Improved leukemia-free survival after postconsolidation immunotherapy with histamine dihydrochloride and interleukin-2 in acute myeloid leukemia: results of a randomized phase 3 trial

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The primary objective of this phase 3 study was to determine whether postconsolidation immunotherapy with interleukin-2 (IL-2) and histamine dihydrochloride (HDC) improved the leukemia-free survival (LFS) of adult patients with acute myeloid leukemia (AML) in complete remission (CR). Three hundred twenty patients with AML (median age, 57 years; range, 18-84 years) were stratified by CR1 or subsequent CR (CR > 1) and randomly assigned to treatment with HDC/IL-2 or no treatment (control). Treatment comprised 10 21-day cycles with IL-2 (16 400 U/kg) plus HDC (0.5 mg); both compounds were administered by subcutaneous injection twice daily. Study arms were balanced for age, sex, previous treatment, leukemic karyotypes, time from CR to inclusion, and frequency of secondary leukemia. Three years after enrollment of the last patient, treatment with HDC/IL-2 was found to improve LFS over control in the study population (CR1 + CR > 1, n = 320; $P < .01$, log-rank test). For patients in CR1 (n = 261), treatment significantly improved LFS ($P = .01$) with 3-year LFS estimates of 40% (HDC/IL-2) compared with 26% (control). Side effects were typically mild to moderate. These results indicate that HDC/IL-2 treatment offers an efficacious and tolerable treatment for patients with AML in remission. (Blood. 2006;108: 88-96)

Introduction

After induction chemotherapy, most patients with acute myeloid leukemia (AML) will achieve complete remission (CR). Despite ensuing consolidation therapies, less than one third of adult patients with AML are permanently cured, and the overriding clinical problem is the high rate of leukemic relapse.[1,2] A small proportion of patients with AML proceed to allogeneic stem cell transplantation (allo-SCT),[3] but there is no consensus about effective relapse-protective therapy beyond the completion of consolidation for patients who do not receive an allo-graft, and the standard of care hence is no treatment. The risk of leukemic relapse along with the limited prospects of long-term survival after a relapse[1,2] call for novel therapeutic strategies to maintain CR in AML.

Although the precise role of the immune system in the control and eradication of leukemic cells remains unresolved, several independent lines of evidence suggest that cytotoxic effector cells such as T cells and natural killer (NK) cells participate in protecting patients with AML against relapse.[4-7] The graft-versus-leukemia (GVL) effect achieved by allo-SCT using HLA-matched donors relies at least in part on the integrity of T cells, as demonstrated inter alia by an increased risk of relapse after T-cell depletion or after the use of immunosuppressive regimens aimed at reducing graft-versus-host disease.[3,8] In addition, recent studies imply that the GVL effect exerted by HLA haplotype-mismatched transplants is mediated by alloreactive NK cells; thus, donor/recipient HLA class I disparities are assumed to trigger grafted NK cells to eliminate recipient leukemic cells and to protect patients against relapse.[9] The notion that NK cells also play a protective role in patients with AML not receiving an allograft is supported by reports suggesting that the functional status of NK cells recovered from patients with AML in remission not receiving a transplant is inversely correlated with risk of relapse.[4-7]

T cells and NK cells with antileukemic activity can be recovered from most patients with AML in remission not receiving a transplant,[6,10] and attempts have been made to pharmacologically

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M.B. was the principal investigator; B.N. was the medical monitor; M.B., K.G., B.N., E.W., and K.H. wrote the study protocol with the assistance of B.S.; K.H. wrote the manuscript with the assistance of M.B., B.N., R.O., J.S., K.G., and A.I.R.; M.B., S.C., J.C., W.K.H., A.D.H., D.E.H., R.O., J.M.R., R.S., and E.A.S. were principal investigators in their respective countries.

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activate these lymphocytes to attack and destroy residual leukemic cells. IL-2, an endogenous T-cell– and NK-cell–activating cytokine, has been administered to patients with AML in nonrandomized trials with the aim of preventing relapse, but the outcome of these studies has been inconsistent,[11-14] and the 2 randomized studies so far reported have failed to demonstrate a reduced frequency of relapse in patients treated with IL-2.[15,16]

The present study evaluated an approach to immunotherapy in AML in which IL-2 is supplemented with histamine dihydrochloride (HDC) to enhance the function of cytotoxic antileukemic lymphocytes. Our study was based on in vitro and in vivo results demonstrating that HDC/IL-2 yields a more efficient lymphocyte-mediated destruction of several human malignant target cells, including freshly recovered AML blasts, than either compound alone.[17-20] Clinical studies in melanoma have suggested that cytotoxic lymphocytes are more efficiently activated by systemic treatment with HDC/IL-2 in vivo than by monotherapy with IL-2.[21] A pilot study in patients with AML suggested that treatment using HDC/IL-2 in first or subsequent CR was safe and feasible,[18,22] and the outcome in terms of relapse protection was considered encouraging.

