REGULAR ARTICLE
blood advances

Randomized trial comparing standard vs sequential high-dose chemotherapy for inducing early CR in adult AML

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Key Points

- High-dose chemotherapy increased early remission and overall and relapse-free survival compared with conventional-dose chemotherapy.
- Allograft performance in high-risk patients and some standard-risk patients significantly improved survival.

Here we evaluated whether sequential high-dose chemotherapy (sHD) increased the early complete remission (CR) rate in acute myelogenous leukemia (AML) compared with standard-intensity idarubicin-cytarabine-etoposide (ICE) chemotherapy. This study enrolled 574 patients (age, 16-73 years; median, 52 years) who were randomly assigned to ICE (n = 286 evaluable) or sHD (2 weekly 3-day blocks with cytarabine 2 g/m^2 twice a day for 2 days plus idarubicin; n = 286 evaluable). Responsive patients were risk-stratified for a second randomization. Standard-risk patients received autograft or repetitive blood stem cell-supported high-dose courses. High-risk patients (and standard-risk patients not mobilizing stem cells) underwent allotransplantation. CR rates after 2 induction courses were comparable between ICE (80.8%) and sHD (83.6%; $P = .38$). sHD yielded a higher single-induction CR rate (69.2% vs 81.5%; $P = .0007$) with lower resistance risk ($P < .0001$), comparable mortality ($P = .39$), and improved 5-year overall survival (39% vs 49%; $P = .045$) and relapse-free survival (36% vs 48%; $P = .028$), despite greater hematotoxicity delaying or reducing consolidation blocks. sHD improved the early CR rate in high-risk AML (odds ratio, 0.48; 95% confidence interval [CI], 0.31-0.74; $P = .0008$) and in patients aged 60 years and less with de novo AML (odds ratio, 0.46; 95% CI, 0.27-0.78; $P = .003$), and also improved overall/relapse-free survival in the latter group (hazard ratio, 0.70; 95% CI, 0.52-0.94; $P = .01$), in standard-risk AML, and postallograft (hazard ratio, 0.61; 95% CI, 0.39-0.96; $P = .03$). sHD was feasible, effectively achieved rapid CR, and improved outcomes in AML subsets. This study is registered at www.clinicaltrials.gov as #NCT00495287.

Introduction

In adult acute myelogenous leukemia (AML), intensive induction chemotherapy is a standard approach for achieving complete remission (CR),[1] as defined by an international panel of experts.[2] CR enables the delivery of consolidation chemotherapy and/or allogeneic hematopoietic stem cell transplantation (HSCT), which can prolong survival and cure the disease.[1,3-5] Because CR is the starting point for

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curative treatment and a strong predictor of survival, it is important to optimize the induction chemotherapy protocol.

Several groups have attempted to improve the standard "3+7" CR induction regimen, which includes 3 days of daunorubicin and 7 days of conventional-dose cytarabine.[6] Large randomized trials have tested high-dose (HD) daunorubicin (or equivalent idarubicin),[7-12] HD cytarabine,[13-17] and nucleoside analogs,[18-20] with variable results. HD cytarabine is a mainstay of postremission consolidation,[21] and is widely used in relapsed/refractory AML. The use of HD cytarabine-based programs improved the rates of CR and relapse-free survival (RFS) in the Australasian Leukemia Study Group trial,[13] as well as among patients aged 45 years or younger in the European Organization for Research and Treatment of Cancer-Gruppo Italiano Malattie Ematologiche dell'Adulto AML 12 study,[17] but not in 3 other trials.[14,16,20]

The German AML Study Group (AMLSG) developed an effective sequential HD regimen for relapsed/refractory AML, which includes cytarabine plus either mitoxantrone or idarubicin.[22-25] Up-front use of this regimen with dose-dense HD cytarabine-mitoxantrone (HAM)–HAM double induction (cytarabine 3 g/m^2 and mitoxantrone) yielded 83% CR and 7% persistent leukemia.[26] A trial extension in the German collaborative intergroup project yielded 76% CR and 13% refractory AML. A parallel East Germany Hematology-Oncology Group trial (NCT01414231) compared continuous vs infusional sequential cytarabine (2 g/m^2) and achieved 74% CR and 14% resistance, showing no improvement over standard ICE (idarubicin-cytarabine-etoposide) induction.[27]

The Northern Italy Leukemia Group performed a phase 2 study using a sequential HD regimen for patients resistant to standard ICE induction (NCT00400673). Of 95 treated patients, 57% achieved CR, with similar rates across different clinico-cytogenetic risk groups.[28] Long-term outcome was significantly improved among patients who entered early CR during course 1 (ie, before receiving any HD chemotherapy).[29] Achieving CR after a single course is considered a favorable prognostic factor,[30-34] with rare exceptions.[35] In 1 large retrospective study (n = 8907), late CR achieved after reinduction therapy predicted significantly poorer outcome.[31] Early responders are typically easier to manage, curable with shorter hospitalization and fewer complications, and can receive earlier postremission therapy and HSCT.

In the present randomized trial, we compared ICE with sequential HD schedule (sHD) in untreated patients, with the aim of improving the early CR rate and evaluating any favorable effect on survival.

Patients and methods

Patients

All patients with newly diagnosed AML or high-risk (HR) myelodysplasia (MDS; marrow blasts > 10%) who presented at the participating institutions during the study period were registered for an outcome assessment project and screened for trial eligibility. Eligible patients had AML or HR MDS that was de novo, secondary to cancer chemoradiotherapy, or developed in a background of MDS or chronic myeloproliferative neoplasm, excluding acute promyelocytic leukemia. Only patients at least 16 years old were included. Because of the high AML prevalence among elderly individuals, we did not initially set an upper age limit for study inclusion, but rather enrolled older patients who were deemed fit enough to receive study medications. Thus, this trial included patients older than 65 years until a study amendment set an age limit of 65 years. All included patients gave their written informed consent. The study was approved by the Institutional Review Boards of the 17 participating Northern Italy Leukemia Group sites, registered as NCT00495287, and conducted in accordance with the Declaration of Helsinki.

AML diagnosis

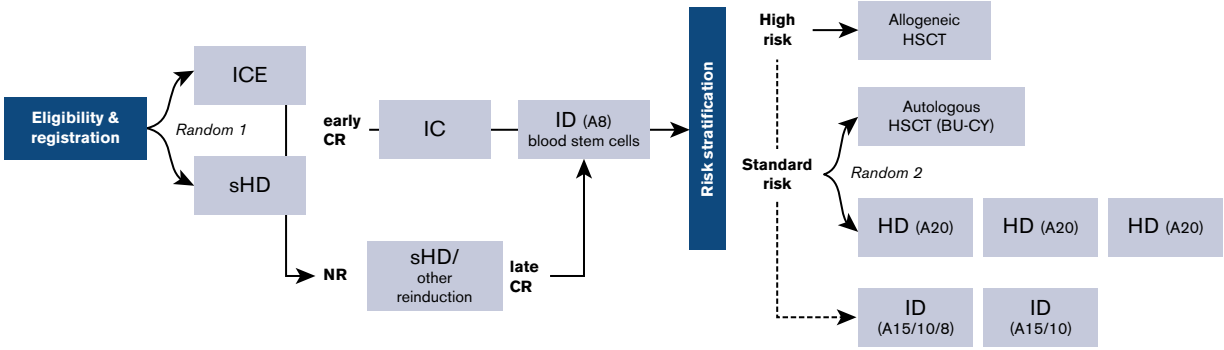
AML diagnosis was locally confirmed by cytogenetics and immunophenotype, following the standard criteria adopted for this study (supplemental File 1). Patient enrollment required central review of diagnostic bone marrow slides and trephine biopsy. *FLT3*,[36] *NPM1*,[37] *CEBPA*, and *MLL* mutations were investigated at the Laboratorio "Paolo Belli," Department of Hematology, Bergamo Hospital, and the Institute of Hematology, Perugia University.

