in 6 (7.2%) of the 83 patients. Molecular analysis revealed *C-KIT* mutations in 19 (25%), *CEBP α* biallelic mutations in 6 (7.9%), *FLT3*-internal tandem duplication (*FLT3-ITD*) in 4 (5.3%), *WT1* in 4 (5.3%), *NPM1* in 2 (2.6%), and *PTPN11* in 2 (2.6%) patients. In the initial risk classification, 10 patients (12.0%) were considered low risk, 56 (67.5%) intermediate risk, and 17 (20.5%) high risk. During the study, another 100 patients were treated with an SDC regimen (Table 2). Compared with the LDC recipients, patients allocated to the SDC group had significantly higher WBC counts at presentation and more patients were classified as having high-risk AML (Table 2).

Induction response and evaluation

All 83 patients receiving LDC were evaluable for response (Figure 1). Seventy (84.4%) had a hematologic response: 58 (69.9%) experienced CR and 12 (14.5%) experienced PR. No deaths as a result of toxicity were observed during this treatment phase. The remaining 13 patients (15.6%) were considered to be

nonresponders (Table 3). Eighty patients received induction II, 58 with the same LDC regimen and 22 with the SDC combination. The remaining 3 patients abandoned treatment before induction II. Of the 22 patients who received SDC for induction II, 13 were refractory to induction I and 9 received SDC because of physician preference. Of the 80 patients evaluable for response to induction II, 71 (88.8%) showed CR, 6 had PR or NR, and 3 abandoned treatment before bone marrow evaluation. Two patients who received SDC for induction II died as a result of TRM. In the final risk assessment after induction II evaluation, 10 patients (13.0%) were classified as having low-risk, 51 patients (66.2%) were classified as having intermediate-risk, and 16 patients (20.8%) were classified as having high-risk AML (Table 3). There was no substantial difference between the initial and definitive risk classifications; only 2 patients in the LDC group who were classified initially as having intermediate risk moved to the high-risk category.

For comparison purposes, the response evaluation data for 100 patients who received SDC for induction are presented in Table 3. There was no significant difference in CR, PR, or NR among patients treated with the LDC or SDC regimen. Because of a shortage of omacetaxine mepesuccinate in China in 2016, mitoxantrone and daunorubicin were substituted for omacetaxine mepesuccinate for induction I and II in the LDC (n = 59) and SDC groups (n = 81), respectively. There were no differences in CR rates among patients who received omacetaxine mepesuccinate or mitoxantrone for induction chemotherapy ($P = .316$).

Clearance of somatic mutations after inductions I and II

We analyzed 208 bone marrow samples from 59 patients (32 treated with 2 courses of LDC and 27 treated with 2 courses of SDC) by whole-exome sequencing (supplemental Table 3). These patients had experienced hematologic remission after induction I and had samples available. In total, 439 somatic mutations of 362 genes were detected at diagnosis: 275 in the LDC group and 164 in the SDC group. The average number of mutations per patient in the LDC group was 8.6 (range, 3-17 mutations) and 6.1 (range, 1-15 mutations) in SDC. The average variant allele frequency (VAF) at diagnosis was 29.1% (range, 5.0%-84.7%) for the LDC group and 29.1% (range, 5.1%-94.7%) for the SDC group ($P = .996$). After induction I, the average VAF decreased to 1.9% for the LDC group and 0.6% for the SDC group ($P < .001$), and after induction II, the average VAF decreased to 0.17% for the LDC group and 0.078% for the SDC group ($P = .052$) (Figure 2A-B).

We also analyzed the clearance of specific nonrandom AML-associated mutations.²⁸⁻³¹ Nine of the most frequently mutated genes in pediatric AML² were detected recurrently in both the LDC and SDC groups (Figure 2C-D). A total of 27 nonrandom AML-associated mutations were detected in the 22 patients in the LDC group, and 26 such mutations were detected in the 18 patients in the SDC group, but all of them were cleared (VAF <2.5%) after induction II. Notably, some of these mutations were undetectable immediately after induction I in both groups (Figure 2C-D).

Postremission therapy

Because the 2 groups had similar treatment responses after induction chemotherapy and because the postremission approach was the same for all patients, we grouped them together for the

Table 3. Response evaluation

	LDC*	SDC	Total	P†
First induction	83	100	183	.811
CR	58 (69.9)	67 (67.0)	125 (68.3)	
PR	12 (14.5)	18 (18.0)	30 (16.4)	
NR	13 (15.6)	15 (15.0)	28 (15.3)	
Second induction	80	96	176	.436
CR	71 (88.8)	83 (86.4)	154 (87.5)	
PR	4 (5.0)	6 (6.3)	10 (5.7)	
NR	2 (2.5)	6 (6.3)	8 (4.5)	
Unavailable‡	3 (3.7)	1 (1.0)	4 (2.3)	
Treatment-related deaths	1§	2	3	1.000
Induction I	0	0	0	
Induction II	1 (1.2)	2 (2.0)	3 (1.6)	
Abandonment	7	7	14	
After induction I	3 (3.6)	4 (4.0)	7 (3.8)	.912
After induction II	4 (4.8)	3 (3.0)	7 (3.8)	

All data are presented as n (%).