In the phase 3 trial reported herein, adult patients with AML in CR received systemic postconsolidation immunotherapy with HDC and IL-2 with the aim of preventing relapse of leukemia. The trial recruited 320 patients with AML in CR who were randomly assigned to receive 18 months of treatment with HDC/IL-2 or no treatment. We account for the primary trial endpoint, ie, the leukemia-free survival (LFS), of all study patients 3 or more years after the last enrollment, along with predefined secondary endpoints, including LFS rates in patients in first (CR1) or subsequent CR (CR > 1), overall survival, and toxicity.

Patients, materials, and methods

Patients

Patients aged 18 years or older with de novo or secondary AML were eligible for enrollment. Inclusion criteria were verified CR; adequate renal, cardiac, and pulmonary functions; and a performance status (according to Eastern Cooperative Oncology Group [ECOG] criteria) of 0 to 1. Any previous induction or consolidation therapy was allowed with the exception of allo-SCT; other exclusion criteria included active peptic ulcer, a history of recent asthma, or previous hypersensitivity reactions. Elapsed time from dates of CR and the completion of consolidation chemotherapy were not to exceed 6 and 3 months, respectively. The characteristics of all participating patients.

Study design and objective

This open-label, randomized, multicenter phase 3 study enrolled patients with AML at 100 centers in Australia, Canada, Europe, Israel, New Zealand, and the United States between June 1998 and October 2000. The patients were enrolled after the completion of induction and consolidation therapies and were randomly assigned to either a treatment or a control arm. Country-specific randomization schedules were produced electronically based on a block size of 4 and stratified by CR status. The investigators received the treatment assignment from a centralized randomization center.

Altogether 320 patients were enrolled in the study, 261 patients in the CR1 group and 59 in the subsequent CR group. The primary objective was to determine the efficacy of postconsolidation maintenance treatment with HDC/IL-2 versus control on the LFS of all patients. The secondary objectives included LFS rates at 12, 24, and 36 months after random assignment, effects of treatment on LFS of patients in CR1 and subsequent CR, overall survival, safety, toxicity, and quality of life. The trial was approved by the ethics committee of each participating institution, and all patients gave written informed consent before enrollment.

Treatment and dosing rationale

The study scheme is presented in Figure 1. Patients in the treatment arm received 10 consecutive 3-week cycles of HDC/IL-2, whereas patients in the control arm received no treatment. The treatment continued for a total of 18 months or until the patients relapsed, died, discontinued therapy because of adverse events, withdrew consent, or became lost to follow-up. Cycles 1 to 3 comprised 3 weeks of treatment and 3 weeks off treatment, and in cycles 4 to 10 the off-treatment periods were extended to 6 weeks. In each cycle, patients in the treatment arm received HDC (Maxim Pharmaceuticals, San Diego, CA) at 0.5 mg subcutaneous twice a day and human recombinant IL-2 (aldesleukin; 16 400 U/kg subcutaneous twice a day; Chiron Corporation, Emeryville, CA). After 18 months of treatment (HDC/IL-2 arm) or observation (control arm), all patients were followed for at least 18 additional months until the study closure date on October 31, 2003 (ie, 3 years after enrollment of the last patient).

To avoid acute toxicity, HDC was administered at a rate not exceeding 0.1 mg per minute. In the event of HDC-related side effects, the injection time was prolonged to 7 to 10 minutes; if toxicity persisted, the dose was reduced by 20%. Reduction of the IL-2 dose was prescribed in case of side effects or inconveniences related to this treatment. The first doses of study drugs were administered under the supervision of the investigator. Subsequent doses were administered by the patients at home. The dose of HDC was predicted to saturate 70% to 80% of phagocyte cell histamine H_2 receptors,[23-25] and the dose of IL-2 had been shown previously to cause significant expansion of cytotoxic lymphocytes with documented antileukemic activity.[18,19,26] The doses and schedules were considered suitable for long-term treatment on the basis of the results obtained in a pilot study.[22]

Definitions and response criteria

Complete remission (CR) was defined as less than 5% blast cells in normocellular bone marrow, without evidence of extramedullary leukemia. Relapse was defined as at least 5% blast cells in the bone marrow or extramedullary leukemia. Leukemia-free survival (LFS) was defined as the time from random assignment to the date of relapse or death from any cause, whichever occurred first. Overall survival was measured from the date of random assignment to death from any cause. Patients who were lost to follow-up or were still alive at the time of data cutoff were censored at the last date they were known to be alive. Patients randomly assigned in subsequent CR (CR > 1) had previously relapsed at least once following CR1 and achieved a new remission. Analysis of safety included patients who received at least one dose of HDC/IL-2 or had measurements in a cycle for the control arm.

For the duration of the trial, patients in the control arm followed the same schedule of clinical and laboratory assessments, including bone marrow investigations when clinically indicated, as those in the treatment arm. All clinical and laboratory assessments were performed locally.

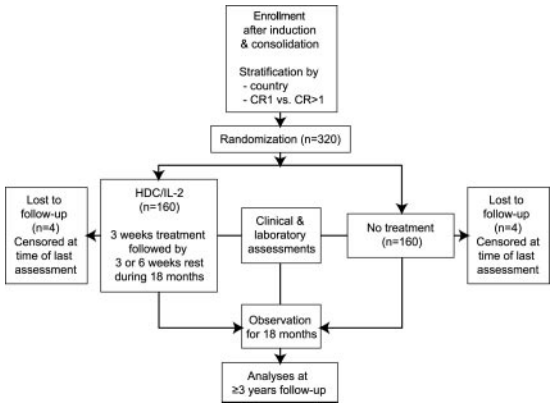

Figure 1. Study scheme.

