Continuous infusion of intermediate-dose cytarabine and fludarabine with idarubicin for patients younger than 60 years with resistant acute myeloid leukemia: A prospective, multicenter phase II study

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We assessed continuous infusion (CI) of fludarabine and cytarabine (FLAG) plus idarubicin for patients under 60-years old with resistant acute myeloid leukemia (AML). Induction chemotherapy consisted of idarubicin (12 mg/m^2 iv infusion over 30 min on Days 1–3), plus fludarabine (30 mg/m2/day) and cytarabine (1,000 mg/m^2/day) on Days 1–5 as a 24-hr CI. G-CSF was added on Days 1–5. The 29 patients enrolled were of median age 40 years (range, 18–57 years); of these, 8 (27.6%) had primary refractory disease, 19 (65.5%) were in early relapse, and 1 each (3.4%) was in multiple relapse and relapse after SCT. In response to induction, 8 patients (27.6%) achieved CR, 2 (6.9%) achieved CRp, and 19 (65.5%) failed treatment; of the latter, 14 had aplasia, three had an indeterminate course, and two showed resistance. Seven patients remain alive, while two were lost to follow-up. Nineteen patients died, 14 of infection, one of toxicity during consolidation, three of relapse after SCT, and two of persistent disease. These findings indicate that although CI of FLAG plus idarubicin was effective for eradicating blasts, it carried a high risk of toxicity. Reduced doses are recommended for CI of FLAG plus idarubicin. Am. J. Hematol. 84:161–166, 2009. © 2008 Wiley-Liss, Inc.

Introduction

The prognosis for patients with acute myeloid leukemia (AML) refractory to first line treatment or in first or subsequent relapse is poor. CR1 duration in relapse patients is the most important prognostic factor correlating with the probability of CR2 and survival [1]. Phase I clinical investigations of fludarabine in AML patients have used a variety of treatment schedules, including bolus infusions, [2] infusions every 12 hr [3], continuous infusions (CIs), [4–6] and loading-dose followed by CI [7–9]. The latter schedule has been used to rapidly achieve and maintain concentrations shown in vitro to achieve maximal inhibition of cell growth.

The original FLAG regimen (fludarabine combined with cytarabine under the support of G-CSF) consists of G-CSF 400 μg/m^2/day 1 day before (patients with WBC count $<$ 50,000/μL) and/or during (all patients) fludarabine 30 mg/m^2/day and cytarabine 2 g/m^2/day for 5 days [10]. CR rates, however, were similar with FLAG and FA (fludarabine plus cytarabine) (63% vs. 53%, $P = 0.50$). As first line treatment, FLAG or FLAG-like regimens have shown significant activities against AML and high risk myelodysplastic syndrome (MDS) [11,12], and FLAG-like regimens were effective for relapsed or refractory AML [13–20]. Patients with refractory AML treated with FLAG or FLAG-like regimens had poorer prognosis (7–64%) than patients with relapsed AML (34–81%) [13–20].

CI of fludarabine and cytarabine (FLAG) have been evaluated in pediatric patients with relapsed acute leukemia, with results indicating that these two agents acts synergistically [21]. Phase I/II studies of idarubicin given with CI fludarabine followed by CI cytarabine have also been performed in children with AML [22,23]. Sequential CI of fludarabine plus cytarabine was found to induce CR in 14 of 20 patients [24]. A modified FLAG regimen, consisting of sequential CI of fludarabine and ara-C plus liposomal daunorubicin, as salvage therapy for refractory or relapsed patients with AML, has shown encouraging efficacy and safety results [25].

We recently evaluated CI of intermediate-dose cytarabine and etoposide, plus idarubicin, or mitoxantrone (CIE or CME), in patients with refractory or relapsed acute leukemia [26,27]. Although CR rates were 36.8 and 46.0%, relapse occurred in the majority of CR patients. The median relapse-free survival and overall survival periods were 177 and 182 days, respectively, suggesting that induction and postremission therapy should be intensified. We therefore investigated the feasibility of CI intermediate dose cytarabine and fludarabine (FLAG) plus idarubicin for patients with resistant AML other than acute promyelocytic leukemia.