*Twenty-two patients received 1 course of LDC for induction I and SDC for induction II.

†Monte Carlo analysis was used for categorical variables with N×3 charts.

‡Two patients abandoned treatment and 1 patient in the LDC/SDC group died of treatment-related toxicity before evaluation of remission induction II; 1 patient died of treatment-related toxicity in the SDC group before evaluation of remission induction II.

§Patient received LDC for induction I and SDC for induction II.

postremission analysis. Of the 176 patients who completed 2 induction courses, 9 were transferred to another hospital for HSCT, 6 underwent HSCT at our center, and 151 received consolidation I (Figure 1; Table 3). Of these 151 patients, 38 underwent HSCT, 4 died as a result of TRM, and 4 abandoned treatment. Of the 104 patients who received consolidation II, 15 underwent HSCT, 1 died as a result of TRM, and 9 completed the planned therapy. Of the 79 patients who received consolidation III, 5 underwent HSCT, 2 died as a result of toxicity, and 72 completed therapy. No patient who received LDC for both induction cycles experienced TRM during induction or consolidation (Table 3).

The overall relapse rate was 18.0%, with 28 (84.8%) of 33 relapses occurring before HSCT. There were 17 relapses in the LDC group (representing 20.5% of the 83 patients in that group) and 16 relapses in the SDC group (representing 16.0% of the 100 patients in that group) ($P = .447$). We analyzed relapse-free survival according to definitive risk classification and treatment group. There was no significant difference in relapse rates between the 2 groups, irrespective of the risk classification (supplemental Figure 1). Sixteen of the 33 patients who relapsed underwent salvage therapy and HSCT; the other 17 refused further therapy.

Eighty-six (47.0%) of the 183 patients underwent HSCT: 74 in first CR and 12 in second CR. There was no significant difference in the number of patients who received HSCT according to treatment group: 38 (45.8%) of the 83 patients in the LDC group received HSCT vs 48 (48.0%) of the 100 patients in the SDC group ($P = .758$) (Table 3). Overall, 11.5% of patients experienced TRM, and 16.4% of deaths were attributed to the disease. Abandonment occurred in 9.8% of the patients and was more common during the remission induction phase.

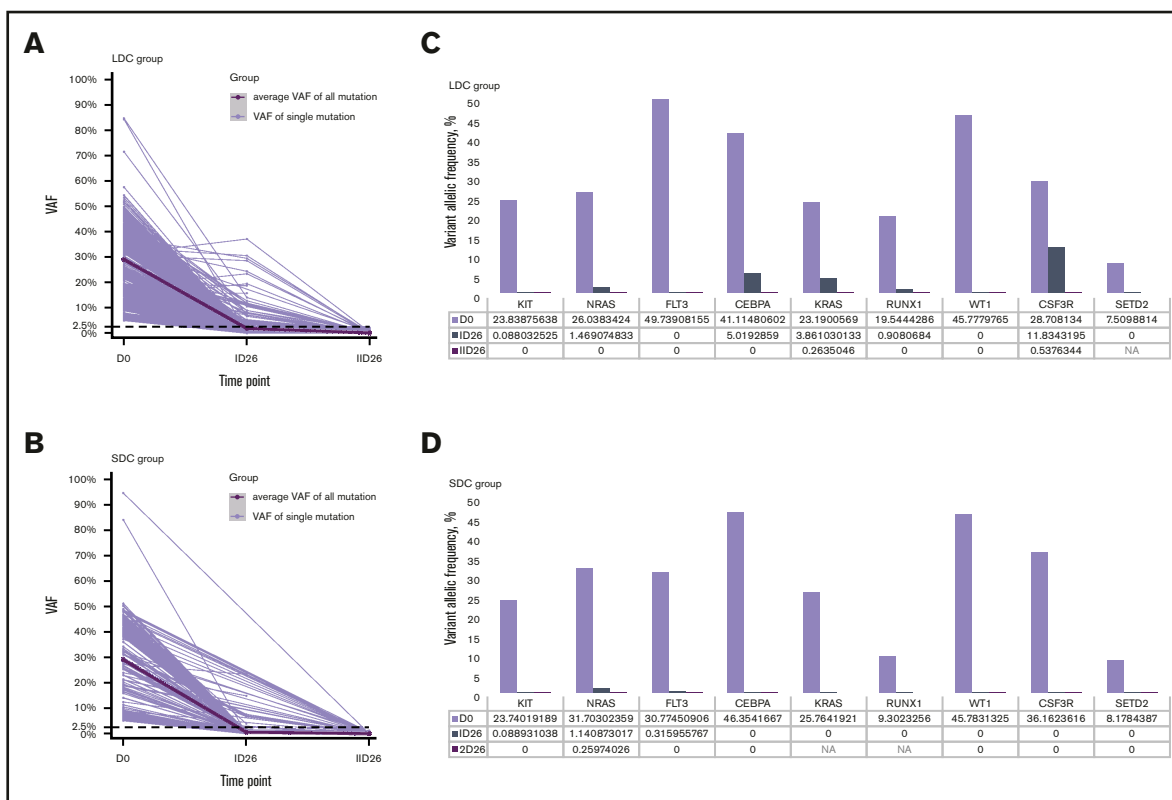


Figure 2. Relative change in VAFs of somatic mutations. (A-B) The change in VAFs of somatic mutations detected at diagnosis and after induction I and induction II in patients who were treated with LDC (A) or SDC (B). The thick black line shows the average VAF of all detected mutations; gray lines show the VAF of each single detected mutation. (C-D) Relative change in VAFs of the specific nonrandom AML-associated mutations at diagnosis and after induction I and induction II in the LDC (C) and SDC (D) groups. Nonrandom AML-associated genes were selected from the top 50 mutated AML-associated genes in pediatric AML and were detected recurrently in both groups. DO, at diagnosis; ID26, after induction I; IID26, after induction II; NA, not available.