Toxicity grading was made according to the National Cancer Institute Common Toxicity Criteria, version 2. Original reports of cytogenetics were classified according to the MRC criteria.[27]

Statistical analyses

Sample size calculation. The number of subjects was initially based on a hypothesized improvement in median LFS of 50% in subjects in CR1 in the HDC/IL-2 arm compared with control subjects. Ninety-six events in CR1 would be needed in each study arm to provide a statistical power of 80%, with a type I error rate of 0.05.[28] For the hypothesized improvement in median LFS of 75% in subjects in subsequent CR, 51 events in each study arm would provide a statistical power of 80%, with a type I error rate of 0.05. In the initial protocol there were 2 primary endpoints; one for patients in CR1 and one for patients in subsequent CR. However, when a total of 320 subjects (261 in CR1 and 59 in CR > 1) had been enrolled, accrual of the necessary number of subjects in subsequent CR did not seem to be feasible. Therefore, further recruitment was terminated in October 2000, and in a protocol amendment the primary endpoint was changed to determination of LFS in the combined population of patients in CR1 and in subsequent CR (ie, all patients randomly assigned into the trial). In October 2000, 320 patents had entered the study; these patients were to be followed for at least 3 years, and it was estimated that a sufficient number of events would occur before that time point.

Efficacy analyses. The primary efficacy analysis, duration of LFS, was thus conducted on the population of all randomly assigned patients. The Kaplan-Meier procedure was used to estimate the LFS and overall survival distributions for all patients (n = 320), as well as for the subgroups of patients in CR1 (n = 261) and in subsequent CR (n = 59). The LFS or survival estimates were compared using a log-rank test. For the log-rank comparisons, all events recorded until the closure date of the study were taken into account. Frequency analyses were performed by using the chi-square test or the Fisher exact test. All statistical analyses of efficacy (LFS and overall survival) were stratified by country and, if applicable, CR stratum (CR1 or CR > 1). The efficacy analyses were performed according to the intent-to-treat principle, and all reported *P* values are 2-sided.

Cox modeling. Cox proportional hazards model regression analyses,[29,30] stratified by country and CR status (CR1 and CR > 1), were performed to assess the effects of possible confounding covariates on LFS. The Cox model included treatment and covariates identified as having a prognostic influence on outcome. The final model included the treatment effect and the variables selected in the univariate models.

Results

Patient characteristics

Three hundred twenty patients with AML in their first (CR1) or subsequent (CR > 1) CR were enrolled. One hundred sixty patients were randomly assigned to the HDC/IL-2 treatment arm and 160 to the control arm. Two hundred sixty-one patients were in CR1, and 59 were in subsequent CR. The 2 arms were evenly distributed with respect to prespecified prognostic factors (Table 1).

Table 1. Patient characteristics

	Control; n = 160	HDC/IL-2; n = 160
Sex		
Men	86 (54)*	86 (54)
Women	74 (46)	74 (46)
Age, y, median (range)		
Overall	54 (18-84)	55 (18-81)
Older than 60 y	59 (37)	66 (41)
CR status		
CR1	132 (82)	129 (81)
CR > 1	28 (18)	31 (19)
FAB classification		
M0/M1/M5/M6	56 (35)	60 (38)
M2/M3/M4	93 (58)	87 (54)
Performance status†		
0	114 (71)	125 (78)
1	46 (29)	35 (22)
WBC count at diagnosis, $\times 10^9$/L		
Fewer than 20	111 (69)	97 (61)
20-100	36 (23)	51 (32)
More than 100	13 (8)	12 (7)
Karyotype‡		
Favorable	13 (8)	14 (9)
Intermediate	95 (59)	95 (59)
Adverse	7 (4)	10 (6)
Unknown	45 (28)	41 (26)
No more than 15% blasts after first induction	144 (90)	147 (92)
Antecedent hematologic disorder§	14 (9)	15 (9)
Previous high-dose AraC‖	108 (68)	105 (66)
Previous auto-SCT	17 (11)	22 (14)
CR - Rx¶, d, median (range)		
Overall	135 (4-553)	147 (6-727)
6 mo or fewer	125 (78)	117 (73)
More than 6 mo	35 (22)	43 (27)
Consolidation - Rx#, d, median (range)		
Overall	64 (14-468)	63 (20-545)
3 mo or fewer	122 (76)	125 (78)
More than 3 mo	35 (22)	34 (21)

FAB indicates French-American-British; WBC, white blood cell; auto-SCT, autologous stem cell transplantation; Rx, random assignment.

*If not otherwise indicated, results are presented as absolute numbers with percentages shown in parenthesis.

†Assessed at the time of random assignment.

‡Classified according to MRC criteria.[27]

§Twelve (control) and 13 (HDC/IL-2) patients had myelodysplastic syndrome prior to AML. Two patients in each arm had other previous hematologic malignancies.

‖At least 2 g/m^2 per day for 3 or more days during induction or consolidation.

¶Time (days) from current CR to date of random assignment.