Results

Patients

From April 2005 to February 2007, 29 patients (19 men, 10 women), of median age 40 years (range, 18–57 years), were enrolled. At enrollment, these patients had a mean WBC of 20,400/μL (range, 470–15,807/μL), a mean peripheral blood blast count of 28.2% (range, 0–90%), and a

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TABLE I. Patient Characteristics

Characteristics	No. of patients (%)
Gender	
Male	19 (65.5)
Female	10 (34.5)
Age (years)	
≤40	15 (51.7)
>40	14 (48.3)
Cytogenetic risk group at diagnosis	
Favorable	7 (24.1)
Intermediate	14 (48.3)
Poor	6 (20.7)
Unknown	2 (6.9)
Front-line induction regimen	
AD	24 (82.8)
AI	4 (13.8)
Other	1 (3.4)
Response to front-line induction	
CR	22 (75.9)
No CR	7 (24.1)
Duration of first CR (months)	
≤12	24 (82.8)
>12	5 (17.2)
Disease status at treatment	
Refractory	8 (27.6)
Relapsed	21 (72.4)
HSCT prior to treatment	
Not done	26 (89.7)
Allogeneic/autologous	3 (10.3)
WBCs at treatment ($\times 10^3/\mu L$)	
≤20	22 (75.9)
>20	7 (24.1)
PB blasts at treatment (%)	
≤40	22 (75.9)
>40	7 (24.1)
BM blasts at treatment (%)	
≤40	13 (44.8)
>40	16 (55.2)

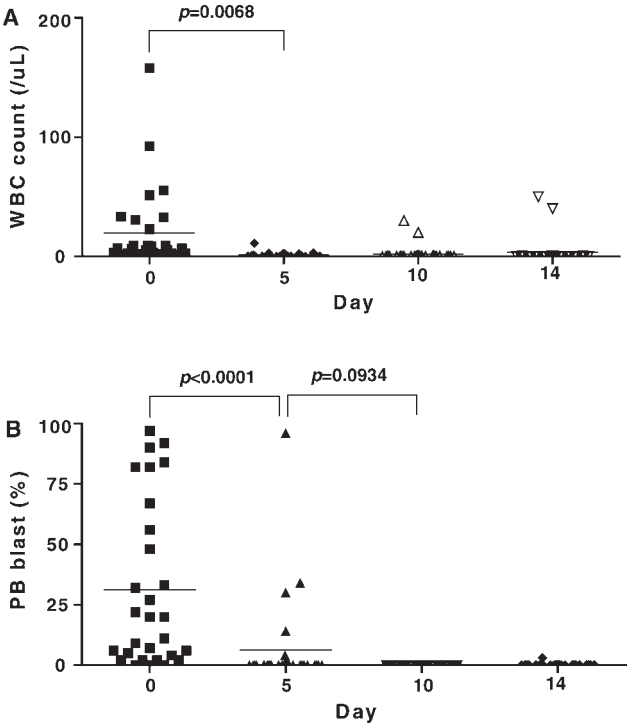

Figure 1. Changes in peripheral blood counts until early evaluation. Changes of peripheral blood white blood cell (WBC) counts (A) and blast counts (B) on Days 0 ($n = 29$), 5 ($n = 29$), 10 ($n = 28$) and 14 ($n = 27$) following induction chemotherapy. Each horizontal bar indicates the mean value. Differences in WBC ($P = 0.0068$) and blasts ($P < 0.0001$) between Day 0 and Day 5 were statistically significant.

mean bone marrow blast count of 54.0% (range 0–95.1%). Three patients (10.3%) had received prior allogeneic or autologous hematopoietic stem cell transplantation (HSCT). Most patients ($n = 24$, 82.8%) had received cytarabine and daunorubicin as induction therapy, and had a duration of CR less than 12 months. Of the 27 patients evaluated for chromosomal abnormalities at diagnosis, 10 had two or more abnormalities, seven had t(8;21) translocations, five had normal chromosomes, three had other single abnormalities, and two had MLL abnormalities. Patient characteristics are summarized in Table I.

