Increased Risk of Leukemia Relapse With High-Dose Cyclosporine A After Allogeneic Marrow Transplantation for Acute Leukemia

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Eighty-one patients with acute myeloid leukemia (ANLL, n = 44) or acute lymphoblastic leukemia (ALL, n = 37), aged 10 to 50 years, were randomized to receive 1 mg/kg per day (n = 41, group A) or 5 mg/kg per day (n = 40, group B) of cyclosporine A (CyA) from day −1 to day +20 after bone marrow transplantation (BMT). All patients received CyA orally thereafter. All patients were prepared with cyclophosphamide (CY) 120 mg/kg and fractionated total body irradiation (TBI), and received unfractinated BMT from an HLA-identical sibling. The two groups were comparable for diagnosis, disease status, French-American-British (FAB) classification, WBC count at diagnosis, cytogenetic abnormalities, extramedullary disease before BMT, donor/recipient age and sex, number of cells infused, and number of days with intravenous (IV) CyA. Median follow-up for surviving patients in group A was 993 v 832 days in group B. Patients in group A had lower serum levels of CyA (295 v 686 ng/mL, P = .004), lower bilirubin levels (1.9 v 2.6 mg/dL, P = .07), lower creatinine levels (0.9 v 1.4 mg/dL, P = .06), and a lower proportion of CD8+ cells in the peripheral blood (PB) within day +21 (19% v 28%, P = .07). First day to 0.5 × 10^9/L neutrophils was comparable in the two groups (13 v 14 days; P = .1). In a Cox model, the actuarial risk of acute graft-versus-host disease (GVHD) grade II+, after stratification for age (< 20 years >) was significantly lower in group B patients (0.54, P = .04). The actuarial risk of developing chronic GVHD was comparable (P = .9). Actuarial transplant-related mortality (TRM) at 240 days was 28% and 26% (P = .8) in group A and B; the major cause of death was GVHD in group A (P = .02) and multiorgan toxicity in group B (P = .07). The actuarial risk of relapse at 2 years overall was 20% in group A and 52% in group B (P = .001); it was 9% v 43%, respectively, for patients in first remission (P = .0001) and 48% v 63% for patients in non-first-complete remission (CR) (P = .1). Actuarial 2-year disease-free survival (DFS) in group A and B was 58% v 32% (P = .02) for all patients, 71% v 35% (P = .01), in first remissions, and 30% v 23% (P = .2) in advanced disease. In conclusion, protection from GVHD-related death with high-dose CyA is offset by significant organ toxicity and leukemia relapse: As a consequence, long-term DFS is clearly superior in patients receiving low-dose CyA after Cy and fractionated TBI.

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Cyclosporine A (CyA) is currently the most commonly used agent for prophylaxis of graft-versus-host disease (GVHD) after allogeneic bone marrow transplantation (BMT). It is used in more than 70% of all patients, either as a single agent, with short-course metothrexate (MTX) or with T-cell depletion. Besides its well-known immunosuppressive effect, CyA also has pronounced side effects on most organs, including kidney, liver, skin, and the CNS, and drug toxicity is a relevant clinical problem. CyA is usually administered intravenously (IV) for the first week after transplant and orally thereafter; the relative bioequivalence of oral to IV doses is 1:3. Schedules of administration of CyA vary between centers and have also changed with time: A dose ranging between 3 and 5 mg/kg per day has been tested in association with methothrexate (MTX). A low dose of 1.5 mg/kg per day has been tested in association with methothrexate (MTX).

Serum and blood levels of CyA can be monitored by several techniques, and most investigators will aim at serum levels of 100 to 250 ng/mL and at blood levels of 300 to 600 ng/mL. Despite a correlation between circulating levels of CyA and GVHD reported by some investigators, a “therapeutic window” has been difficult to identify because of a threefold variation in bioavailability noted among individual patients. The present study was designed to compare two different doses of IV CyA in patients with acute leukemia undergoing matched allogeneic marrow transplants. The endpoints of the study were (1) CyA toxicity, (2) transplant-related mortality, and (3) leukemia relapse.