#Time (days) from the completion of consolidation to date of random assignment.

Overall trial results

Eight patients, 4 in each study arm, were lost to follow-up or withdrew their consent; these patients were censored at the time of last assessment. All other patients had an LFS follow-up exceeding 3 years. The median follow-up of living patients was 47.3 months (range, 1-68 months) in the control arm and 46.7 months (range, 1-66 months) in the HDC/IL-2 arm.

LFS. A total of 221 relapses (not including any deaths prior to relapse) occurred in the study; 119 in the control arm and 102 in the HDC/IL-2 treatment arm. The Kaplan-Meier distribution curves for the entire study population (n = 320) demonstrated a significant improvement of LFS for the HDC/IL-2 arm compared with the control arm ($P < .01$, log-rank test; Table 2; Figure 2). One hundred seventy-three relapses occurred in patients in CR1; 97 in the control arm, and 76 in the HDC/IL-2 arm. In the CR1 subgroup of patients, the Kaplan-Meier distribution curves demonstrated a significantly improved LFS ($P = .01$; Table 2; Figure 3) for patients treated with HDC/IL-2, whereas no significant difference regarding LFS was observed in patients in subsequent CR ($P = .4$, log-rank test; Table 2). The estimated LFS rates at 12, 24, and 36 months for the entire study population (CR1 + CR > 1) as well as for the CR1 and subsequent CR subgroups are shown in Table 2. In the population of all study patients, 49 in the HDC/IL-2 treatment arm and 31 in the control arm remained in uninterrupted CR at the closure date of the study. Forty-six patients in CR1 in the treatment arm and 27 in the control arm remained in CR1 at the closure date.

Table 2. Leukemia-free survival (LFS)

	Log-rank test*	Hazard ratio	95% CI†	97.5% CI†	LFS rate ± SE, %‡		
					12 mo	24 mo	36 mo
All patients	0.01	0.71	0.54-0.92	0.52-0.96			
Control, n = 160					42 ± 3.9	29 ± 3.6	24 ± 3.4
HDC/IL-2, n = 160					48 ± 4.0	41 ± 3.9	34 ± 3.8
Patients in CR1	0.01	0.69	0.51-0.93	0.49-0.97			
Control, n = 132					45 ± 4.3	32 ± 4.1	26 ± 3.8
HDC/IL-2, n = 129					52 ± 4.4	45 ± 4.4	40 ± 4.4
Patients in subsequent CR	0.40	0.79	0.43-1.46	0.39-1.60			
Control, n = 28					30 ± 8.8	15 ± 6.9	15 ± 6.9
HDC/IL-2, n = 31					29 ± 8.2	23 ± 7.5	10 ± 5.3

*Statistical analysis using the log-rank test, stratified by country and, if applicable, CR stratum.
†Confidence intervals for the hazard ratios of the treatment arms.
‡LFS rates are Kaplan-Meier estimates at each time point.

Overall survival and outcome after relapse. The treatment did not affect overall survival in a statistically significant manner, neither in the entire patient population ($P = .2$; Table 3; Figure 4) nor in the subgroups of patients in CR1 ($P = .2$; Table 3; Figure 5) or subsequent CR ($P > .5$; Table 3). In the population of all patients, the median time between relapse and death was 9.0 and 9.5 months in the treatment and control arms, respectively. For patients in CR1, the median times from relapse to death were 9.2 (HDC/IL-2) and 9.5 (control) months.

Seven patients in the study, 4 in the control arm and 3 in the HDC/IL-2 treatment arm, died of AML without a previously captured relapse date. Four patients, all in CR1, died of causes unrelated to leukemia. Thus, 2 patients in the control arm died of unknown causes at 9 and 21 months after random assignment, respectively. In the treatment arm, the causes of death were pneumonia (at 28 months after randomization), and multiorgan failure secondary to sepsis (at 40 months).

Cox models. Univariate analysis of Cox proportional hazards modeling of potential prognostic factors for LFS in the study population revealed that age older than 60 years, adverse karyotype, AML of FAB classes M0/M1/M5/M6, percentage of bone marrow blasts exceeding 15% after first induction, and time from CR to random assignment of fewer than 6 months were associated with an adverse prognosis with respect to LFS. In addition, treatment with high-dose cytarabine during induction or consolidation treatments was associated with a longer duration of LFS (Table 4). In the multivariate analysis, 2 factors, that is, age (≥ 60 versus < 60) and karyotype (adverse versus other), were found to have a significant effect on LFS. The multivariate Cox proportional hazards model using the backward selection procedure demonstrated that the treatment effect observed was unaffected by demographic or prognostic factors. Thus, the multivariate analysis revealed a significant superiority of HDC/IL-2 within the population of all study patients with an adjusted P value of .006 (Table 5). An analysis for the subgroup of patients in CR1 yielded a correspondingly adjusted P value of .01 (Tables 4 and 5).

Compliance and toxicity

Three hundred seventeen patients were included in the safety population, 157 in the HDC/IL-2 treatment arm and 160 in the control arm. In the treatment arm, patients received a median of 6 (range, 1-10) 3-week cycles of HDC/IL-2, and of 49 nonrelapsed patients, 45 (92%) completed all 10 scheduled cycles.