Induction chemotherapy

Induction chemotherapy was completed in 27 (93.1%) patients. The scheduled treatment was stopped in one patient on Day 2 due to poor compliance and in another patient on Day 3 due to azotemia. There were no dose reductions or delayed dose administration during induction chemotherapy.

Toxicities during induction chemotherapy

Following induction chemotherapy, 13 (44.8%), 13 (44.8%), 10 (34.5%), and 5 (17.2%) patients showed recovery to absolute neutrophil count (ANC) $\geq$ 500/μL, ANC $\geq$ 1,000/μL, platelets $\geq$ 50,000/μL and platelets $\geq$ 100,000/μL, respectively. The median times to recovery to ANC $\geq$ 500/μL, ANC $\geq$ 1,000/μL, platelets $\geq$ 50,000/μL and platelets $\geq$ 100,000/μL were 29 days (range, 25–46 days), 32 days (range, 26–47 days), 31.5 days (range, 23–67 days) and 43 days (range, 34–52 days), respectively. Febrile neutropenia occurred in 27 (93.1%) patients with a median duration of 7 days (range, 1–27 days). There were no other grade 3/4 nonhematological toxicities. Antibiotics, antifungal agents, and antiviral agents were administered to 27 (91.7%), 26 (86.2%), and 2 (8.3%) patients, respectively. Patients received a median 27 units (range, 4–261 units) of platelet concentrate and 10.5 units (range, 2–148 units) of packed red cells. None of the patients received WBC transfusions, but 25 patients (86.2%) received G-CSF support.

Responses to induction therapy

Elimination of peripheral WBCs and blood blasts was rapid, occurring within 5 days of induction chemotherapy (see Fig. 1). Changes in peripheral blood counts are summarized in Table II, with difference in WBCs ($P = 0.0068$) and blasts ($P < 0.0001$) between Days 0 and 5 being of statistical significance. Peripheral blasts were not detected in any patient by Day 10. Early bone marrow evaluation in 21 patients showed that mean blast count in peripheral blood was 0.1% (range, 0–3.0%) and mean blast count in bone marrow was 3.9% (range, 0–64.6%); 18 of these 21 (85.7%) patients had fewer than 5% blasts in bone marrow. Mean bone marrow cellularity was 10% (range, 0–60%).

Table III summarizes response to induction therapy. CR was achieved by six patients (20.7%) and CRp by 3 (10.3%), making the overall response rate 31%. Median days required for CR was 48 (range 37–63 days). In contrast, 20 patients showed treatment failure, most commonly due to aplasia (14 patients, 48.3%). There were no statistically significant clinical factors affecting CR, including early relapse versus refractory ($P = 0.625$), HSCT in first CR versus non-HSCT ($P = 1.000$), number of prior induction therapy regimens ($P = 0.337$), CR duration ≤12 versus > 12 months ($P = 1.000$), age ≤40 versus > 40 years ($P = 1.000$), >40% versus ≤40% peripheral blasts ($P = 1.000$),

TABLE II. Elimination of Peripheral Blood White Blood Cells and Blasts until Early Evaluation of Induction Therapy

Day	WBCs (/μL), mean (range)	ANC (/μL), mean (range)	Blasts (%), mean (range)
0 (n = 29)	19597.6 (470–15,8070)	3749.5 (0.0–29,696)	31.3 (0.0–97)
5 (n = 29)	1111.0 (0.0–11,100)	923.8 (0.0–11,100)	6.2 (0.0–96)
10 (n = 28)	1863.9 (0.0–30,000)	10.1 (0.0–188)	0 (0.0–0.0)
14 (n = 27)	3404.8 (0.0–50,000)	4.3 (0.0–54)	0.1 (0.0–3)

WBC, white blood cell; ANC, absolute neutrophil count.