Trial design and CR induction, randomization 1

The study protocol (supplemental File 2), its background (supplemental File 3.1), and the trial amendments (supplemental File 3.2) are reported online. Amendment 3 limited enrollment to patients 65 years old or younger, as insufficient accrual of older patients (n = 35) led to inconclusive results in randomization 1. For CR induction (randomization 1), patients were stratified according to age (≤60 or >60 years) and were randomly assigned to undergo standard ICE or sHD. Patients who achieved CR underwent risk-adapted postremission therapy (Figure 1). Both induction groups received a cumulative idarubicin dose of 36 mg/m^2, previously defined in a dose-escalation study.[38] All patients received granulocyte colony-stimulating factor (G-CSF), which is safe and effective in reducing absolute neutropenia duration and related complications after chemotherapy.[39,40] In both study groups, G-CSF administration started on day 11 (ie, after the second sHD block). The current sHD regimen was patterned after the administration schedule of cytarabine 3 g/m^2 (more activity than 1 g/m^2) developed by the AMLSG for relapsed/refractory AML.[23] To limit toxicity, particularly the risk for early death because of infection associated with cytarabine 3 g/m^2, patients in the sHD group received cytarabine 2 g/m^2 (1 g/m^2 if >65 years of age).[24] Adopting a 2-step induction strategy derived from the previous trial, patients who were unresponsive to course 1 received an augmented sHD course with cytarabine 3 g/m^2 (2 g/m^2 when >60 years of age) or other salvage therapy at the discretion of the treating physicians. Patients who did not achieve CR after 2 courses were removed from the study, as were patients who were assigned to HSCT as salvage instead of chemotherapy.

Risk-oriented postremission therapy and random 2

Patients who achieved CR were risk stratified according to cytogenetics,[41-43] *FLT3* mutations, and selected clinical risk factors (HR MDS, AML secondary to MDS/myeloproliferative neoplasm, therapy-related AML, minimally differentiated AML, erythroleukemia, megakaryoblastic leukemia, primary isolated granulocytic sarcoma, white blood cell count, >50 × 10^9/L, and late CR). The HR group comprised patients with unfavorable or unknown cytogenetics or with intermediate/normal cytogenetics plus either *FLT3* and *MLL* mutations or another clinical risk feature. This group was scheduled to receive HSCT from siblings or unrelated donors. The standard-risk (SR) group comprised patients with favorable cytogenetics and those with intermediate/normal cytogenetics and lacking *FLT3* mutations or any clinical risk factors. SR patients, along with HR patients unable to receive allogeneic HSCT, underwent randomization 2. Some



Chemotherapy course	Drugs	Dosing, route and infusion time (h, hours)	Days	Notes
ICE	Idarubicin Cytarabine Etoposide	12 mg/m^2/d, IV over 0.5 h 100 mg/m^2/bd, IV over 0.5 h 100 mg/m^2/d, IV over 1 h	1-3 1-7 1-5	G-CSF from day 11 to neutrophil recovery
sHD	Cytarabine Idarubicin	2 g/m^2/bd, IV over 3 h 18 mg/m^2/d, IV over 0.5 h	1-2, 8-9 3,10	**Course 1 (random 1):** cytarabine 1 g/m^2 if age >65 years; **Course 2 (reinduction):** cytarabine 3 g/m^2 (2 g/m^2 if age >60 years); G-CSF from day 11 to neutrophil recovery
IC	Idarubicin Cytarabine	10 mg/m^2/d, IV over 0.5 h 100 mg/m^2/bd, IV over 0.5 h	1-3 1-7	G-CSF from day 11 to neutrophil recovery
ID (A8)	Cytarabine	1 g/m^2/bd, IV over 2 h	1-4	G-CSF twice daily from day 8 to neutrophil recovery and blood stem cell harvest
HD (A20) Age ≤ 65 and mobilizer	Idarubicin Cytarabine	8 mg/m^2/d, IV over 0.5 h 2 g/m^2/bd, IV over 3 h	1,2 1-5	Autologous blood stem cell rescue on day 6 (1-2 x10^6/kg CD34+ cells); G-CSF from day 8 to neutrophil recovery
ID (A15/10) Age >65 and/or non-mobilizer	Idarubicin Cytarabine	10 mg/m^2/d IV over 0.5 h (A10) 1 g/m^2/bd, IV over 2 h (A10) 1.5 g/m^2/bd, IV over 2 h (A15)	1 1-5 1-5	A15: autologous blood stem cell rescue on day 6 (1-2 x10^6/kg CD34+ cells); G-CSF from day 8 to neutrophil recovery

Figure 1. Design of the Northern Italy Leukemia Group AML trial 02/06. ICE was compared with sHD induction chemotherapy. After a common 2-step CR induction and early consolidation phase, patients received 1 of 2 types of final consolidation, depending on risk. Patients at HR received allogeneic HSCT. Patients at SR, 65 years of age or younger, and mobilizing blood stem cells underwent a second randomization to either standard BU-CY-conditioned autologous HSCT or up to 3 repetitive blood stem cell-supported HD courses, denoted "A20" after the cumulative cytarabine (arabinosylcytosine, A) dose of 20 g/m^2. SR patients unable to proceed to HD consolidation could receive allogeneic HSCT, and HR patients unable to receive HSCT could undergo HD consolidation. Patients older than 65 years and/or not mobilizing blood stem cells underwent 1 to 2 additional age-adapted ID consolidation courses, denoted "A15," "A10," or "A8" after the cumulative cytarabine dose (dashed line).

patients received intravenous busulfan (BU; 0.8 mg/kg every 6 hours for 4 days) and cyclophosphamide (CY; 60 mg/kg daily for 2 days), followed by blood stem cell autotransplantation. Others underwent repetitive blood stem cell-supported HD chemotherapy courses (cumulative cytarabine 20 g/m^2 plus idarubicin 16 mg/m^2). Blood stem cells were mobilized with intermediate-dose (ID) cytarabine and G-CSF. Patients who displayed poor mobilization ($<2 \times 10^6$/kg CD34$^+$) and patients older than 65 years were consolidated with age-adapted ID chemotherapy courses (Figure 1) or were considered for HSCT despite SR status.

Objectives, definitions, and statistics

The primary study endpoint was whether sHD significantly reduced the risk for chemoresistance and increased the early CR rate compared with standard ICE chemotherapy. In the prior study, 22% of patients exhibited ICE-resistant AML (n = 129/581), of whom 57% were effectively rescued by sHD (n = 54/95)[28] (supplemental File 3.1). Extrapolating these data for the sample size calculation for the current study, we estimated that we needed at least 250 patients per group to demonstrate a 38% relative risk reduction (RRR) for unresponsive disease, with sHD given as investigational course 1, with 80% power and 0.05 α error. We used standard endpoint definitions. CR was defined as more than 1.0×10^9/L neutrophils and at least 100×10^9/L platelets, regenerating marrow with evidence of trilineage hematopoiesis, blast cells less than 5% (no Auer rods), and no extramedullary leukemia. CR included CR with incomplete hematological recovery, defined as fewer than 1.0×10^9/L neutrophils and/or fewer than 100×10^9/L platelets.[5] Resistant AML was defined as less than CR/CR with incomplete hematological recovery, with persistence of AML blasts in the bone marrow and/or blood smears. Response was assessed by evaluation of bone marrow on day 28, or later if clinically indicated. Day 14 bone marrow examination was not planned in this study.