Survival and prognostic factors

With a median follow-up of 50 months (range, 4-85 months), the 5-year probabilities of EFS, OS, and CIR for the 183 children were $63.1\% \pm 5.8\%$, $72.3\% \pm 4.7\%$, and $19.3\% \pm 3.0\%$, respectively (Figure 3A). Comparing the LDC and SDC groups, the 5-year probabilities of OS, EFS, and CIR were $72.7\% \pm 6.9\%$ vs $72.5\% \pm 6.2\%$ (Figure 3B), $61.4\% \pm 8.7\%$ vs $65.2\% \pm 7.4\%$, and $20.5\% \pm 4.5\%$ vs $17.6\% \pm 3.9\%$, respectively (Figure 3C). Because 22 patients allocated to the LDC group received the standard regimen for induction II, we performed an analysis taking into account the induction regimen actually received. For patients treated for induction with the LDC/LDC, LDC/SDC, or SDC/SDC regimens, there were no significant differences in 5-year OS ($72.5\% \pm 8.1\%$, $72.7\% \pm 13.1\%$, and $72.5\% \pm 6.2\%$, respectively; $P = .993$) (Figure 3D), EFS ($62.3\% \pm 1.0\%$, $59.1\% \pm 17.7\%$, and $65.2\% \pm 7.4\%$, respectively; $P = .701$), or CIR ($23.0\% \pm 5.4\%$, $13.6\% \pm 7.5\%$, and $17.6\% \pm 3.9\%$, respectively; $P = .502$). To explore the impact of initial WBC count on outcome, we analyzed the survival in patients with WBC counts of $<100 \times 10^9/L$ and found no significant difference between the LDC and SDC groups in terms of OS ($73.3\% \pm 5.1\%$ vs $79.7\% \pm 4.7\%$, respectively; $P = .332$) or EFS ($61.0\% \pm 5.6\%$ vs $70.0\% \pm 5.4\%$, respectively; $P = .194$).

In a univariable analysis, WBC count, FAB subtype, definitive risk classification, and HSCT were associated with OS. FAB subtype,

definitive risk classification, and treatment group were associated with EFS (supplemental Table 4). Multivariable regression analysis for the entire cohort showed that induction treatment had no significant impact on OS, EFS, or CIR. HSCT was significantly associated with OS (hazard ratio, 0.390; $P = .007$). Definitive risk classification was also an independent predictor for OS and EFS (Table 4). Because significantly more SDC recipients than LDC recipients had WBC counts of $\geq 100 \times 10^9/L$ (25 vs 6 recipients; $P = .01$), we analyzed the SDC group without these patients. Cox regression analysis revealed that chemotherapy intensity during induction had no significant association with survival in patients with WBC counts of $<100 \times 10^9/L$. Risk classification and HSCT remained significantly associated with outcome in the cohort with WBC counts of $<100 \times 10^9/L$ (supplemental Table 5). We also analyzed outcomes according to definitive risk classification and treatment group. There was no significant difference in survival between the 2 groups, irrespective of the risk classification (supplemental Figure 1).

Selected toxicities and treatment cost

Supplemental Table 6 shows selected treatment-related complications among patients receiving the LDC or SDC regimen. Patients receiving LDC for induction I had a significantly shorter period of neutrophil and platelet recovery than did SDC recipients (median, 12 vs 20 days [$P < .001$] and 11 vs 16 days [$P < .001$],