**MATERIALS AND METHODS**

*Design of the study.* Eligible for study were patients with acute leukemia aged 10 to 50 years undergoing allogeneic BMT from an HLA-identical sibling in our unit. Patients were randomized to receive CyA 1 or 5 mg/kg per day from day −1 to day +20 post-BMT after stratification for disease (acute lymphoblastic leukemia/acute nonlymphoblastic leukemia, ALL/ANLL) and disease status (first and non-first remission). All patients received CyA orally 12 mg/kg per day thereafter for 100 days; the dose was then gradually reduced until it was discontinued at 1 year if possible. At the time of recurrence of leukemia, CyA was usually discontinued. No other agent was used for GVHD prophylaxis. The physician on call in the unit was free to increase the dose of CyA after Cy and fractionated TBI. From the Department of Hematology, Trapianti di Midollo Osseo, Ospedale San Martino Genova, Italy.

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comparable for diagnosis (ANLL vs ALL), disease status (first vs non-first complete remission), FAB classification, WBC count at diagnosis, cytogenetic abnormalities at diagnosis (yes/no), extramedullary disease before BMT (yes/no), donor/recipient age and sex, and number of cells infused. The distribution of the FAB subclassification for 44 patients with ANLL in group A and B was FAB1, n = 3, n = 7; FAB2 n = 3, n = 6; FAB3 n = 5, n = 5; FAB4 n = 6, n = 3; and FAB5 n = 4, n = 2, respectively. The median follow-up in group A and B is 983 and 632 days, respectively, for survivors and 80 and 180 days, respectively, for deceased patients (Table 1).

All patients were prepared with CY 120 mg/kg and TBI as already described.11 TBI was given in three fractions (3.3 Gy/day for 3 days) to ANLL patients and in six fractions (2 Gy twice daily for 3 days) to ALL patients. The actual dose of TBI received by the patients was reanalyzed retrospectively according to thermoluminescent dosimeters (TLD) recordings and was comparable in the two groups (Table 1). All patients received unseparated BM. An autopsy was performed in all deceased patients to confirm the clinical diagnosis, in particular to assess whether GVHD was a contributory cause of death.

Peripheral blood mononuclear cells (PBMCs). PBMCs were obtained at day +14 and +21 post-BMT, run on a Ficoll-Hypaque gradient, and processed for immunologic and enzymatic markers as described previously.13 Cells were incubated with antibodies to CD3, CD4, and CD8 antigens or irrelevant negative control antibody for 10 minutes at room temperature, washed twice in phosphate-buffered saline (PBS) and incubated 10 minutes at room temperature with 10 μL goat anti-mouse FITC-conjugated Ig ( Coulter, Hialeah, FL). Cells were then washed three times: the percentage of reactive cells was determined by flow microhemotronic analysis with an Epics Profile (Contron, Milano, Italy) after correction for background staining with the irrelevant antibody.

Cytotoxicological markers were a naphthyl-acetate esterase (ANAE) and acid phosphatase (AP). Preparation of PBMCs with a Shandon cytocentrifuge, cytochemical staining techniques, and scores for T/non-T cells have been described previously.14 Twenty-eight patients were studied: 13 in group A (7 ANLL and 6 ALL, 8 of whom were in first and 5 of whom were in non-first CR) and 15 in group B (9 ANLL and 6 ALL, 10 of whom were in first and 5 of whom were in non-first CR).

Statistical analysis. Survival curves were calculated by the method of Kaplan and Meier.15 Data were also analyzed by contingency tables, log-rank, and rank-sum Mann-Whitney and Fisher tests. The multivariate Cox model was also used separately for expression of CD3, CD4, and CD8 antigens or irrelevant negative control antibody for 10 minutes at room temperature, washed twice in phosphate-buffered saline (PBS) and incubated 10 minutes at room temperature with 10 μL goat anti-mouse FITC-conjugated Ig ( Coulter, Hialeah, FL). Cells were then washed three times: the percentage of reactive cells was determined by flow microhemotronic analysis with an Epics Profile (Contron, Milano, Italy) after correction for background staining with the irrelevant antibody.