Randomization 2 compared long-term RFS (time from date of CR to relapse or death in remission) between SR treatment groups. Secondary objectives included overall survival (OS; from study

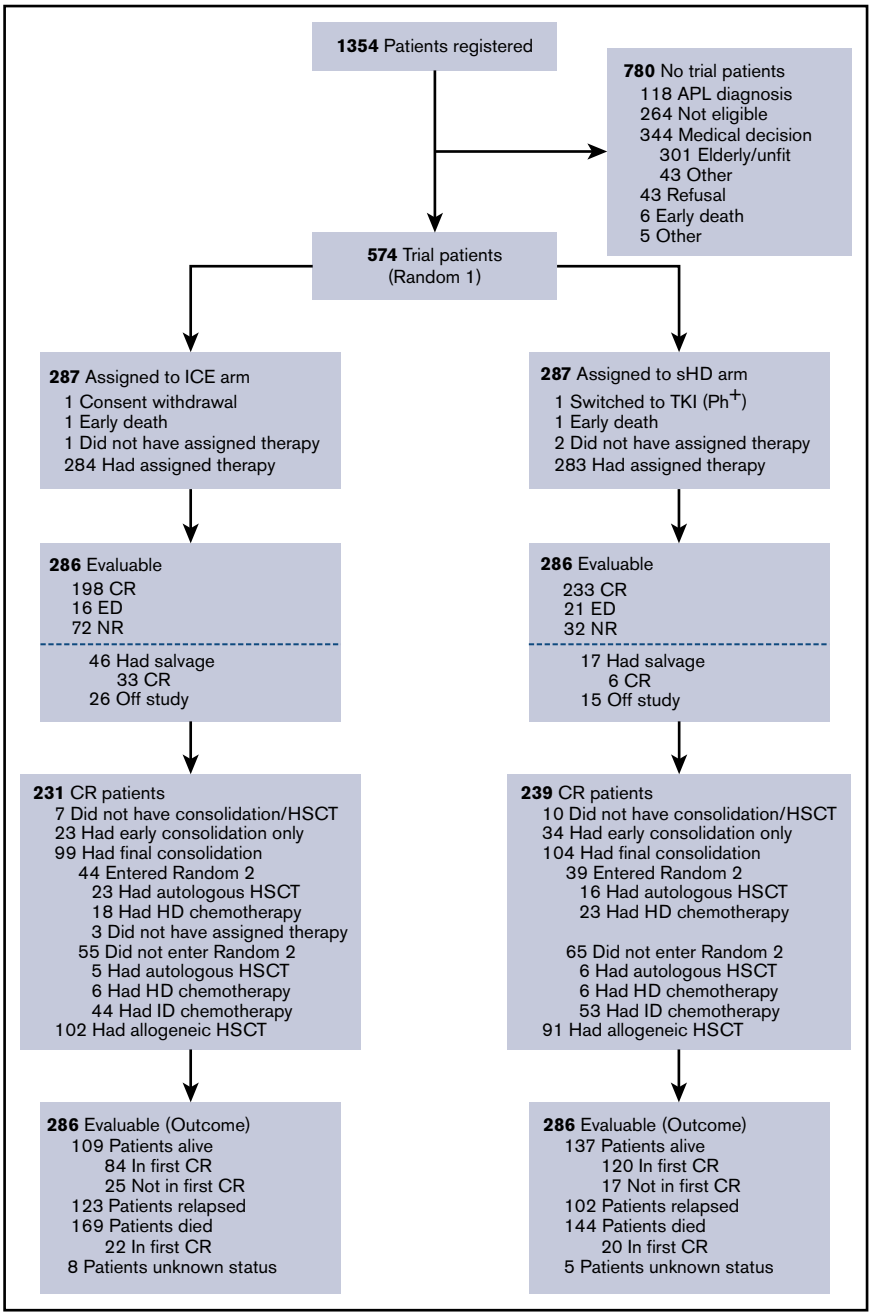


Figure 2. CONSORT diagram illustrating patient selection, study flow, successive treatment steps, and patient outcome, according to the randomization group. APL, acute promyelocytic leukemia; ED, early death; NR, nonresponder; TKI, tyrosine-kinase inhibitor.

enrollment to death), event-free survival (from enrollment to induction failure, relapse, or death in remission),[2] cumulative incidence of relapse, and the feasibility and efficacy of study treatments in different age and risk groups. To evaluate prognostic effects not assessable by the original Northern Italy Leukemia Group risk classification, we performed post hoc prognostic analysis, using the European LeukemiaNet (ELN) 2010 genetic risk stratification,[44] including the results of centralized analysis of *CEBPA* and *MLL* mutations in cryopreserved AML cells. Therapy-related complications were graded using common toxicity criteria (CTC; http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf).

Statistical analyses were performed following the intention-to-treat principle. Baseline patient characteristics were compared between treatment groups, using a χ^2 or Fisher's exact test (categorical variables) or the Mann-Whitney *U* test (continuous variables). We assessed the relationship between CR achievement and study group, using the χ^2 test. The RRR indicated the proportion of ICE failures (resistance/death) that could have been avoided with sHD. Survival was estimated using the Kaplan-Meier method, and long-term outcomes compared using the log-rank test. Relapse incidence was assessed using cumulative incidence function, considering death as a competing event. Subgroup and multivariate analyses with logistic regression and Cox models were performed to estimate odds ratios with 95% confidence intervals (CIs) for CR achievement, and hazard ratios with 95% CIs for survival outcomes. In multivariate analysis, HSCT was considered

Table 1. Demographics and main clinico-diagnostic characteristics of 572 patients randomly assigned to receive induction chemotherapy with either ICE or sHD

Patient characteristics	All patients (N = 572)	ICE (N = 286)	sHD (N = 286)	*P*
Age (at randomization), median (range), y	52 (16-73)	53 (16-73)	50 (18-72)	.34
>60 y, n (%)	140 (24.5)	73 (25.5)	67 (23.4)	.55
Sex				
Male, n (%)	301 (52.6)	156 (54.5)	145 (50.7)	.35
Diagnosis, n (%)				
AML morphological subtype*				.82
SR	478 (83.6)	240 (83.9)	238 (83.2)	
HR	94 (16.4)	46 (16.1)	48 (16.8)	
HR MDS	6 (1.0)	3 (1.0)	3 (1.0)	
AML category				.80
De novo	494 (86.4)	248 (86.7)	246 (86.0)	
Non de novo†	78 (13.6)	38 (13.3)	40 (14.0)	
ECOG PS, n (%)				.01
0	292 (51.1)	130 (45.5)	162 (56.6)	
1	232 (40.6)	134 (46.9)	98 (34.3)	
2	42 (7.3)	19 (6.6)	23 (8.0)	
3	6 (1.0)	3 (1.0)	3 (1.0)	
Clinical features, n (%)				
Hepatomegaly	42 (7.3)	20 (7.0)	22 (7.7)	.74
Splenomegaly	65 (11.4)	29 (10.1)	36 (12.6)	.35
Extramedullary involvement	71 (12.4)	34 (11.9)	37 (12.9)	.70
Hematology, median (range)				
Hemoglobin, g/dL	9.1 (3-15.8)	9.3 (3.6-14.8)	8.9 (3-15.8)	.82
WBC, $\times 10^9$/L	10.5 (0.5-990)	12.3 (0.5-990)	9.2 (0.6-260)	.10
Platelets, $\times 10^9$/L	53 (2-852)	53 (2-815)	53.5 (3-852)	.74
Peripheral blood blast cells, %	47.5 (0-100)	46 (0-100)	48 (0-100)	.94
Bone marrow blast cells, %	77 (0-100)	80 (6-100)	74.5 (0-100)	.18
Cytogenetics, n (%)				
Favorable	52 (9.1)	23 (8.0)	29 (10.1)	.59
t(8;21)‡	20 (3.5)	10 (3.5)	10 (3.5)	
inv(16)§,‖	32 (5.6)	13 (4.5)	19 (6.6)	
Intermediate				
Normal karyotype	272 (47.6)	142 (49.7)	130 (45.5)	.31
Abnormal¶	9 (1.6)	4 (1.4)	5 (1.7)	.08
Adverse#	170 (29.7)	82 (28.7)	88 (30.8)	.52
Other	12 (2.1)	6 (2.1)	6 (2.1)	1.0
Unknown	57 (10.0)	29 (10.1)	28 (9.8)	.88

After amendment 3 (September 2009), patients older than 65 years were no longer enrolled into the study.