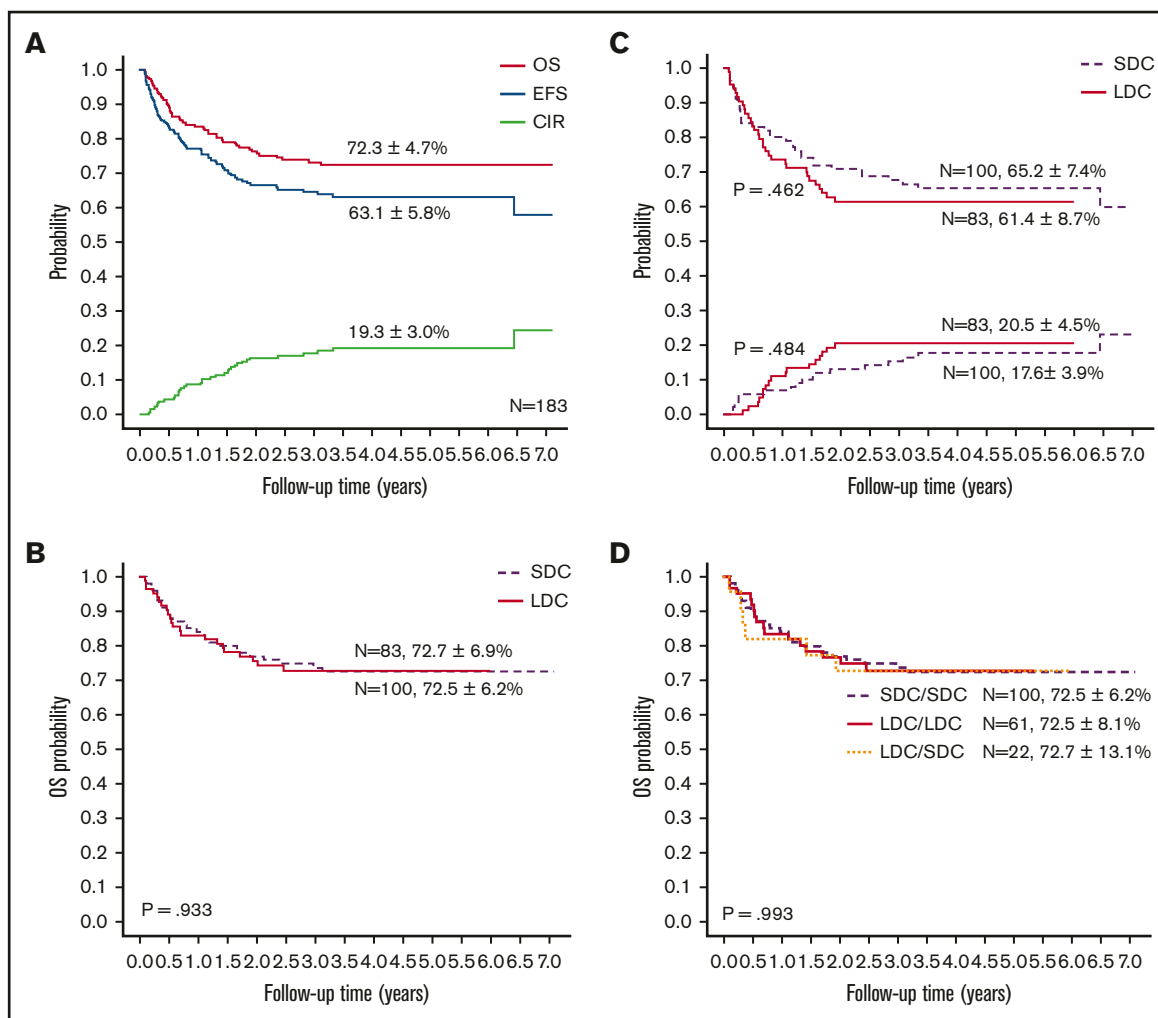


Figure 3. Outcomes of study patients according to treatment. (A) OS, EFS, and CIR for all study patients. OS (B) and EFS (upper graph lines) and CIR (lower graph lines) (C) according to treatment group. (D) OS according to the actual treatment received in induction.

respectively). Of the SDC recipients, 91% had grade 3 or 4 febrile neutropenia compared with 61.4% in the LDC group ($P < .001$). Similarly, patients receiving LDC for induction II had a shorter period of neutropenia and thrombocytopenia, fewer episodes of fever and neutropenia, and fewer episodes of grade 3 or 4 pneumonia and perianal abscess when compared with LDC recipients.

The median number of packed red blood cell and platelet units transfused was significantly lower for the LDC group than for the SDC group for induction I and II (supplemental Table 7). The overall cost of treatment for induction I and induction II, including blood products, antibiotics, and other expenses, was significantly higher for the SDC group than for the LDC group (\$7256.50 vs \$6278.90; $P = .01$).

Discussion

Our study expands the evidence that ~70% of children with de novo AML experience CR after a single course of the LDC regimen.²⁰ After receiving a second induction course with the same low-intensity regimen or with a standard induction regimen, ~90% of the patients experienced CR. The CR rates in this

cohort were comparable to those in children with AML treated with 2 courses of a conventional induction regimen at the same institution or at institutions participating in international studies of pediatric AML.^{1,11}

Minimally myelosuppressive regimens have been used in adults with relapsed AML or in those with de novo AML who were too frail to receive standard induction therapy.³²⁻³⁵ A combination of low-dose cytarabine with low-dose aclarubicin administered concurrently with G-CSF was first used in Japan.³⁶ A variation of this regimen that substitutes omacetaxine mepesuccinate for aclarubicin has been used extensively in China^{37,38} and with different anthracyclines in other regions.³⁹⁻⁴¹ The mechanisms underlying the efficacy of LDC plus G-CSF remain elusive.⁴²⁻⁴⁷

Studies in elderly patients with AML have shown surprisingly high rates of CR and low treatment-related toxicity.^{32,37,48,49} After experiencing CR, patients were usually treated with reduced-intensity consolidation. As expected, relapse was noted in most elderly patients within 8 to 12 months.⁵⁰ The low-dose cytarabine and low-dose anthracycline plus G-CSF regimens were effective at inducing remission and were well tolerated in elderly patients