TABLE III. Response to Induction Therapy

Response	No. of patients (%)
Objective response	9 (30.0)
CR	6 (20.7)
CRp	3 (10.3)
Treatment failure	20 (69.0)
Resistant	3 (10.3)
Aplasia	14 (48.3)
Indeterminate course	3 (10.3)

CR, complete remission; CRp, complete remission without platelet recovery.

>40% versus ≤40% bone marrow blasts (P = 1.000), and favorable versus less favorable risk group (P = 0.158). Three patients were salvaged after the failure of induction therapy, two by mitoxantrone-combination chemotherapy and one by autologous HSCT. Among seven patients with t(8;21), four patients achieved CR and three died of aplasia during induction therapy.

Postremission therapy

Among patients who had achieved CR, three received 0 consolidation cycles, four received 1 cycle and two received 2 cycles. Consolidation treatment was halted due to HSCT in six patients (one autologous, five allogeneic), death during consolidation therapy in one patient, infection in one patient and poor performance status in one patient. HSCT was performed in seven patients (six in CR and one resistant). All four patients with t(8;21) who had achieved CR during induction therapy received one or two consolidation therapies. Among them, one patient died during second consolidation and two patients could perform HSCT.

Follow-up and survival

At follow-up, 23 (79.3%) patients were alive with disease, five (17.2%) showed no evidence of disease, and one (3.4%) was of unknown disease status, being lost to follow-up after attaining CR. Three patients relapsed, with a median time to relapse (TTR) of 11.48 months [95% confidence interval (CI), 4.89–18.07 months] (see Fig. 2). The 6-month and 1-year TTR rates were 83.8 and 41.7%, respectively. Two patients were censored from TTR, one each due to death and loss to follow-up. Relapse after HSCT occurred in 1/1 (100%) patient receiving autologous and 2/5 (40%) patients receiving allogeneic HSCT. At last follow-up, seven patients were alive, 20 patients were dead and two patients were lost to follow-up. Death occurred during induction aplasia in 14 patients; during salvage chemotherapy in two patients; after autologous HSCT in two patients; during consolidation in one patient; and after allogeneic HSCT in one patient. The causes of death were infection in 15 (bacterial infection in 13, fungal infection in one and unidentified infection in one) patients, interstitial pneumonia in one patient, cerebral hemorrhage in one patient, and pulmonary failure in three patients. Median OS was 2.47 months (95% CI, 0.83–4.10 months) (Fig. 3A), and median OS of the eight patients who achieved CR was 11.38 months (95% CI, 4.89–18.07 months) (Fig. 3B). The

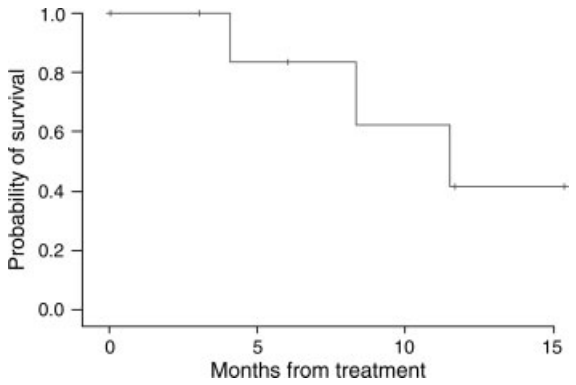

Figure 2. Time to relapse. Kaplan-Meier curve of time to relapse. Three patients relapsed, with a median TTR of 11.48 months [95% confidence interval (CI), 4.89–18.07 months].