All patients in the treatment arm and 95% of those in the control arm experienced adverse events (AEs). Table 6 shows adverse events reported by more than 5% of patients in any of the 2 arms. The events which were significantly more prominent in the HDC/IL-2 arm included IL-2–related side effects such as injection site reactions, fever, fatigue, and myalgia, along with side effects related to HDC such as palpitations, flushing, and headache. There were no cases of grade 3 or 4 hypotension, nor were there any cases of capillary leakage syndrome or renal insufficiency. The incidence of AEs resulting in dose reduction or treatment interruption was

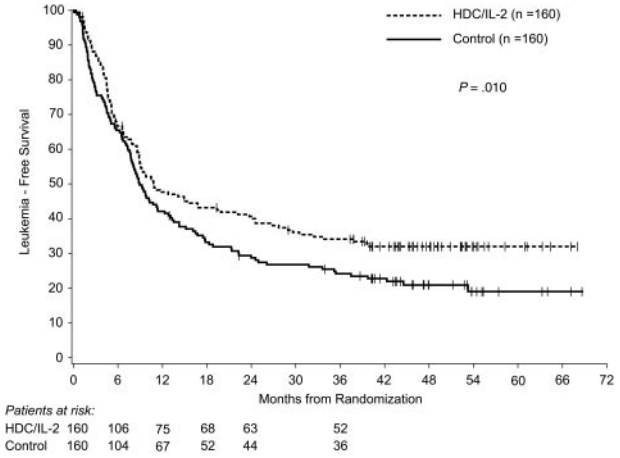

Figure 2. LFS of all patients randomly assigned (n = 320). Statistical analysis was performed by use of the log-rank test, stratified by country and CR stratum.

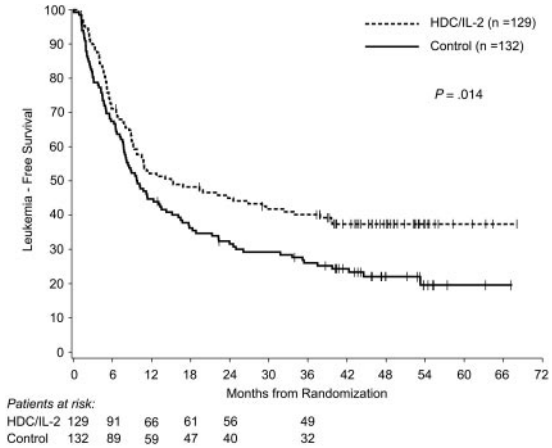

Figure 3. LFS of patients in CR1 at randomization (n = 261). Statistical analysis was performed by use of the log-rank test, stratified by country.

Table 3. Overall survival (OS)

	Log-rank test*	Hazard ratio	95% CI†	97.5% CI†	OS rate ± SE, %‡		
					12 mo	24 mo	36 mo
All patients	0.21	0.82	0.61-1.11	0.58-1.16			
Control, n = 160					70 ± 3.6	51 ± 4.0	44 ± 4.0
HDC/IL-2, n = 160					78 ± 3.3	55 ± 4.0	48 ± 4.0
Patients in CR1	0.16	0.78	0.56-1.09	0.53-1.15			
Control, n = 132					73 ± 3.9	53 ± 4.4	46 ± 4.4
HDC/IL-2, n = 129					80 ± 3.5	61 ± 4.3	55 ± 4.4
Patients in subsequent CR	> 0.5	1.00	0.52-1.93	0.47-2.13			
Control, n = 28					59 ± 9.5	41 ± 9.5	33 ± 9.1
HDC/IL-2, n = 31					68 ± 8.4	32 ± 8.4	19 ± 7.1

*Statistical analysis using the log-rank test, stratified by country and, if applicable, CR stratum.
†Confidence intervals for the hazard ratios of the treatment arms.
‡OS rates are Kaplan-Meier estimates at each time point.

26%, the most common reasons being local inflammatory reactions at the injection sites (7.1%) or fever (5.1%).

Thirteen patients (8.3%) in the HDC/IL-2 arm discontinued treatment because of AEs not related to relapse. The causes for discontinuation or early termination were neutropenia (n = 3), asthenia, polyarthritis, acute congestive heart failure, bronchospasm, venous thrombosis/renal-cell cancer, hepatobiliary disorder, hypersensitivity with local reaction/flush, gastrointestinal hemorrhage, nausea/vomiting, and thrombocytopenia.

The incidence of serious adverse events (SAEs) was 18.8% and 17.8% in the control and HDC/IL-2 arms, respectively. Most SAEs were relapse related. Seven patients (4.5%) in the treatment arm had 9 treatment-related SAEs. These events were fever (n = 3), congestive heart failure, dehydration, endocarditis, grand mal convulsion, polyarthritis, and aspergillosis. Patients with treatment-related SAEs or dose modifications were not censored for any parameters of efficacy. There were no treatment-related deaths.