Table 4. Multivariable Cox regression of OS, EFS, and CIR in 183 patients

Factor	OS			EFS			CIR		
	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
WBC × 10⁹/L									
<100	Reference			Reference			Reference		
≥100	1.483	0.691-3.186	.312	1.052	0.515-2.148	.889	0.758	0.305-1.883	.550
FAB subtype									
M1/M2	Reference			Reference			Reference		
M4/M5	0.994	0.411-2.406	.990	0.932	0.465-1.867	.842	2.008	0.844-4.777	.115
Other	2.118	0.800-5.612	.131	1.586	0.715-3.519	.256	1.158	0.354-3.793	.808
Definitive risk									
Low	Reference			Reference			Reference		
Intermediate	2.156	0.483-9.631	.314	2.566	0.765-8.609	.127	2.286	0.484-10.805	.297
High	7.831	1.629-37.637	.010	6.201	1.689-22.769	.006	3.900	0.769-19.783	.100
HSCT									
No	Reference			Reference			Reference		
Yes	0.390	0.197-0.769	.007	0.693	0.397-1.212	.198	1.054	0.514-2.162	.886
Induction treatment									
SDC/SDC	Reference			Reference		—	Reference		
LDC/LDC	1.387	0.674-2.857	.375	1.439	0.788-2.628	.236	1.630	0.801-3.317	.178
LDC/SDC	1.141	0.368-3.542	.819	1.115	0.437-2.843	.820	0.738	0.214-2.545	.630

HR, hazard ratio.

with AML. Because there was no evidence that G-CSF promoted leukemia growth, we hypothesized that children with AML who were at increased risk of death as a result of toxicity or treatment abandonment for socioeconomic reasons would benefit from this approach, which would allow time to manage the initial clinical complications and address the socioeconomic needs of the patients' families. However, in contrast to the postremission strategy used with elderly patients, we used a risk-adapted postremission approach that included cycles of conventional intensive chemotherapy and HSCT when indicated. The 5-year OS was 72% for patients treated with 2 courses of LDC in induction and with adapted-risk intensive postremission therapy and HSCT in their first or second CR. This rate was not significantly different from that of patients who received conventional induction therapy and risk-adapted postremission treatment at the same institution. The OS rates for our low-dose cohort were also comparable to those reported for recent international clinical trials in the field of pediatric AML.⁹⁻¹¹

The advantages of the LDC regimen over SDC include the shorter period of neutropenia and thrombocytopenia and the lower frequency of severe (grade 3 or 4) episodes of febrile neutropenia, mucositis, pneumonia, bleeding, and other infectious processes. Consistent with these findings, the use of blood products was significantly lower in patients who received the LDC regimen for both induction I and II. Finally, the overall cost of the 2 induction courses for LDC recipients was significantly lower than that for SDC recipients. Interestingly, the cost of induction I did not differ between the 2 cohorts, possibly reflecting the preferential inclusion of patients with comorbidities at presentation of AML in the LDC group. Therefore, children with AML who present

with infectious or nutritional comorbidities require intensive supportive care (supplemental Table 8) that includes prolonged hospitalization irrespective of the treatment intensity during remission induction. Finally, exposure to anthracyclines is lower among patients treated with LDC. However, whether a lower cumulative dose of anthracyclines in remission induction is associated with less short- and long-term cardiotoxicity remains elusive.

To gain insights into the quality of response after induction, we compared the clearance of somatic mutations in a subset of patients who had bone marrow samples available for testing and who received 2 induction courses of LDC or 2 courses of SDC. VAF <2.5% has been used to indicate response in AML, corresponding to 5% mutated cells, assuming the mutations occur heterozygously.^{28,29} After induction I, the average VAF was significantly higher for the LDC recipients than for the SDC recipients. After induction II, there was no significant difference in the average VAF for the 2 groups. We also examined the clearance of nonrandom AML-associated mutations^{29-31,51} by treatment regimen and by using a criterion of VAF <1%.³⁰ The VAFs of all genes selected were <1% after the second induction course, irrespective of the treatment arm.

Together, these observations suggest that in most cases of pediatric AML, the leukemia cell burden at diagnosis is very sensitive to chemotherapy and can be substantially reduced by low myelosuppressive approaches to a degree that permits robust reconstitution of the normal hematopoietic system. However, on the basis of adult AML data, minimally myelosuppressive regimens do not eradicate AML^{52,53}; hence, postremission intense myelosuppressive therapy or HSCT is necessary for cure. A critical question

not yet addressed by randomized studies is whether the initial intensity of induction chemotherapy influences the CIR. Indirect evidence that the intensity of induction might not be critical for eradicating AML comes from studies showing that increasing the intensity and complexity of remission induction regimens has not changed the ~50% relapse rates in patients who do not undergo HSCT.^{10,54} The definitive answer to this question is even more relevant for patients in low-resource settings in which supportive care may be deficient and in which families cannot afford the treatment cost. In these situations, TRM or abandonment rates during induction are very high. Finally, for any child with AML, the intensity of current regimens is associated with severe toxicity that may have long-term deleterious effects on growth, development, and organ integrity.^{8,55}