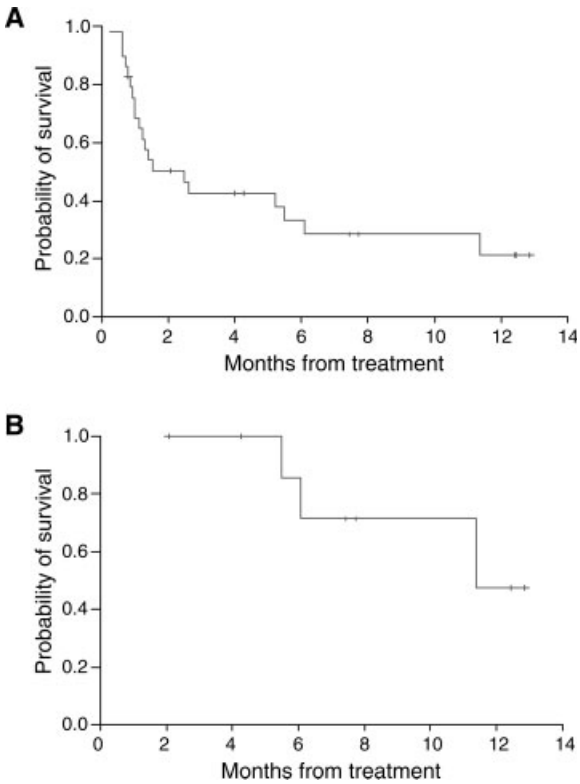

Figure 3. Overall survival. Kaplan-Meier curve of overall survival (OS) in all patients (A) and in patients who achieved complete remission (B). Median OS was 2.47 months [95% confidence interval (CI), 0.83–4.10 months] in all patients and 11.38 months (95% CI, 4.89–18.07 months) in the eight patients who achieved CR.

6-month and 1-year OS rates of all patients were 33.2 and 21.3%, respectively. The 6-month and 1-year OS rates in CR patients were 77.1 and 42.9%, respectively.

Discussion

Cytarabine remains one of the most effective agents in the treatment of AML. Once infused, cytarabine must be phosphorylated to its active 5′-triphosphate, ara-CTP. CI schedules of ara-C have been used clinically to exploit its S-phase specificity and to avoid the detrimental effects of its rapid clearance from leukemic blasts. Incorporation of a 72-hr infusion of higher dose cytarabine into combination chemotherapy regimens should proceed cautiously, with a starting dose of 6 g/m^2/72 hr [28]. Attempts to overcome cytarabine resistance have included increasing the duration of exposure by CI as well as by increasing the dosage [26,27].

CR2 rates of regimens used to treat relapsed or refractory AML have been shown to vary from 8% to 89% [29]. Most regimens that result in a CR2 rate >60% include combinations of ara-C and mitoxantrone [30–38]. We sought to replace etoposide and mitoxantrone with fludarabine, by CI of this agent plus intermediate-dose cytarabine, in addition to idarubicin, to treat patients with relapsed or refractory acute leukemia.

The amount of ara-CMP incorporated in vitro into human leukemia cell DNA has been found to predict loss of cellular clonogenicity, [39–41] with the major determinant of ara-CMP incorporation into DNA being the product of intracellular ara-CTP concentration and time [41]. This observation has been confirmed in patients in vivo, where the intracellular pharmacokinetics of ara-CTP and clinical response to high-dose ara-C, given either intermittently or by CI, showed statistically significant correlations [42–44]. Similarly, very low incorporation of ara-C into DNA in vitro was predictive of an adverse outcome with subsequent ara-C-based therapy in vivo [45]. Arabinosyl-2-fluoroadenine (F-ara-A) increased the rate of ara-CTP accumulation in leukemia cells when incubated. Moreover, infusion of fludarabine prior to intermittent infusion of intermediate-dose cytarabine increased ara-CTP accumulation in circulating leukemic blasts of AML and chronic lymphocytic leukemia (CLL) patients receiving combination regimens [46,47], a biochemical modulation due to the influence of F-ara-ATP on the rate of ara-CTP accumulation [47,48]. As F-ara-A has a terminal half-life in plasma of ~12 hr, [49,50] a plateau concentration in plasma would not be reached for at least 2 days after the start of a CI. A loading dose (LD) followed by CI of fludarabine, however, can achieve plateau plasma concentration more rapidly, avoiding the high peak levels that may be associated with neurotoxicity [51]. We therefore administered a fludarabine LD followed by CI.