Discussion

The treatment of AML (excluding acute promyelocytic leukemia) in adults begins with induction therapy using combinations of anthracyclines and cytarabine, which results in CR in most patients.[3,31,32] The induction phase of treatment is followed by intensive consolidation chemotherapy, usually in the form of high-dose cytarabine. These induction/consolidation regimens result in long-term disease-free survival rates of 25% to 35%, or less, with an unfavorable karyotype of the leukemic clone and age older than 60 years as major determinants of poor prognosis.[33-38] For patients with AML in CR not receiving an allograft who are beyond the consolidation phase, there is no treatment available that clearly prolongs CR or prevents relapse. Postconsolidation maintenance chemotherapy, which is common in the treatment of acute lymphoblastic leukemia, has not proven beneficial in AML,[39-43] and most patients will not receive further therapy. The choice of comparator arm in the present study, therefore, was based on the notion that no treatment after the completion of consolidation is the current standard of care.

The decision not to include additional conceivable comparators in the trial, the most apparent candidate being an IL-2 alone arm, was based on an assessment of the efficacy and toxicity reported in previous trials using monotherapy with IL-2 as maintenance therapy for patients with AML in CR. The available documentation about the putative efficiency of IL-2 to prevent relapse in AML was inconsistent at the onset of the present phase 3 trial,[11-14,44,45] and severe toxicity from IL-2 treatment had been described.[46] Blaise et al[44] reported 2 treatment-related deaths in patients with AML receiving IL-2 in a randomized phase 2 trial, which failed to show superiority of IL-2 treatment over no treatment. Although subsequent studies emphasize that IL-2 can be safely administered to patients with AML in CR,[47] the combination of inconsistent therapeutic efficiency and presumed toxicity of monotherapy with IL-2 called for caution regarding the use of a comparator arm of IL-2 alone in this study.

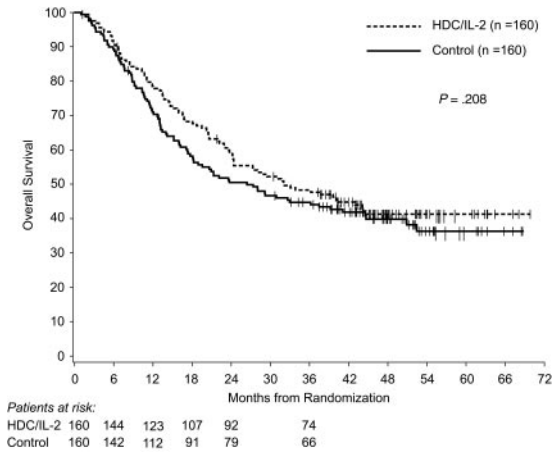

Figure 4. Overall survival of all patients randomized (n = 320). Statistical analysis was performed by use of the log-rank test, stratified by country and CR stratum.

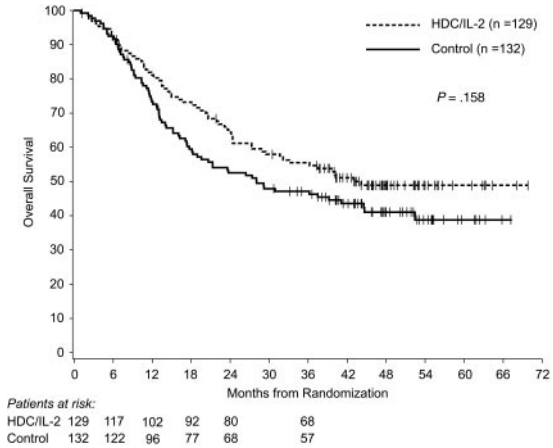

Figure 5. Overall survival of patients in CR1 at randomization (n = 261). Statistical analysis was performed by use of the log-rank test, stratified by country.

Table 4. Univariate Cox proportional hazards analysis of leukemia-free survival for all participating patients (n = 320) and patients in CR1 (n = 261)

Variable	Hazard ratio	95% CI	P*
All patients			
Age, y (≤ 60 vs > 60)†	0.66	(0.50-0.87)	< .01
Sex (men vs women)	1.32	(1.00-1.74)	.05
FAB class: M2/M3/M4 (no vs yes)‡	1.38	(1.05-1.81)	.02
Karyotype (MRC): adverse (no vs yes)‡	0.48	(0.28-0.84)	.01
Percentage blast cells (≤ 15% vs > 15%)	0.57	(0.33-0.99)	< .05
High-dose cytarabine therapy (no vs yes)	1.41	(1.04-1.92)	.03
Months from current CR to random assignment (≤ 6 vs > 6)	1.51	(1.08-2.11)	.02
Patients in CR1			
Age, y (≤ 60 vs > 60)†	0.61	(0.45-0.83)	< .01
FAB class: M2/M3/M4 (no vs yes)‡	1.49	(1.10-2.02)	.01
Karyotype (MRC): adverse (no vs yes)‡	0.48	(0.27-0.84)	.01
History of antecedent hematologic disorder (no vs yes)	0.65	(0.44-0.97)	.03
High-dose cytarabine therapy (no vs yes)	1.550	(1.10-2.18)	.01
Months from current CR to random assignment (≤ 6 vs > 6)	1.57	(1.09-2.27)	.02

*P values were calculated using the Wald test and stratified by country and, if applicable, CR stratum. Variables with a P value for the hazard ratio ≤ 0.1 were retained from the backward selection model.