+, gene rearrangement or mutation; ITD, internal tandem duplication; PM, point mutation; NILG, Northern Italy Leukemia Group; sHD, sequential HD cytarabine/idarubicin; WBC, white blood cell count.

*HR: minimally differentiated AML, erythroleukemia, megakaryoblastic leukemia, undifferentiated/bilineal/biphenotypic acute leukemia, acute panmyelosis with myelofibrosis, myeloid sarcoma, AML with multilineage dysplasia, HR MDS (marrow blast cells, 10%-20%); SR, all other morphological and diagnostic subsets.

†AML after myelodysplastic or chronic myeloproliferative syndromes, related to another therapy (chemotherapy/radiotherapy for another cancer), or preceded by an antecedent hematologic disorder (unexplained cytopenia).

‡Total number with either method: 27/572 (4.7%).

§inv(16), t(16;16), del(16q).

‖Total number with either method: 42/572 (7.3%).

¶Abnormalities included +6, +11, +13, +22, del(12p), t(9;11), -Y.

#Adverse abnormalities included −5/del(5q), −7/del(7q), t(11;19)/t(11q23), and MLL gene rearrangements, t(9;22), abn 3q,9q,11q,12p,20q,21q,17p, iso(17q), +8, +21, t(3;3), t(3;5); inv(3), t(6;9), t(6;11), and complex karyotype with 3 or more unrelated clonal markers.

**Eleven patients with concurrent favorable and adverse cytogenetics were included in the adverse group, as per protocol design.

Table 1. (continued)

Patient characteristics	All patients (N = 572)	ICE (N = 286)	sHD (N = 286)	*P*
Genetics, n (%)				
MLL-rearrangement	24/397 (6.0)	10/200 (5.0)	14/197 (7.1)	.65
FLT3-ITD+	103/569 (18.1)	55/284 (19.4)	48/285 (16.8)	.58
FLT3-PM+	40/555 (7.2)	22/275 (8.0)	18/280 (6.4)	.36
NPM1+	167/551 (30.3)	87/277 (31.4)	80/274 (29.2)	.68
CBFB-MYH11+‖	41/557 (7.4)	16/278 (5.8)	25/279 (9.0)	.33
AML1/ETO+‡	27/558 (4.8)	15/277 (5.4)	12/281 (4.3)	.45
CEBPA+	19/361 (5.3)	8/182 (4.4)	11/179 (6.1)	.60
NILG risk group, n (%) **				.13
SR	156 (27.3)	70 (24.5)	86 (30.1)	
HR	416 (72.7)	216 (75.5)	200 (69.9)	
ELN 2010 risk group, n (%)				.56
Favorable	166 (29.0)	82 (28.7)	84 (29.4)	
Intermediate 1	147 (25.7)	80 (28.0)	67 (23.4)	
Intermediate 2	70 (12.2)	36 (1.6)	34 (11.9)	
Adverse	135 (23.6)	60 (21.0)	75 (26.2)	
Unknown	54 (9.4)	28 (9.8)	26 (9.1)	

After amendment 3 (September 2009), patients older than 65 years were no longer enrolled into the study.

+, gene rearrangement or mutation; ITD, internal tandem duplication; PM, point mutation; NILG, Northern Italy Leukemia Group; sHD, sequential HD cytarabine/idarubicin; WBC, white blood cell count.

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**Eleven patients with concurrent favorable and adverse cytogenetics were included in the adverse group, as per protocol design.

a time-dependent variable. *P* values were 2-sided and not adjusted for multiple comparison. The significance level was fixed at 5%. Statistical analyses were performed with SAS software version 9.4.

Results

Patients

Figure 2 shows patient disposition and study flow. Between January 2007 and March 2012, we registered and screened 1354 patients for eligibility, of whom 574 were enrolled and randomized. Among these patients, 572 were evaluable: 286 in each study group. Two trial patients were excluded from analysis: 1 withdrew consent and 1 had Philadelphia-positive AML and was switched to tyrosine kinase inhibitors. Five patients did not complete the allocated treatment, including 2 in the ICE group (1 early death, 1 medical decision) and 3 in the sHD group (1 early death; 2 other chemotherapy), but were included in the analysis based on treatment intention. The median patient age was 52 years (range, 16-73 years). Diagnostic characteristics and HR proportions were balanced between study groups, except that the sHD group included a higher incidence of patients with an optimal performance score (Table 1). Of the 1354 patients registered in the prospective outcome study project, 780 were excluded from the trial for the following reasons: diagnosis of acute promyelocytic leukemia (n = 118), trial ineligibility (n = 264), medical decisions related to higher age and/or clinical unfitness (n = 209; median age, 64 years; range, 19-89 years), refusal (n = 43), early death (n = 6), unspecified reason (n = 5), and (starting September 2009) age older than 65 years (n = 135; supplemental File 3.2). The outcomes of all 1354 registered patients are available online (supplemental File 3.3).

CR induction and randomization 1 results

In randomization 1, the CR rate was significantly higher with sHD (n = 233, 81.5%) than ICE (n = 198, 69.2%; $P = .0007$) because of a markedly lower incidence of refractory AML (11.2% vs 25.2%; $P < .0001$) and a comparable early death rate ($P = .39$; Table 2). sHD treatment yielded a RRR of 0.40 (95% CI, 0.19-0.55), matching the predicted primary study endpoint (RRR, 0.38). Based on the course 1 refractory responses, course 2 reinduction chemotherapy was administered to 46/72 patients in the ICE-resistant group and 17/32 patients in the sHD-resistant group, achieving late CR in 33 (71.7%) and 6 (35.3%) patients, respectively, with augmented sHD (n = 25/36, 69.4%) or other chemotherapy (n = 14/27, 51.9%). After 2 induction courses, the overall CR rates were 80.8% in the ICE group and 83.6% in the sHD group ($P = .38$). In addition, CR was achieved in 12 of 13 course-1-resistant patients who underwent off-study allogenic HSCT.

Table 2. Main CR induction and trial results, according to randomization group

	ICE group (n = 286)	sHD group (n = 286)	RRR*/hazard ratio (95% CI)	P
CR induction course 1 (random 1), (%)				
CR†	198 (69.2)	233 (81.5)	0.40 (0.19-0.55)	.0007
NR	72 (25.2)	32 (11.2)		<.0001
ED	16 (5.6)	21 (7.3)		.39
CR induction course 2, n/N (%)				
CR	33/46 (71.7)	6/17 (35.3)	−1.29 (−3.09 to −0.28)	.008
NR	12/46 (26.1)	11/17 (64.7)		.008
ED	1/46 (2.2)	0		1.0
Total CR (courses 1+2), n (%)‡	231 (80.8)	239 (83.6)	0.15 (−0.22 to 0.40)	.38
Relapse and survival estimates				
5-y incidence of relapse (95% CI), %	55 (48-61)	44 (37-50)	0.77 (0.59-0.99)	.046
5-y incidence of death in CR (95% CI), %	10 (6-14)	8 (5-12)	0.87 (0.48-1.60)	.66
Overall survival				
Median, y	2.2	4.5		
5-y rate (95% CI), %	39 (33-45)	49 (42-55)	0.79 (0.63-0.99)	.045
Survival of CR patients				
Median, y	3.6	N/A		
5-y rate (95% CI), %	46 (39-52)	54 (47-61)	0.81 (0.62-1.06)	.12
Relapse-free survival				
Median, y	1.5	3.4		
5-y rate (95% CI), %	36 (29-42)	48 (41-54)	0.77 (0.60-0.97)	.028

N/A, not achieved.

*Proportion of failures that would be avoided with sHD in patients treated with ICE.

†Treatment groups were well distributed by study center (n = 17; $P = .5$). By Cochran-Mantel-Haenszel χ^2 test using center as stratification factor, the higher CR rate with sHD at random 1 remained statistically significant ($P = .001$). Similarly, stratified Cox models confirmed the treatment effect on OS (hazard ratio, 0.78; 95% CI, 0.62-0.99; $P = .041$) and relapse-free survival (hazard ratio, 0.75; 95% CI, 0.59-0.96; $P = .022$).