The 30% CR rate observed in this study was discouraging. The major cause of treatment failure was aplasia, suggesting our dose intensity was too high. The dose-limiting toxicity of this agent has been shown to be myelosuppression, especially leukopenia [9]. The maximum tolerated dose (MTD) and recommended starting dose for phase II trials was 20 mg/m^2 by IV push followed by a 48-hr CI of 30 mg/m^2/24 hr for 48 hr. This schedule maintained a continuous plasma 2-F-ara-A concentration of 0.2–1.0 μmol/L for 48 hr, the concentration shown to inhibit cellular growth in vitro. In pediatric patients, [21] the MTD of CI fludarabine and cytarabine was 10 μM fludarabine for 48 hr followed by 72 hr of 15 μM ara-C. In phase I/II trials, idarubicin was administered with CI fludarabine followed by CI cytarabine [22,23]. The MTD was a fludarabine LD of 10.5 mg/m^2 followed by a CI of 30.5 mg/m^2/24 hr for 24 hr, followed by cytarabine LD of 390 mg/m^2 and CI of 101 mg/m^2/h for 72 hr. With CI fludarabine and ara-C, idarubicin was tolerable at a dose of 12 mg/m^2/day for 3 days. In another trial, fludarabine was administered at an LD of 10 mg/m^2 over 15 min on Day 0 followed by a CI of 20 mg/m^2/24 hr for 72 hr, whereas cytarabine was administered at an LD of 390 mg/m^2 over 15 min 3.5 hr after fludarabine and then as a CI over 96 hr at 1,440 mg/m^2/24 hr [24].

Although the fludarabine-induced increase in the rate of ara-CTP accumulation appeared to be dependent on the cellular concentration of F-ara-ATP, ara-CTP accumulation was not further augmented by peak cellular F-ara-ATP concentrations greater than 15 μM [52]. These findings suggest that a lower dose of fludarabine may result in cellular F-ara-ATP concentrations sufficient to maximize the rate of ara-CTP accumulation. Furthermore, the finding, that G-CSF administered along with fludarabine-ara-C was associated with a 40% increase in F-ara-ATP accumulation, indicated that a lower dose of fludarabine may effectively modulate ara-CTP metabolism [10,53]. The minimal dose of fludarabine required for the maximal modulation of ara-CTP in human leukemia blasts during therapy has been evaluated [54], showing that a lower dose of fludarabine, administered concurrently with cytarabine and G-CSF, would be adequate to control leukemia.

A shorter duration of infusion, 4 rather than 5 days, is indicated, because the former exposure time was shown sufficient to maximally augment ara-C anabolism for at least 24 hr after fludarabine [8], allowing the dose of fludarabine to be further reduced. Moreover, due to the prolonged cytopenia caused by idarubicin, it may be better to reduce the dose intensity of this agent.

We observed a rapid elimination of peripheral blood blasts by Day 10, indicating that our CI strategy is highly effective in controlling leukemic cells, even in patients with resistant AML. In addition, early evaluation, on Day 14, was important in evaluating the efficacy of this regimen. We observed no statistically significant clinical factors affecting CR. Only the favorable risk group showed marginal benefits from this regimen. This, however, may have been due to the high rate of treatment-related deaths.

Postremission therapy in AML is very important because the duration of CR2 is usually short. In patients with resistant AML, induction chemotherapy should be used as bridging therapy to achieve maximal CR before performing HSCT. We found, however, that two of five patients receiving allogeneic HSCT and one of one receiving autologous HSCT relapsed. This implies that CR2 may have large minimal residual disease in spite of consolidation therapy.

In conclusion, we found that a regimen consisting of continuous FLAG with idarubicin failed to improve efficacy in patients with resistant AML, due to the high rate of treatment failure associated with aplasia. The doses of cytarabine, fludarabine, and idarubicin should be reduced in the setting of CI.

Patients and Methods

Patient eligibility. Patients <60-years old with resistant AML, other than acute promyelocytic leukemia, were eligible. This included patients in primary resistance, defined as failure to achieve CR after initial induction chemotherapy that included standard dose cytarabine; those in early relapse, occurring after first CR lasting less than 12 months; those with multiple relapses; and those in relapse after allogeneic HSCT.