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INCREASED RISK OF LEUKEMIA RELAPSE

Table 2. CyA Dose, Serum Levels, Toxicity, Survival, and Relapse

<table>
<thead>
<tr>
<th>CyA Programmed Dose</th>
<th>1 mg/kg</th>
<th>5 mg/kg</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>41</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Mean dose received/day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day −1/+10*</td>
<td>1.02 ± 0.38</td>
<td>4.08 ± 1.1</td>
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<tr>
<td>Day +11/+20</td>
<td>1.87 ± 1.15</td>
<td>2.58 ± 1.2</td>
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<tr>
<td>Total days IV CyA</td>
<td>27 ± 8</td>
<td>24 ± 6</td>
<td>.02</td>
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<tr>
<td>Bilirubin (mg/dL)†</td>
<td>1.9 ± 1.2</td>
<td>2.6 ± 2.6</td>
<td>.07</td>
</tr>
<tr>
<td>Creatinine (mg/dL)†</td>
<td>0.9 ± 0.1</td>
<td>1.4 ± 0.4</td>
<td>.06</td>
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<tr>
<td>Serum levels (ng/mL)†</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Day −1/+10</td>
<td>295 ± 290</td>
<td>686 ± 776</td>
<td>.004</td>
</tr>
<tr>
<td>Day +11/+20</td>
<td>465 ± 562</td>
<td>650 ± 688</td>
<td>.1</td>
</tr>
<tr>
<td>Alive</td>
<td>24</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Dead</td>
<td>17</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Of GVHD</td>
<td>9</td>
<td>2</td>
<td>.02</td>
</tr>
<tr>
<td>Of multiorgan toxicity</td>
<td></td>
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<td>.07</td>
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<tr>
<td>Hepatitis ± VOD</td>
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<td>2</td>
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<tr>
<td>Interstitial pneumonitis</td>
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<tr>
<td>Infections</td>
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<td>1</td>
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<tr>
<td>Congestive heart failure</td>
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<tr>
<td>Neurotoxicity</td>
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<td>1</td>
<td></td>
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<tr>
<td>Capillary leak syndrome</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Of relapse (n/at risk)</td>
<td>6/30</td>
<td>15/30</td>
<td>.01</td>
</tr>
<tr>
<td>Follow-up surviving patients, days: median (minimum–maximum)</td>
<td>983 (318–1,411)</td>
<td>632 (263–1,314)</td>
<td>.04</td>
</tr>
<tr>
<td>Follow-up deceased patients, days: median (minimum–maximum)</td>
<td>80 (10–300)</td>
<td>180 (20–1,290)</td>
<td>.06</td>
</tr>
</tbody>
</table>

*From transplant. †Mean value of highest level per patient. ‡Mean value of all determination.

reanalyzed for a correlation either with the dose of CyA administered or with the time (days) post-BMT: A positive linear correlation was found between CD8 expression and the dose of CyA administered (P = .005) and between macrophage ANAE staining and days post-BMT (P = .006).

Acute GVHD grade 0 to I, II, and III to IV developed in 16, 18, and 7 patients in group A and in 22, 15, and 3 patients in group B (P = .7). The actuarial risk of developing grade II+ acute GVHD at 100 days was 61% (47% to 76%, 95% confidence limits) and 45% (31% to 62%, 95% confidence limits) in groups A and B, respectively (P = .1).

In a Cox model, however, when we stratified for age (< 20 years >) and CyA dose (group A/B), both age [risk 2.29 for older patients, confidence interval (CI) 1.14 to 4.59, P = .01] and CyA dose (risk 0.54 for 5 mg/kg, CI 0.29 to 1.00, P = .04) had significant impact on the risk of developing acute GVHD. Chronic GVHD was absent, limited, extensive in 26, 6, and 6 and in 21, 7, and 6 patients in groups A and B, respectively (P = .8). The actuarial risk of developing chronic GVHD was 39% and 40% in the two groups (P = .9).

Transplant-related mortality (TRM) and causes of death. TRM at 240 days was 28% and 26% in group A and B (P = .8), (Fig 1). In patients aged less than 20 years, actuarial TRM was 15% versus 29% in group A and B, P = .3. In patients aged more than 20 years, actuarial TRM was 40% versus 24% P = .3, in groups A and B, respectively.