‡Including CR with incomplete hematological recovery: 3 in ICE group and 8 in sHD group ($P = .12$).

CR induction randomization 1 toxicity

In randomization 1, sHD more frequently led to blood cytopenia, fever, and infections related to treatment-emergent toxicity, as well as hepatic and metabolic complications, but did not increase early mortality (Table 3). From treatment day 1, recovery to a neutrophil count higher than 0.5×10^9/L took 21 days with ICE vs 27 days with sHD ($P < .0001$), and recovery to a platelet count higher than 20×10^9/L took 21 days with ICE vs 29 days with sHD; $P < .0001$). Infection rates were 82% with ICE and 90.5% with sHD ($P = .003$), with sHD associated with high incidences of bacterial and *Aspergillus* spp. etiology, sepsis, and pneumonia. Most hepatic, metabolic, and cutaneous adverse events were CTC grade 2 or lower. Only 2 to 5 episodes in each CTC category scored higher with sHD than ICE.

Postremission therapy

As a result of greater myelosuppression and related toxicities, significantly fewer patients with sHD completed early postremission consolidation (n = 166/239, 69.5%) compared with ICE patients (186/231, 80.5%; $P = .005$). Compared with ICE, sHD required longer intercycle intervals and displayed inferior $CD34^+$ blood cell mobilization (supplemental File 3.4). Of the 470 total CR patients, 193 (41%; 20% of SR and 50% of HR; 102 ICE vs 91 sHD, $P = .18$) received allogeneic HSCT as their final treatment. Another 103 patients (22%; 41% of SR and 13% of HR) received HD treatment under randomization 2, of whom 83 were randomized and 80 actually underwent either autologous HSCT or repetitive blood stem cell-supported HD courses. Of the remaining patients, 97 (21%) received ID chemotherapy courses, 60 (12.8%) underwent only early consolidation because of early relapse or toxicity, and 17 (3.8%) received no postremission therapy.

Long-term results

After a median follow-up of 4.9 years (range, 0.2-8.4 years), 204 patients (35.6%) were alive in first CR (84 ICE [29.3%] and 120 sHD [41.9%]; $P = .002$), 42 (7.3%) survived in second/later CR, 313 (54.7%) died, and 13 (2.3%) were lost to follow-up. The 5-year cumulative incidences of relapse were 55% (ICE) and 44% (sHD; $P = .046$). Compared with ICE, sHD was associated with improved 5-year OS (39% [median, 2.2 years] vs 49% [median, 4.5 years]; $P = .045$), 5-year event-free survival (29% [median, 1.2 years] vs 40% [median, 1.6 years]; $P = .019$), and 5-year RFS (36% [median, 1.5 years] vs 48% [median, 3.4 years]; $P = .028$; Table 2; Figure 3). We further examined the survival effects of allogeneic HSCT or randomization 2 therapy. When the 193 patients who underwent allogeneic HSCT in first CR were censored at the time of transplant, sHD no longer conveyed a detectable significant survival advantage (supplemental File 3.5). However, when separately considering the 2 study groups, sHD-treated patients (n = 91)

Table 3. Induction toxicity according to randomization group

Toxicity type	ICE group (n = 286)	sHD group (n = 286)	*P*
Hematologic toxicity (recovery time), median days (range) from treatment day 1			
Neutrophil count, $\times 10^9$/L			
>0.5	21 (8-54)	27 (13-114)	<.0001
>1.5 (G-CSF stopped)	22 (10-57)	28 (14-66)	<.0001
Platelet count, $\times 10^9$/L			
>20	21 (10-58)	29 (15-76)	<.0001
>50	23 (11-56)	32 (17-90)	<.0001
Fever and infections (clinical picture and etiology), n (%)			
Fever >38°C	222	254	.0002
Days with fever, median (range)	5 (1-24)	5 (1-40)	
FUO	121 (42.6)	121 (42.8)	.97
Bacteremia	45 (15.8)	80 (28.3)	.0004
Sepsis	40 (14.1)	63 (22.3)	.01
Pneumonia	59 (20.8)	83 (29.3)	.01
Other involved site	73 (25.7)	85 (30.0)	.25
Gastrointestinal system	31 (10.9)	30 (10.6)	.90
Skin	48 (16.9)	59 (20.8)	.22
Urinary system	1 (0.4)	2 (0.7)	.99
Central nervous system	1 (0.4)	3 (1.1)	.61
Bacterial	77 (27.1)	122 (43.1)	.0001
Gram$^+$	56 (19.7)	94 (33.2)	.0003
Gram$^-$	28 (9.9)	44 (15.5)	.04
Fungal	24 (8.5)	47 (16.6)	.003
Aspergillus spp.	17 (6.0)	37 (13.1)	.004
Proven	2 (0.7)	5 (1.8)	
Probable	13 (4.6)	21 (7.4)	
Possible	2 (0.7)	11 (3.9)	
Candida spp.	3 (1.1)	7 (2.5)	.33
Other	4 (1.4)	5 (1.8)	.99
Parasitic	2 (0.7)	0	.48
Viral	15 (5.3)	16 (5.7)	.84
Unknown	123 (43.3)	108 (38.2)	.21
Other toxicity (type/organ), CTC grading, n			
Hemorrhage			.16
Any grade	41	53	
Grade >2	15	16	
Cardiovascular system			.87
Any grade	19	18	
Grade >2	9	8	
Coagulation			.36
Any grade	12	8	
Grade >2	8	6	
Hepatobiliary system			.01
Any grade	65	91	
Grade >2	23	28	
Metabolism			.04
Any grade	51	70	
Grade >2	18	22	
Kidney			.28
Any grade	9	5	
Grade >2	3	3	
Amylase			1.0
Any grade	4	2	
Grade >2	1	1	
Central/peripheral nervous system			.78
Any grade	7	6	
Grade >2	2	3	
Gastrointestinal system			.42
Any grade	125	134	
Grade >2	39	46	
Skin			.0003
Any grade	37	71	
Grade >2	5	7	
Lungs			.13
Any grade	25	36	
Grade >2	10	18	
Allergy			.73
Any grade	19	21	
Grade >2	2	4	

FUO, fever of unknown origin

exhibited significantly better posttransplantation outcomes than ICE-treated patients (n = 102): 5-year survival of 69% vs 55% ($P = .06$) and RFS of 66% vs 49% ($P = .03$; supplemental File 3.6). Finally, among randomization 2 patients (n = 83 evaluable; 44 ICE and 39 sHD), 5-year OS and RFS did not significantly differ between the 2 groups (supplemental File 3.7.1). Projected RFS was 50% (median, 2.6 years) in the blood stem cell-supported HD chemotherapy group (n = 42, 103 total HD courses; median, 3 courses; range, 1-3 courses) and 36% (median, 1.48 years) in the BU-CY autotransplantation group (n = 41; $P = .31$). We observed a trend of better survival with repetitive HD courses than with autotransplantation in patients with sHD ($P = .05$; supplemental File 3.7.2), but no superiority compared with ICE-treated patients. Compared with HR patients, SR patients had a higher 5-year RFS (51% vs 30%; $P = .03$). Pancytopenic death rates were 2.4% with autotransplantation vs 2.5% with HD courses (1% among 103 total HD courses). The cumulative incidence of bacterial infections and metabolic, neurologic, and cutaneous toxicity was greater in the repetitive HD chemotherapy group (supplemental File 3.8).