†Of the dichotomized groups, the second group is always the baseline.

‡If any of the subgroups of FAB classes or leukemic karyotypes was retained by the selection procedure, then all groups were retained in the final model.

A possible explanation as to why IL-2 therapy has not met the expectations in AML may be that the cytotoxic lymphocytes of patients with leukemia are frequently in a suppressed state and unresponsive to activation. A plethora of mechanisms have been proposed to account for the dysfunctional antileukemic lymphocytes in AML, including the production of T-cell– and NK-cell–inhibitory factors by AML blasts,[48] a deficient expression of NK-cell receptors on leukemic cells,[49] inhibition of antileukemic lymphocytes by mononuclear phagocytes,[4] and an impaired stimulatory interaction between the CD28 antigen expressed by T cells and contact antigens on AML blasts.[50]

The administration of HDC to patients with AML in CR aims at protecting leukemia-reactive T cells and NK cells from suppression, thereby potentially making IL-2 therapy more efficacious. Specifically, HDC targets a mechanism of inhibition of cytotoxic lymphocytes induced by normal and leukemic phagocytes.[20,51-54] In vitro studies demonstrate that HDC in conjunction with IL-2, but neither compound used as a single agent, activates antileukemic lymphocytes to attack and destroy human AML blasts in the presence of phagocytic cells.[18,19,22,55] Moreover, studies in patients with melanoma suggest that treatment with HDC/IL-2 yields a more efficient activation of cytotoxic lymphocytes in vivo than monotherapy with IL-2.[10,21]

This trial met the primary endpoint and thus showed a significantly improved LFS for patients receiving HDC/IL-2 as compared with the current standard of care (n = 320; $P < .01$, log-rank test). The study arms were well balanced for demographics and potential prognostic factors such as age, sex, previous high-dose cytarabine treatment, previous autologous stem cell transplantation, leukemic karyotypes, time from CR to inclusion, and frequency of antecedent hematologic disorder. Cox modeling did not reveal any imbalances between treatment and control arms, which could have explained the improvement of LFS in patients receiving HDC/IL-2, and the adjusted P values in the multivariate analyses were .006 for all patients (n = 320) and .01 for the subgroup of patients in CR1 (n = 261).

The benefit of HDC/IL-2 appeared to result from a reduced relapse risk in patients in CR1, and the sustained difference in LFS between treated and untreated patients in CR1 suggests that the effect of HDC/IL-2 was to prevent rather than to delay relapse. The percentage of patients remaining in CR1 at 3 years after random assignment was 26% and 40% for control and treated patients, respectively, thus representing a greater than 50% improvement of the likelihood of LFS. The results obtained in the subgroup of patients in subsequent CR confirm the poor prognosis of patients with relapsed AML[2,56] in that only 7 of 59 patients in subsequent CR were disease-free at 36 months. Thus, in contrast to the favorable treatment effect observed in patients in CR1, no apparent long-term benefit was observed for patients in subsequent CR.

Overall survival was not significantly affected by treatment, but it should be emphasized that the trial was not powered for differences in survival, and that an analysis of the time point selected for LFS (3 years after last enrollment) was not expected to yield significant results in terms of survival. In addition, the survival after a relapse was longer than that previously reported. Thus, although other investigators typically report a median time from relapse to death of 4 to 5 months,[2,56] the patients in the current trial lived for a median of at least 9 months in both arms, which further limited the possibility of establishing the putative effect of treatment on overall survival at the time of analysis. The reason for this discrepancy may be that the current trial recruited patients after the completion of consolidation rather than immediately after CR,

Table 5. Multivariate Cox proportional hazards analysis of leukemia-free survival for all participating patients (n = 320) and patients in CR1 (n = 261)

Variable	Hazard ratio	95% CI	P*
All patients			
Treatment (HDC/IL-2 vs control)†	0.69	(0.52-0.90)	< .01
Age, y (≤ 60 vs > 60)	0.66	(0.50-0.88)	< .01
Sex (men vs women)	1.31	(0.99-1.73)	.06
FAB class			
M0/M1/M5/M6 (no vs yes)	1.23	(0.73-2.07)	.44
M2/M3/M4 (no vs yes)	1.67	(0.99-2.80)	.05
Karyotype (MRC)			
Favorable (no vs yes)	0.88	(0.50-1.57)	.67
Intermediate (no vs yes)	1.03	(0.74-1.45)	.84
Adverse (no vs yes)	0.50	(0.27-0.93)	.03
Patients in CR1			
Treatment (HDC/IL-2 vs control)†	0.67	(0.50-0.91)	.01
Age, y (≤ 60 vs > 60)	0.75	(0.52-1.07)	.12
FAB class			
M0/M1/M5/M6 (no vs yes)	1.04	(0.57-1.89)	.90
M2/M3/M4 (no vs yes)	1.66	(0.91-3.04)	.10
Karyotype (MRC)			
Favorable (no vs yes)	0.75	(0.40-1.40)	.37
Intermediate (no vs yes)	0.96	(0.65-1.42)	.86
Adverse (no vs yes)	0.52	(0.27-0.99)	< .05
History of antecedent hematologic disorder (no vs yes)	0.76	(0.49-1.16)	.20
High-dose cytarabine therapy (no vs yes)	1.43	(0.97-2.12)	.08
Months from current CR to random assignment (≤ 6 vs > 6)	1.48	(1.01-2.16)	< .05

*Included in this model are variables selected from a backward selection process. P values were calculated using the Wald test and stratified by country and, if applicable, CR stratum.