The major cause of death was GVHD in group A (9 of 41 v 2 of 40 patients in group B, P = .02) and multiorgan toxicity (without clinical and pathologic evidence of GVHD) in group B (8 of 40 v 2 of 41 in group A, P = .07) (Fig 2, Table 2).

Disease-free survival (DFS). Two-year DFS in groups A and B was 58% versus 32% (P = .02) for all patients (Fig 3), 71% versus 35% (P = .01) in first remission (Fig 4), and 35% versus 23% (P = .2) in advanced disease. A multivariate Cox analysis of DFS indicated a borderline increased
risk of failure for female recipients ($P = .03$), and for high-dose CyA ($P = .07$); other variables—remission status ($P = .1$), time from diagnosis to BMT ($P = .12$), donor sex ($P = .14$), acute GVHD ($P = .68$), recipient age ($P = .82$), and donor age ($P = .9$)—were not predictive.

Relapse. The actuarial risk of relapse at 2 years overall was 20% in group A and 52% in group B ($P = .001$) (Fig 5); it was 9% versus 43%, respectively, for patients in first CR ($P = .0001$), and 48% versus 63% for patients in non-first CR ($P = 0.1$). A late relapse in a patient with ANLL grafted in first remission contributed to the 73% actuarial risk of relapse for first-remission patients at 4 years, which is probably overestimated. In patients with ANLL, the 2-year actuarial relapse rate overall was 13% vs 40% ($P = .002$), and in patients with ALL it was 26% vs 60% ($P = .01$). In multivariate Cox analysis on relapse, patients receiving CyA 5 mg/kg had a 9.15-fold relative risk of relapse as compared with patients receiving 1 mg/kg (95% confidence limits 2.34 to 35.84; $P = .001$). Other variables proved not to be significant prognostic factors: donor age ($P = .08$) and sex ($P = .2$), recipient age ($P = .7$) and sex ($P = .9$), total dose of TBI received (less or equal/more than 9 Gy, $P = .4$), chronic GVHD (yes vs no, $P = .5$), diagnosis (ANLL vs ALL) ($P = .3$), and disease status (first remission vs others) ($P = .3$).

**DISCUSSION**

The first endpoint of this study was compliance of patients with and toxicity of two regimens of IV CyA administered post-BMT as GVHD prophylaxis. As expected, the compliance with the low-dose schedule was better: patients received the prescribed dose until day +10, which was then moderately increased (1.8 mg/kg per day) between day +11 and +20 because of the occurrence of clinically evident acute GVHD. On the contrary, some patients in the high-dose schedule had their dose reduced within day +10 and mostly after day +11 because of high creatinine and bilirubin levels. Therefore, the actual difference in CyA dose between the two groups was concentrated in the first 10 days post-BMT (fourfold) and was less so after day +11 (1.3-fold). Serum levels also were 2.32-fold higher in the high-dose CyA group within day +10, and 1.39-fold higher between day +11 and day +20. We therefore are comparing two groups of patients with acute leukemia, stratified for diagnosis and disease status, who received CyA in significantly different doses mainly in the first 10 days post-BMT. The question is whether this difference influences the outcome of the transplant.

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**Fig 3.** DFS for all patients randomized to receive 1 or 5 mg/kg per day CyA. An advantage is apparent for the low-dose CyA group ($P = .02$). Tick marks indicate patients surviving disease-free.

**Fig 4.** DFS at 4 years for first remission patients only: 28 patients (ANLL 16, ALL 12) receiving 1 mg/kg per day CyA have a significantly higher risk of relapse compared with patients receiving 5 mg/kg per day CyA ($P = .01$). Tick marks indicate patients surviving disease-free.

**Fig 5.** Actuarial risk of leukemia relapse for all patients randomized: Patients receiving 5 mg/kg per day CyA had a significantly higher risk of relapse ($P = .001$).
Although the actuarial risk of dying of transplant-related complications was quite similar in the two groups (28% and 26%), the causes of failure were different: GVHD was the main cause of death for patients receiving low-dose CyA, whereas organ toxicity was the predominant cause of failure in the high-dose CyA group. This confirmed that CyA is effective in preventing acute GVHD but is also associated with considerable multiorgan toxicity when used in high doses.