Prognostic analysis

Early CR rates were significantly higher with sHD than ICE in several groups, including the large reference group of patients aged 60 years or less with de novo AML, the HR AML group, adverse genetic/cytogenetic subsets, and the *NPM1* wild-type AML group

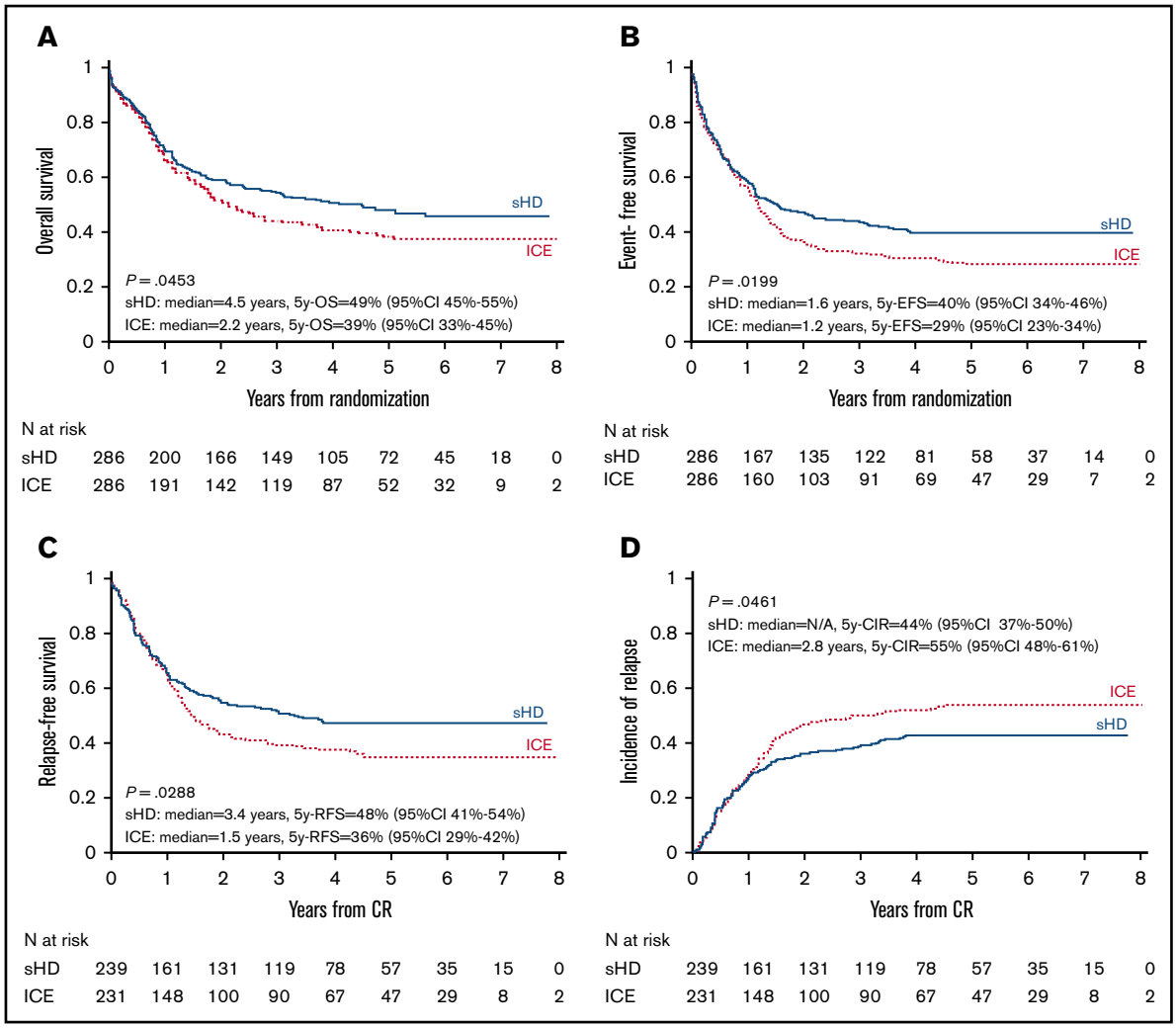

Figure 3. Kaplan-Meier survival analyses, according to the randomization group. (A) OS. (B) Event-free survival (EFS). (C) RFS. (D) Cumulative incidence of relapse (CIR).

(Figure 4). Although the sHD group had a higher incidence of favorable Eastern Cooperative Oncology Group performance score (ECOG PS 0-1), this regimen was associated with an improved early CR rate even among patients with an ECOG PS higher than 1. Some smaller patient populations showed nearly significant (P = .05-.09) CR differences between treatments, including patients with HR morphological variants, high leukocyte counts, age older than 60 years, and/or non de novo AML. The assumption that an improved early CR rate would positively affect OS was validated in both SR and HR groups (supplemental File 3.9), excluding center-related effects on early CR rate, OS, and RFS (Table 2). sHD improved OS and/or RFS in patients aged 60 years or younger with de novo AML, with SR AML, and with core-binding factor-positive AML. Post hoc analysis with the ELN 2010 genetic risk stratification revealed that sHD improved OS and/or RFS in the favorable group, including patients with the *NPM1*-mutated/*FLT3* wild-type genotype or mutated *CEBPA* with a normal karyotype other than core-binding factor AML (supplemental File 3.10).

Multivariate analysis including randomization 1 chemotherapy and the allogeneic HSCT results described earlier confirmed that both sHD induction chemotherapy and allogeneic HSCT had independent, favorable, prognostic effects on survival. This effect was not detectable with autotransplantation in patients entering randomization 2 (supplemental File 3.7.2). Older age, poor ECOG performance score, intermediate/adverse cytogenetics, and *FLT3* mutations negatively affected survival probability, and *NPM1* mutations were consistently associated with better outcome (Table 4). We repeated this analysis with the study risk model and the ELN 2010 genetic risk stratification. After removing the single risk factors included in either risk classification, both sHD regimen and the SR or ELN favorable risk profile were associated with better CR, OS, and RFS.

Discussion

In this randomized study, we compared the sHD regimen with standard ICE chemotherapy, which was considered at least as effective as a HD daunorubicin "3+7" regimen for inducing CR in adult AML.[9,11] This controlled study evolved from a prior AML investigation in which we tested sequential HD reinduction for ICE-unresponsive patients,[29,38] obtaining similar results across all cytogenetic risk groups,[28] and evaluated blood stem cell-supported HD consolidation courses (NCT00400673) in lieu of autotransplantation.[45-47]