All patients were between 15 and 60 years of age, had a performance status of two or less by ECOG performance scale, had not been treated with a high cumulative dose of anthracycline, had no other major illnesses or organ failure incompatible with combination chemotherapy, and had adequate hepatic (serum bilirubin <3.0 mg/dL, AST and ALT <three times the upper normal limit), renal and cardiac function. Patients with hepatic dysfunction due to leukemic infiltration were not excluded. Patients with extramedullary relapse(s) only were excluded. All patients provided written informed consent, and the study protocol was approved by the institutional review board of each participating hospital.

Treatment. All patients were supported by prophylactic antibiotics, including ciprofloxacin (500 mg bid), fluconazole (200 mg qd), and acyclovir (200 mg bid). Induction chemotherapy consisted of CI fludarabine and cytarabine plus idarubicin, following granulocyte-colony stimulating factor (G-CSF) priming. Induction chemotherapy consisted of idarubicin (12 mg/m^2 iv infusion over 30 min on Days 1–3), plus fludarabine (30 mg/m^2/day) and cytarabine (1,000 mg/m^2/day) on Days 1–5 as a 24-hr CI. On Day 1, however, 10 mg/m^2 fludarabine was administered as an IV push instead of a CI, starting 4 hr prior cytarabine. Subcutaneous G-CSF (Lenograstim, 400 μg/m^2) was administered every 24 hr on Days 1–5. Patients who achieved partial remission after the first course of induction chemotherapy were eligible for a second course at least 4 weeks later. G-CSF 250 μg/day was also administered to all patients with hypocellular bone marrow, defined as fewer than 5% blasts on Day 14 or later, until absolute neutrophil count was ≥1,000/μL. Patients who achieved CR were administered 3 cycles of consolidation chemotherapy, which consisted of the same regimen but without idarubicin. Patients were eligible for allogeneic or autologous hematopoietic cell transplantation during or after consolidation chemotherapy.

Toxicity and response evaluation. Blood was drawn every day until full hematologic recovery. Blood cell counts were performed daily, and blood chemistry and electrolytes were sampled twice weekly or more frequently as necessary. All patients were monitored for the occurrence of adverse events, including chemotherapy-induced toxicities. Toxicity was graded according to NCI CTCAE version three. Time to ANC and platelet recovery, transfusions required, frequency of febrile episodes, and use of antibiotics were also monitored. Bone marrow examinations were performed on Day 14 and to identify CR when ANC was >1,000/μL and platelets >100,000/μL without the support of platelet transfusions. Responses were evaluated according to the criteria of the International Working Group [55]. Relapse was defined as bone marrow with >25% blasts or evidence of extramedullary leukemic involvement. Patients with bone marrow in partial remission were classified as in relapse if they had significant splenomegaly, hepatomegaly, lymphadenopathy, mediastinal mass, or central nervous system involvement. CR and CRp were regarded as objective responses to treatment. Overall survival (OS) was calculated as the first day of study treatment to death from any cause, TTR was the interval between CR and relapse.

Statistics. The two-stage minimax design for phase II clinical trials was used. According to this model, the largest response probability, P_0, if true, would indicate that the efficacy of treatment does not warrant further investigation, and the smallest response probability, P_1, if true, would indicate that the efficacy of treatment warrants further evaluation. The reference response rates and acceptable error probabilities were $P_0 = 0.30$, $P_1 = 0.50$, $\alpha = 0.05$, and $\beta = 0.20$. Under these conditions, the total sample size was 30 patients and the decision markers for this two-stage design trial were calculated as: a Stage 1 sample size of 19; a critical value for Stage 1 of 6; and a critical value for Stage 2 of 16. Paired *t*-test was used to compare peripheral blood cell counts during induction therapy. To define the clinical factors affecting CR, major clinical parameters were dichotomized and validated by Fisher's exact test. The Kaplan-Meier method was used to estimate survival.

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