Because one important question is whether low-dose CyA, with increased risk of GVHD-related death, can be given to older patients, we analyzed actuarial transplant-related mortality after stratification for age (20 years). Although we could not find statistically significant differences, there was a trend for increased TRM in older patients who received the low-dose CyA regimen; this was not true of patients receiving high-dose CyA. Thus, when evaluating an older patient for the low-dose CyA schedule, one should take into consideration the higher risk of transplant-related death; however, one should also consider that the ultimate outcome of transplantation DFS, survival, and surviving the transplant will not help patients who will later relapse.

In this study, patients receiving high-dose CyA had a very high probability of leukemia relapse, despite our inability to identify differences in distribution of diagnosis, disease status, FAB subtypes, speed of engraftment, and total dose of TBI received, which we had previously shown to have an important prognostic value in predicting recurrence of leukemia. The higher risk of relapse was observed most frequently in first-remission patients who received high-dose CyA: their relapse rate at 2 years (43%) is not different from our historic control group of 28 first-remission ANLL and ALL patients receiving CyA 5 mg/kg per day (41%, \( P = .07 \)). In patients with advanced leukemia, the difference was less impressive and not statistically significant. The increased risk of relapse was observed both in patients with ANLL (\( P = .002 \)) and in patients with ALL (\( P = .01 \)).

We conclude from these data that high-dose CyA administered in the first 10 days post-BMT does affect the outcome of the transplant by exposing the patient to an increased risk of leukemia relapse. Because fewer patients died of GVHD with this regimen, one might ask whether the negative effect is mediated through a loss of GVHD or through other mechanisms.

Protocols aimed at reducing acute GVHD, including T-cell depletion and MTX plus cyclosporine, have led to an increased risk of leukemia relapse. This is believed to result from a diminution or loss of biologic activity of the infused BM and from mediation of an antileukemia effect by donor lymphocytes known as graft-versus-leukemia effect (GVL). Three distinct components have been proposed for GVL: (1) antileukemia activity associated with clinically evident GVHD, or GVHD effect; (2) antileukemia activity independent of clinically evident GVHD, or allogeneic effect; and (3) antileukemia activity independent of GVHD and modified by T-cell depletion, or T-cell effect.

In the present study, one could test for components 1 and 3: As to the first, the risk of acute GVHD after adjustment for age was greater in patients receiving low-dose CyA, who also had a lower risk of relapse. The risk of chronic GVHD was similar in the two groups. Thus, the GVHD effect in this study, if relevant, would be mainly a result of the acute form of the disease. As to the “T-cell component” of GVL, CyA reversibly inhibits allogeneic T-cell responses by inhibiting the activation cascade necessary for immune functions, particularly lymphokine production. It may be that the production of interleukin-2, \( \gamma \)-interferon, and/or other lymphokines by donor T cells is crucial in the first days post-BMT, immediately after high-dose chemoradiotherapy, and that this function is significantly depressed in patients receiving high-dose CyA. Work is in progress to test this hypothesis. Finally, patients who received low-dose CyA had a significantly lower proportion of CD8+ cells in the PB, suggesting a possible difference in the early immune reconstitution in the two groups.

We showed that patients treated with high-dose CyA during the first 10 days post-BMT transplantation had a decrease in acute GVHD incidence and mortality as compared with patients who received low-dose CyA, but chronic GVHD was not significantly affected. Sustained hematopoietic engraftment was noted in all evaluable patients in both groups. DFS was significantly superior in patients who received low-dose CyA, not because of a lower TRM, but mainly because of a lower rate of leukemia relapse. These data suggest that early administration of a more intense regimen of CyA post-BMT, after preparation with cyclophosphamide and fractionated TBI, appears to result in a high incidence of leukemic relapse.

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Increased risk of leukemia relapse with high-dose cyclosporine A after allogeneic marrow transplantation for acute leukemia

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