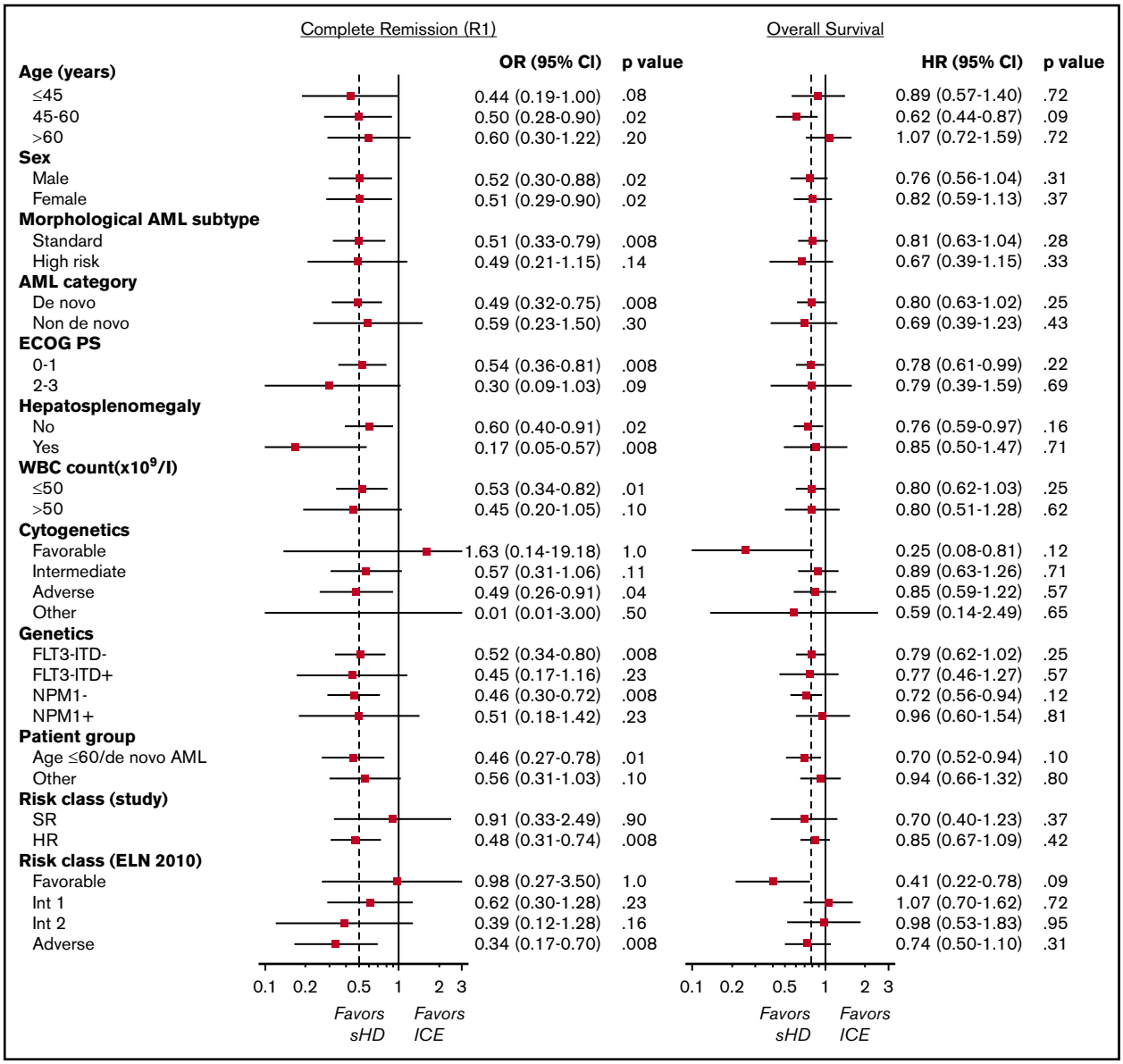

Figure 4. Forest plot of study groups. Effects of treatments on CR and OS, according to the main patient and disease characteristics.

Our present results indicated that sHD yielded a significantly better early induction response and survival than ICE. This study included 572 evaluable patients aged at least 16 years (median age, 52 years). More than 20% had very HR AML or HR MDS and/or were aged older than 60 years, and more than 70% were classified as HR according to the mixed functional clinico-cytogenetic prognostic model used to distinguish between patients eligible for allogeneic HSCT (HR) and others (SR). The primary study endpoint (CR after course 1) showed therapeutic benefit with sHD (early CR of 81.5% vs 69.2%; $P = .0007$) that was attributable to a reduction of chemo-resistant AML from 25% to 11%. The sHD and ICE groups exhibited comparable early mortality, consistently below 10% and lower than reported in German trials with similar regimens.[14,26] After confirming these results in most HR subsets, including patients with adverse ECOG PS, we further demonstrated the feasibility of the current sHD schedule. Although sHD lacked double induction with recycling on day 21, as in the AMLSG trial,[48] it did not favor higher resistance rates. After 23 ICE-resistant patients entered CR with augmented sHD, the gap between study groups (resulting from early CR) was eventually closed. The prognostic advantage related to early CR was confirmed by comparative survival analysis including late CR patients. Retreatment of sHD-resistant patients was more difficult, with poor results after augmented sHD. These very HR patients should be considered for new experimental therapies and/or immediate HSCT salvage.

Induction mortality was not higher with sHD, but sHD showed higher hematologic and infectious toxicity than ICE, which hampered postremission therapy, contributing to deficient patient accrual for randomization 2. However, despite the trend of inferior transplantability, sHD provided a lower relapse incidence and better RFS than the

Table 4. Multivariate analysis of factors that influence CR, OS, and RFS, based on study group, individual prognostic variables including key therapeutic steps (study group, allogeneic HSCT), and combined risk classification models (prospective study classification and post hoc ELN 2010 risk stratification)

	All patients		Complete remission		Overall survival		Relapse-free survival	
	N	CR, n (%)	OR (95% CI)	*P*	Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*
Individual prognostic factors								
Study group								
ICE	286	231 (80.8)	1.00		1.00		1.00	
sHD	286	239 (83.6)	2.11 (1.38-3.23)	.0006	0.71 (0.57-0.89)	.004	0.70 (0.55-0.89)	.004
HSCT*								
No	379	—	—	—	1.00		1.00	
Yes	193	—	—	—	0.33 (0.25-0.44)	<.0001	0.32 (0.23-0.43)	<.0001
Age, y								
≤45	179	163 (91.1)	1.00		1.00		1.00	
45-60	253	209 (82.6)	0.65 (0.38-1.11)	.11	1.25 (0.93-1.69)	.14	0.93 (0.69-1.25)	.62
>60	140	98 (70.0)	0.50 (0.28-0.90)	.02	1.42 (1.03-1.95)	.03	0.81 (0.57-1.16)	.25
Sex								
Male	301	245 (81.4)	1.09 (0.71-1.68)	.69	0.84 (0.67-1.06)	.15	0.76 (0.59-0.98)	.04
Female	271	225 (83.0)	1.00		1.00		1.00	
Morphological AML subtype								
SD	478	403 (84.3)	1.00		1.00		1.00	
HR	94	67 (71.3)	0.62 (0.37-1.06)	.08	1.12 (0.83-1.53)	.46	1.14 (0.79-1.65)	.49
AML category								
De novo	494	418 (84.6)	1.00		1.00		1.00	
Non de novo	78	52 (66.7)	0.63 (0.35-1.11)	.10	1.39 (1.00-1.92)	.05	1.48 (0.99-2.19)	.05
ECOG PS								
0-1	524	438 (83.6)	1.00		1.00		1.00	
2-3	48	32 (66.7)	0.48 (0.23-1.02)	.05	1.26 (0.85-1.85)	.25	0.85 (0.52-1.39)	.53
Hepatosplenomegaly								
No	487	397 (81.5)	1.00		1.00		1.00	
Yes	85	73 (85.9)	1.49 (0.77-2.88)	.23	1.34 (0.97-1.85)	.08	1.58 (1.14-2.20)	.006
WBC count, $\times 10^9$/L								
≤50	443	365 (82.4)	1.00		1.00		1.00	
>50	129	105 (81.4)	0.60 (0.33-1.07)	.08	1.35 (1.01-1.82)	.04	1.33 (0.96-1.82)	.08
Cytogenetics								
Favorable	52	50 (96.2)	1.00		1.00		1.00	
Intermediate	281	246 (87.5)	0.20 (0.05-0.74)	.01	2.53 (1.44-4.45)	.001	1.91 (1.16-3.14)	.01
Adverse	170	118 (69.4)	0.10 (0.03-0.36)	.0005	5.63 (3.22-9.84)	<.0001	4.68 (2.80-7.83)	<.0001
Other	12	11 (91.7)	0.22 (0.03-1.62)	.13	5.46 (2.27-13.15)	.0002	3.80 (1.57-9.15)	.003
Genetics								
FLT3-ITD$^-$	466	380 (81.5)	1.00		1.00		1.00	
FLT3-ITD$^+$	103	88 (85.4)	0.52 (0.28-0.97)	.03	1.90 (1.40-2.57)	<.0001	1.88 (1.36-2.62)	.0002
NPM1 wild-type	384	299 (77.9)	1.00		1.00		1.00	
NPM1 mutated	167	152 (91.0)	4.52 (2.40-8.55)	<.0001	0.56 (0.42-0.76)	.0001	0.64 (0.47-0.86)	.003

Variations in the prognostic significance of individual risk factors outside the combined risk models are reported in the footnotes

OR, odds ratio.

*Time-dependent variable.