†Of the dichotomized groups, the second group is always the baseline.

Table 6. Adverse events

	Control,* n = 160			HDC + IL-2,* n = 157			
	Grades 1 and 2	Grade 3	Grade 4	Grades 1 and 2	Grade 3	Grade 4	*P*†
Blood							
Thrombocytopenia	13.8	9.4	0	13	16	1.3	.16
Eosinophilia	0	0	0	18	1.9	0	< .001
Neutropenia	5.0	3.1	0	6.4	5.7	0	.27
Leukopenia NOS	11	1.9	0	7.0	0.6	0.6	.20
Anemia	5.0	0.6	0	5.7	1.3	0	> .5
Cardiac							
Tachycardia NOS	1.3	0	0	14	0	0	< .001
Palpitations	1.9	0	0	8.3	0	0	.01
Gastrointestinal							
Nausea	9.4	0	0	31	1.3	0	< .001
Dyspepsia	3.8	0	0	15	0.6	0	< .001
Vomiting	5	0	0	15	0.6	0	.003
Diarrhea	10	0	0	18	1.9	0	.02
Abdominal pain	3.8	0	0	6.4	0	0	.32
General							
Fatigue	16	1.3	0	42	1.3	0	< .001
Pyrexia	16	1.3	0	41	2.5	0	< .001
Pain NOS	2.5	0	0	7.7	0	0	.04
Weakness	6.9	0	0	7.6	0	0	> .5
Chest pain	5.7	0.6	0	8.9	0	0	.40
Injection sites							
Erythema	NA	NA	NA	38	0	0	NA
Granuloma	NA	NA	NA	44	0	0	NA
Rigors	NA	NA	NA	18	0	0	NA
Pruritus	NA	NA	NA	22	0.6	0	NA
Infections							
Upper respiratory	14	0	0	18	0	0	.36
Sinusitis	5.7	0	0	5.8	0	0	> .5
Metabolic and nutrition							
Anorexia	3.1	0	0	8.3	0.6	0	.03
Musculoskeletal							
Myalgia	1.9	0	0	19	0	0	< .001
Arthralgia	14	0	0	23	1.3	0	.02
Back pain	12	0	0	13	0.6	0	> .5
Pain in limb	6.9	0	0	12	0	0	.13
Nervous system							
Headache	14	0	0	51	7	0	< .001
Dizziness	8.8	0	0	23	0.6	0	< .001
Dysgeusia	0	0	0	13	1.3	0	< .001
Psychiatric							
Insomnia	6.2	0	0	7.0	0.6	0	>.5
Depression	3.1	0.6	0	7.7	0.6	0	.10
Anxiety	8.8	0.6	0	5.1	0	0	.19
Respiratory							
Cough	14	0	0	27	0.6	0	.003
Pharyngitis	15	0	0	15	0.6	0	> .5
Nasal congestion	1.3	0	0	12	0	0	< .001
Dyspnea NOS	3.2	0	0	16	0.6	0	< .001
Rhinitis	1.9	0	0	5.7	0	0	.08
Rhinorrhea	2.5	0	0	6.3	0	0	.11
Epistaxis	2.5	0	0	5.7	0	0	.17
Sinus congestion	0.6	0	0	5.8	0	0	.01
Skin							
Rash NOS	4.4	0	0	12	0.6	0	.01
Sweating	1.3	0	0	9.5	0	0	< .001
Urticaria	0	0	0	5.1	0	0	.003
Vascular							
Flushing	0	0	0	87	1.3	0	< .001
Hypotension NOS	2.5	0	0	13	0	0	< .001

NOS indicates not otherwise specified; NA, not applicable.

*Data are presented as the percentage of the safety population reported by at least 5% of patients in any of the control or HDC/IL-2 arms.

†Statistical analysis using Fisher exact test.

thereby excluding patients with poor prognosis who relapse shortly after attaining CR.

The toxicity of the regimen was acceptable, as evidenced by the findings that (1) the patients could safely administer HDC/IL-2 themselves at home without medical supervision, and (2) only 13 patients (8%) discontinued treatment because of adverse events not related to relapse. Severe IL-2–related toxicities such as events related to capillary-leak syndrome or renal insufficiency were not observed, probably because the dose of IL-2 was considerably lower than that used in most previous studies.[11-14,44,45] In accordance with the experience from other trials,[55,57] the side effects associated with HDC were transient, did not require treatment, and were without sequelae. Despite that the toxicities associated with treatment were typically mild to moderate, dose reductions were necessary in 26% of patients, mostly because of fever and local inflammatory reactions that subsided or were ameliorated after reduction of the IL-2 dose.

In conclusion, this phase 3 trial demonstrated a significant improvement of LFS in patients with AML receiving postconsolidation immunotherapy with HDC/IL-2 compared with the current standard of care. The HDC/IL-2 treatment appears to offer an efficacious and tolerable treatment for patients with AML in remission.

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