One limitation of our observational study was the exclusion of patients who presented with WBC counts of $\geq 50 \times 10^9/L$ from the LDC group in the initial phase of the study. We were concerned that G-CSF could cause leukocytosis or leukostasis in children with high WBC counts at presentation. If the study progressed and there was no evidence that G-CSF was promoting leukocytosis, patients with WBC counts of $> 50 \times 10^9/L$ at diagnosis received the LDC regimen after treatment with cytarabine alone for cytoreduction. Another limitation of our study was the lack of a randomized comparison group. For comparison, we used published data from international collaborative groups and from a cohort of patients who were admitted to the same institution during the study period. The clinical and biological characteristics of our 2 cohorts were similar except for the significantly higher WBC count in the SDC group. We compared the outcomes of patients with WBC counts of $< 100 \times 10^9/L$ who were treated with the SDC regimen with those of LDC recipients. The outcome data did not differ between the 2 cohorts. A randomized study of LDC plus G-CSF vs SDC for untreated children with AML is ongoing. Finally, pediatric oncologists in LMICs must determine the strengths and weaknesses of their centers and adapt the treatment accordingly. The International Society of Pediatric Oncology has recently published guidelines on adapting AML therapy in LMICs.⁵⁶ The impact of the low-intensity therapy for AML in places with limited supportive care is currently not known. It is important to consider that even a minimally myelosuppressive regimen for induction remission of AML may be associated with prolonged neutropenia. Moreover, improved outcomes require intensive postremission chemotherapy or HSCT for high-risk AML subtypes.

In conclusion, the results of this study suggest that the long-term survival rates of children who receive 1 or 2 courses of the low-intensity therapy regimen followed by risk-adapted standard postremission therapy that includes access to HSCT are comparable to those reported in international AML studies. Further investigation is

necessary to determine the role of this approach in countries with limited resources.

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Authorship

Contribution: S.H., R.C.R., and Q.-f.W. were the principal investigators of this study and take responsibility for the integrity of data and the accuracy of data analysis; Y.H., A.C., R.C.R., S.H., and Q.-f.W. designed the study and wrote the manuscript; A.C., S.J., X.Z., and Q.-f.W. designed the mutation clonal detection and performed sequencing; Jianqin Li, J. Ling, J.F., Y.Y., and J.P. provided study materials or samples; Y.H., A.C., S.J., and X.Z. helped collect samples and analyze sequencing data; Y.H., F.F., and C.C. performed the statistical analyses; Y.H., A.C., and R.C.R. prepared the figures and tables; Y.H., L.G., L.F., and S.C. collected clinical data; H.H., P.X., J. Lu, Y.W., and Jie Li provided advice on clinical study design and participated in clinical data collection; and all authors reviewed and approved the submitted manuscript.

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References

1. Elgarten CW, Aplenc R. Pediatric acute myeloid leukemia: updates on biology, risk stratification, and therapy. *Curr Opin Pediatr*. 2020;32(1):57-66.
2. Bolouri H, Farrar JE, Triche T Jr., et al. The molecular landscape of pediatric acute myeloid leukemia reveals recurrent structural alterations and age-specific mutational interactions. *Nat Med*. 2018;24(1):103-112.
3. Bill M, Nicolet D, Kohlschmidt J, et al. Mutations associated with a 17-gene leukemia stem cell score and the score's prognostic relevance in the context of the European LeukemiaNet classification of acute myeloid leukemia. *Haematologica*. 2020;105(3):721-729.
4. Estey EH. Acute myeloid leukemia: 2019 update on risk-stratification and management. *Am J Hematol*. 2018;93(10):1267-1291.