†The study risk classification incorporated: morphological AML subtype, AML category, hepatosplenomegaly, WBC, *FLT3* mutations, and cytogenetics. In addition to SR risk class, the modified prognostic model identified significant associations for improved CR (sHD group, $P = .0008$; age ≤60 years, $P = .01$; mutated *NPM1*, $P < .0001$), OS (HSCT, $P < .0001$; sHD group, $P = .05$; mutated *NPM1*, $P = .0006$), and RFS (HSCT, $P < .0001$; sHD group, $P = .003$; mutated *NPM1*, $P = .007$). All other factors did not reach statistical significance ($P > .05$).

‡The ELN 2010 risk stratification incorporated: cytogenetics and genetics. In addition to the favorable risk class, the modified prognostic model identified significant associations for improved CR (sHD group, $P = .0004$; age ≤ 60 years, $P = .03$), OS (HSCT, $P < .0001$; sHD group, $P = .003$; no hepatosplenomegaly, $P = .009$; WBC, ≤50 $\times 10^9$/L, $P = .02$), and RFS (HSCT, $P < .0001$; sHD group, $P = .001$; no hepatosplenomegaly, $P = .0002$; WBC, ≤50 $\times 10^9$/L, $P = .02$). All other factors did not reach statistical significance ($P > .05$).

Table 4. (continued)

	All patients		Complete remission		Overall survival		Relapse-free survival	
	N	CR, n (%)	OR (95% CI)	P	Hazard ratio (95% CI)	P	Hazard ratio (95% CI)	P
Risk stratification models								
Study risk classification†								
SR	156	145 (92.9)	1.00		1.00		1.00	
HR	416	325 (78.1)	0.31 (0.18-0.55)	<.0001	3.52 (2.58-4.80)	<.0001	3.22 (2.37-4.38)	<.0001
ELN 2010 risk stratification‡								
Favorable	166	159 (95.8)	1.00		1.00		1.00	
Intermediate 1	147	119 (81.0)	0.18 (0.09-0.39)	<.0001	3.55 (2.46-5.11)	<.0001	2.78 (1.95-3.95)	<.0001
Intermediate 2	70	58 (82.9)	0.21 (0.09-0.52)	.0006	3.81 (2.46-5.90)	<.0001	4.53 (2.95-6.96)	<.0001
Adverse	135	92 (68.1)	0.11 (0.05-0.24)	<.0001	6.88 (4.73-9.99)	<.0001	5.69 (3.89-8.33)	<.0001

Variations in the prognostic significance of individual risk factors outside the combined risk models are reported in the footnotes
OR, odds ratio.
*Time-dependent variable.
†The study risk classification incorporated: morphological AML subtype, AML category, hepatosplenomegaly, WBC, *FLT3* mutations, and cytogenetics. In addition to SR risk class, the modified prognostic model identified significant associations for improved CR (sHD group, $P = .0008$; age ≤60 years, $P = .01$; mutated *NPM1*, $P < .0001$), OS (HSCT, $P < .0001$; sHD group, $P = .05$; mutated *NPM1*, $P = .0006$), and RFS (HSCT, $P < .0001$; sHD group, $P = .003$; mutated *NPM1*, $P = .007$). All other factors did not reach statistical significance ($P > .05$).
‡The ELN 2010 risk stratification incorporated: cytogenetics and genetics. In addition to the favorable risk class, the modified prognostic model identified significant associations for improved CR (sHD group, $P = .0004$; age ≤ 60 years, $P = .03$), OS (HSCT, $P < .0001$; sHD group, $P = .003$; no hepatosplenomegaly, $P = .009$; WBC, $\leq 50 \times 10^9$/L, $P = .02$), and RFS (HSCT, $P < .0001$; sHD group, $P = .001$; no hepatosplenomegaly, $P = .0002$; WBC, $\leq 50 \times 10^9$/L, $P = .02$). All other factors did not reach statistical significance ($P > .05$).

control group, consistent with the idarubicin/HD cytarabine/fludarabine group of the Medical Research Council 15 trial.[49] These results suggested that early treatment intensity had an overall higher effect than the number and interval of subsequent chemotherapy courses. Both the current sHD and idarubicin/HD cytarabine/fludarabine regimens included idarubicin plus cytarabine 2 g/m^2, with G-CSF, and the latter regimen added fludarabine.[49,50] Together, these findings suggest that sHD may be a valid alternative to the classic "3+7" or ICE regimens for inducing early CR, particularly in HR patients, and to standard reinduction for patients unresponsive to a conventional first course, in keeping with recent ELN recommendations.[1]

Separate analysis was required to assess suitability for allogeneic HSCT and its effects. HSCT was applicable to 50% of HR patients, and multivariate analysis confirmed that HSCT was an essential component of curative treatment of many of these patients. However, sHD patients had a significantly better posttransplantation course than ICE patients, indicating a positive interaction between HD induction and postinduction HSCT. Recent HSCT studies have demonstrated the usefulness of MRD analysis for predicting the risk for transplantation failure.[51-53] Our present randomization 1 results might suggest that sHD-treated patients may harbor less residual AML cells before HSCT compared with ICE-treated patients. Although a formal pretransplantation MRD study was not planned, at randomization 1, we performed an immunophenotypic study of early peripheral blast cell clearance.[54] These data will be separately reported, and could confirm that the sHD regimen had greater activity in achieving early blast cell reduction.

One main limitation of this study was that induction course 2 was not delivered as homogeneously as planned. Among course-1-resistant patients, 43% did not receive the augmented sHD regimen because of medical decisions. However, this finding did not affect the analysis of randomization 1 results and did not hamper the outcome comparison between early vs late CR patients. In addition, CR was not centrally assessed but was evaluated using standard criteria.[5] Another limitation was that randomization 2 failed because of lower-than-expected patient accrual. This step required the harvest of 2 to 6 $\times$ 10^6/kg CD34$^+$ blood cells, which was successful in only 45.9% of CR cases. We previously adopted the use of repetitive HD consolidation courses, supported with 1 to 2 $\times$10^6/kg CD34$^+$ blood stem cells (NCT00400673), to avoid prolonged pancytopenia-related complications and ensure dose-dense consolidation[55,56] compared with similarly effective schedules.[57] Compared with BU-CY-conditioned autotransplantation, we found that the HD consolidation modality was feasible, with minimal mortality risk, although infectious morbidity was increased as a result of the cumulative effects of multiple HD courses. This type of consolidation preserved fertility and showed noninferior outcomes compared with autotransplantation. As a consequence, we consider this protocol for younger patients who are excluded from allogeneic HSCT.

Another limitation of this study was that we lacked details regarding new highly relevant genetic markers.[58,59] Nevertheless, our univariate and multivariate prognostic analyses revealed factors associated with the best outcomes. We defined HR categories according to cytogenetics (with results comparable to other adult AML series), adverse clinical characteristics, and *FLT3* mutations. We also studied *NPM1*, *MLL*, and *CEPBA* gene mutations according to the ELN 2010 genetic risk stratification, which allowed analysis refinements in many cases. Both approaches confirmed that sHD was superior to ICE in achieving CR (particularly in HR subsets) and prolonging OS and RFS. The best results were observed in SR or favorable-risk (ELN) groups, and in the typical subset of patients aged 60 years or younger with de novo AML. The improved CR rate with sHD in the HR group did not affect survival in the general intention-to-treat analysis. However, this combination provided high RFS and survival rates in the subgroup of CR patients proceeding to HSCT.

Overall, our present findings highlighted the inappropriateness of a uniform postremission therapy, with HSCT as the sole therapeutic option, among patients with aggressive disease behavior. In this HR setting, and for AML in general, we might further improve results by targeting specific molecular lesions.[60,61]

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Authorship

Contribution: R.B. and A.R. designed and performed research, analyzed and interpreted data, and wrote the manuscript; T.I., G.G., F.M., I.C., D.M., E.T., L.D.P., C.C., E.B., F.C., M.B., A.M.S., E.T., L.C., P.C., A.C., D.F., P.Z., O.S., E.A., S.C., A.B., B.F., and E.M.P. performed research and collected data; A.M., C.B., and E.O. analyzed and interpreted data and performed statistical analysis; and C.P. analyzed and interpreted data, performed statistical analysis, and wrote the manuscript.

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