5. Klein K, de Haas V, Kaspers GJL. Clinical challenges in de novo pediatric acute myeloid leukemia. *Expert Rev Anticancer Ther.* 2018;18(3):277-293.
6. Creutzig U, Zimmermann M, Reinhardt D, Dworzak M, Stary J, Lehrnbecher T. Early deaths and treatment-related mortality in children undergoing therapy for acute myeloid leukemia: analysis of the multicenter clinical trials AML-BFM 93 and AML-BFM 98. *J Clin Oncol.* 2004;22(21):4384-4393.
7. Klein K, van Litsenburg RRL, de Haas V, et al. Causes of early death and treatment-related death in newly diagnosed pediatric acute myeloid leukemia: Recent experiences of the Dutch Childhood Oncology Group. *Pediatr Blood Cancer.* 2020;67(4):e28099.
8. Chow EJ, Ness KK, Armstrong GT, et al. Current and coming challenges in the management of the survivorship population. *Semin Oncol.* 2020;47(1):23-39.
9. Rasche M, Zimmermann M, Borschel L, et al. Successes and challenges in the treatment of pediatric acute myeloid leukemia: a retrospective analysis of the AML-BFM trials from 1987 to 2012. *Leukemia.* 2018;32(10):2167-2177.
10. Reedijk AMJ, Klein K, Coebergh JWW, et al. Improved survival for children and young adolescents with acute myeloid leukemia: a Dutch study on incidence, survival and mortality. *Leukemia.* 2019;33(6):1349-1359.
11. Rubnitz JE, Lacayo NJ, Inaba H, et al. Clofarabine can replace anthracyclines and etoposide in remission induction therapy for childhood acute myeloid leukemia: the AML08 multicenter, randomized phase III trial. *J Clin Oncol.* 2019;37(23):2072-2081.
12. Rafiee R, Chauhan L, Alonzo TA, et al. ABCB1 SNP predicts outcome in patients with acute myeloid leukemia treated with gemtuzumab ozogamicin: a report from Children's Oncology Group AAML0531 Trial. *Blood Cancer J.* 2019;9(6):51.
13. Lins MM, Mello MJG, Ribeiro RC, De Camargo B, de Fátima Pessoa Militão de Albuquerque M, Thuler LCS. Survival and risk factors for mortality in pediatric patients with acute myeloid leukemia in a single reference center in low-middle-income country. *Ann Hematol.* 2019;98(6):1403-1411.
14. Kulkarni KP, Marwaha RK. Childhood acute myeloid leukemia: an Indian perspective. *Pediatr Hematol Oncol.* 2011;28(4):257-268.
15. Gupta S, Bonilla M, Valverde P, et al. Treatment-related mortality in children with acute myeloid leukaemia in Central America: incidence, timing and predictors. *Eur J Cancer.* 2012;48(9):1363-1369.
16. Hafez HA, Solieman RM, Bilal D, Hashem M, Shalaby LM. Early deaths in pediatric acute leukemia: a major challenge in developing countries. *J Pediatr Hematol Oncol.* 2019;41(4):261-266.
17. Begum M, Islam A, Rahman AA, Akter M, Alam ST, Tasmeen R. Abandonment and outcome of childhood acute myeloid leukemia in a tertiary level hospital. *Mymensingh Med J.* 2018;27(1):95-102.
18. Huang LB, Guan XQ, Zhang YC, Zhang XL, Ke ZY, Luo XQ. Current status of diagnosis and prognosis of infant acute leukemia in China. *Pediatr Blood Cancer.* 2009;53(6):973-977.
19. Hong D, Zhou C, He H, Wang Y, Lu J, Hu S. A 10-year follow-up survey of treatment abandonment of children with acute myeloid leukemia in Suzhou, China. *J Pediatr Hematol Oncol.* 2016;38(6):437-442.
20. Hu Y, Chen A, Zheng X, et al. Ecological principle meets cancer treatment: treating children with acute myeloid leukemia with low-dose chemotherapy. *Natl Sci Rev.* 2019;6(3):469-479.
21. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics.* 2009;25(14):1754-1760.
22. McKenna A, Hanna M, Banks E, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res.* 2010;20(9):1297-1303.
23. Cibulskis K, Lawrence MS, Carter SL, et al. Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. *Nat Biotechnol.* 2013;31(3):213-219.
24. Larson DE, Harris CC, Chen K, et al. SomaticSniper: identification of somatic point mutations in whole genome sequencing data. *Bioinformatics.* 2012;28(3):311-317.
25. Koboldt DC, Zhang Q, Larson DE, et al. VarScan 2: somatic mutation and copy number alteration discovery in cancer by exome sequencing. *Genome Res.* 2012;22(3):568-576.
26. Freifeld AG, Bow EJ, Sepkowitz KA, et al; Infectious Diseases Society of America. Clinical practice guideline for the use of antimicrobial agents in neutropenic patients with cancer: 2010 update by the Infectious Diseases Society of America. *Clin Infect Dis.* 2011;52(4):e56-e93.
27. Averbuch D, Orasch C, Cordonnier C, et al; ECIL4, a joint venture of EBMT, EORTC, ICHS, ESGICH/ESCMID and ELN. European guidelines for empirical antibacterial therapy for febrile neutropenic patients in the era of growing resistance: summary of the 2011 4th European Conference on Infections in Leukemia. *Haematologica.* 2013;98(12):1826-1835.
28. Kico JM, Miller CA, Griffith M, et al. Association between mutation clearance after induction therapy and outcomes in acute myeloid leukemia. *JAMA.* 2015;314(8):811-822.
29. Jongen-Lavrencic M, Grob T, Hanekamp D, et al. Molecular minimal residual disease in acute myeloid leukemia. *N Engl J Med.* 2018;378(13):1189-1199.
30. Morita K, Kantarjian HM, Wang F, et al. Clearance of somatic mutations at remission and the risk of relapse in acute myeloid leukemia. *J Clin Oncol.* 2018;36(18):1788-1797.
31. Pan L, Li Y, Zhang HY, et al. DHX15 is associated with poor prognosis in acute myeloid leukemia (AML) and regulates cell apoptosis via the NF-κB signaling pathway. *Oncotarget.* 2017;8(52):89643-89654.
32. Shen Y, He A, Wang F, et al. Granulocyte colony stimulating factor priming chemotherapy is more effective than standard chemotherapy as salvage therapy in relapsed acute myeloid leukemia. *Med Clin (Barc).* 2018;151(9):339-344.
33. Thomas X, Le Jeune C. Treatment of elderly patients with acute myeloid leukemia. *Curr Treat Options Oncol.* 2017;18(1):2.
34. Pollyea DA, Amaya M, Strati P, Konopleva MY. Venetoclax for AML: changing the treatment paradigm. *Blood Adv.* 2019;3(24):4326-4335.

35. DiNardo CD, Jonas BA, Pullarkat V, et al. Azacitidine and venetoclax in previously untreated acute myeloid leukemia. *N Engl J Med.* 2020;383(7): 617-629.
36. Yamada K, Furusawa S, Saito K, et al. Concurrent use of granulocyte colony-stimulating factor with low-dose cytosine arabinoside and aclarubicin for previously treated acute myelogenous leukemia: a pilot study. *Leukemia.* 1995;9(1):10-14.
37. Xie M, Jiang Q, Li L, et al. HAG (homoharringtonine, cytarabine, G-CSF) regimen for the treatment of acute myeloid leukemia and myelodysplastic syndrome: a meta-analysis with 2,314 participants. *PLoS One.* 2016;11(10):e0164238.
38. Hu X, Fu W, Wang L, et al. HAG regimen improves survival in adult patients with hypocellular acute myeloid leukemia. *Oncotarget.* 2016;7(3):3623-3634.
39. Takahashi W, Nakamura Y, Tadokoro J, et al. [CAG-GO therapy for patients with relapsed or primary refractory CD33-positive acute myelogenous leukemia] [in Japanese]. *Rinsho Ketsueki.* 2012;53(1):71-77.
40. Ma X, Wang J, Xu Y, et al. Dose-enhanced combined priming regimens for refractory acute myeloid leukemia and middle-and-high-risk myelodysplastic syndrome: a single-center, retrospective cohort study. *OncoTargets Ther.* 2016;9:3661-3669.
41. Minakata D, Fujiwara S, Ito S, et al. A low-dose cytarabine, aclarubicin and granulocyte colony-stimulating factor priming regimen versus a daunorubicin plus cytarabine regimen as induction therapy for older patients with acute myeloid leukemia: A propensity score analysis. *Leuk Res.* 2016;42:82-87.
42. Kitagawa J, Hara T, Tsurumi H, et al. Cell cycle-dependent priming action of granulocyte colony-stimulating factor (G-CSF) enhances in vitro apoptosis induction by cytarabine and etoposide in leukemia cell lines. *J Clin Exp Hematop.* 2010;50(2):99-105.
43. Saba F, Soleimani M, Kaviani S, et al. G-CSF induces up-regulation of CXCR4 expression in human hematopoietic stem cells by beta-adrenergic agonist. *Hematology.* 2015;20(8):462-468.
44. De La Luz Sierra M, Gasperini P, McCormick PJ, Zhu J, Tosato G. Transcription factor Gfi-1 induced by G-CSF is a negative regulator of CXCR4 in myeloid cells. *Blood.* 2007;110(7):2276-2285.
45. Souza LM, Boone TC, Gabilove J, et al. Recombinant human granulocyte colony-stimulating factor: effects on normal and leukemic myeloid cells. *Science.* 1986;232(4746):61-65.
46. Zhang H, Goudeva L, Immenschuh S, et al. miR-155 is associated with the leukemogenic potential of the class IV granulocyte colony-stimulating factor receptor in CD34⁺ progenitor cells. *Mol Med.* 2015;20(1):736-746.
47. Ehlers S, Herbst C, Zimmermann M, et al. Granulocyte colony-stimulating factor (G-CSF) treatment of childhood acute myeloid leukemias that overexpress the differentiation-defective G-CSF receptor isoform IV is associated with a higher incidence of relapse. *J Clin Oncol.* 2010;28(15): 2591-2597.
48. Wei G, Ni W, Chiao JW, Cai Z, Huang H, Liu D. A meta-analysis of CAG (cytarabine, aclarubicin, G-CSF) regimen for the treatment of 1029 patients with acute myeloid leukemia and myelodysplastic syndrome. *J Hematol Oncol.* 2011;4(1):46.
49. Minakata D, Fujiwara SI, Ikeda T, et al. Relationship between white blood cell count elevation and clinical response after G-CSF priming chemotherapy for acute myeloid leukemia. *Int J Hematol.* 2017;106(3):411-417.
50. Zhang WG, Wang FX, Chen YX, et al. Combination chemotherapy with low-dose cytarabine, homoharringtonine, and granulocyte colony-stimulating factor priming in patients with relapsed or refractory acute myeloid leukemia. *Am J Hematol.* 2008;83(3):185-188.
51. Madan V, Han L, Hattori N, et al. ASXL2 regulates hematopoiesis in mice and its deficiency promotes myeloid expansion. *Haematologica.* 2018;103(12): 1980-1990.
52. Podoltsev NA, Stahl M, Zeidan AM, Gore SD. Selecting initial treatment of acute myeloid leukaemia in older adults. *Blood Rev.* 2017;31(2):43-62.
53. Burnett AK. Treatment of older patients with newly diagnosed AML unfit for traditional therapy. *Clin Lymphoma Myeloma Leuk.* 2018;18(9):553-557.
54. Karlsson L, Forestier E, Hasle H, et al. Outcome after intensive reinduction therapy and allogeneic stem cell transplant in paediatric relapsed acute myeloid leukaemia. *Br J Haematol.* 2017;178(4):592-602.
55. Getz KD, Sung L, Ky B, et al. Occurrence of treatment-related cardiotoxicity and its impact on outcomes among children treated in the AAML0531 clinical trial: a report from the Children's Oncology Group. *J Clin Oncol.* 2019;37(1):12-21.
56. Bansal D, Davidson A, Supriyadi E, et al. SIOP PODC adapted risk stratification and treatment guidelines: Recommendations for acute myeloid leukemia in resource-limited settings. *Pediatr Blood Cancer.* 2019;e28087.