Background and Objectives. To analyze the results of standard versus alternative myeloablative conditioning regimens in allogeneic hematopoietic stem cell transplantation for high-risk acute leukemia.

Design and Methods. From October 1986 to February 2000, 104 consecutive patients (male: n = 63; median age: 21, range 1.3-44.2 years) with high-risk acute leukemia underwent a non-T-cell depleted graft from an HLA-identical sibling following a standard or alternative myeloablative conditioning regimen. Sixty patients were affected by acute lymphoblastic leukemia (ALL) and 44 by acute myeloid leukemia (AML); the phase at transplant was ≥ 2nd complete remission (CR) in 76, untreated 1st relapse with < 20% blasts in 11, refractory leukemia or overt resistant relapse in 17. Pre-transplant regimens consisting of either 12 Gy fractionated total body irradiation (TBI) or 16 mg/kg busulphan (BU) combined with cyclophosphamide (CY) were defined standard (n = 38), whereas all other myeloablative regimens (TBI plus 60 mg/kg etoposide and three-drug combinations) were considered alternative (n = 66).

Results. No significant differences in terms of baseline characteristics, incidence and severity of either acute or chronic graft-versus-host disease (GVHD) were observed between the two groups, but a significantly higher proportion of patients prepared with an alternative regimen were not evaluable for chronic GVHD or overt resistant relapse (p = 0.026). Sixty-six patients died, 38 of relapse, 26 of transplant-related mortality (TRM) and 2 of other causes. Thirty-eight patients are still alive with a follow-up ranging from 0.7 to 13.8 years (median, 7.1 years); only 1 of 39 patients who relapsed after transplant is alive in CR at 5.7 years from relapse. At the medi-
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CY have long represented the classical conditioning regimens used worldwide to prepare patients to receive an allogeneic SCT. However, from the EBMT retrospective analysis no substantial difference was found in the outcome of patients with intermediate acute leukemia undergoing an allogeneic bone marrow transplant after TBI or BU-based conditioning. Although some non-randomized studies on the use of an intensified conditioning have reported an improving outcome for high-risk patients, the search for regimens alternative to the standard (TBI-CY or BU-CY) aiming at reducing the RR without increasing transplant-related mortality (TRM) has proven to be difficult.[11] Only one randomized trial has compared the classical BU-CY combination to an alternative regimen consisting of fractionated TBI followed by high-dose etoposide (VP16) in patients with advanced leukemia.[12] When adjusted for age, no significant difference in outcome was found between the two treatment groups. Considering the current debate on the use of non-myeloablative conditioning regimens it is important to keep in mind that, as compared to chronic myeloid leukemia (CML), the graft-versus-leukemia (GVL) effects in acute myeloid leukemia (AML) are less pronounced and even less in acute lymphoid leukemia (ALL).[13-14] Therefore, myeloablative regimens should, in principle, be more appropriate in preparing patients with acute leukemia, particularly if transplanted in an advanced phase of the disease. However, in this setting, it remains an open question whether or not the use of more intensive regimens alternative to the standard ones is preferable. We report here the results obtained in our institute retrospectively comparing standard versus alternative myeloablative conditioning regimens in allogeneic hematopoietic stem cell transplants for high-risk acute leukemia over a period of several years.

Design and Methods

Patients

From October 1986 to February 2000, 104 consecutive patients with high-risk acute leukemia received a non-T-cell depleted graft from an HLA-identical sibling. Part of this patient series has been described previously.[15] Of the 104 patients, 60 were male. The median age was 21 years (range, 1.3-44.2 years). The stem cell source was bone marrow in 98 cases and peripheral blood in 6.

Conditioning regimens

The following regimens used to prepare patients for SCT were considered standard:

- TBI-CY (n = 7): fractionated 12 Gy TBI over 3 days (2 Gy twice daily) followed by CY 120 mg/kg over 2 days;
- BU-CY2 (n = 24): BU 16 mg/kg over 4 days followed by CY 120 mg/Kg over 2 days;
- BU-CY4 (n = 7): BU 16 mg/kg over 4 days followed by CY 200 mg/Kg over 4 days.

The following regimens were considered alternative:

- VP16-TBI (n = 43): etoposide 60 mg/kg total dose, administered by continuous i.v. infusion over 3 days, followed by fractionated 12 Gy TBI over 3 days;
- Three-drug regimens (n = 23): fractionated TBI-CY or BU-CY combined with idarubicin 42 mg/m² over 2 days (n = 17) or VP16 20 mg/kg (n = 4) or Ara-C 2 g/m² twice daily over 2 days (n = 2).

Graft-versus-host disease prophylaxis

All patients received cyclosporine (CsA). A short-course of methotrexate (MTX) was given to 41 patients. Low doses of 6-methylprednisolone (PDN) were added to CsA and CsA-MTX in 9 and 8 patients, respectively. Acute and chronic graft-versus-host disease (GVHD) was diagnosed and graded according to the established criteria.[16] All engrafted patients surviving with a full donor chimerism at least 100 days after transplant were considered evaluable for chronic GVHD.

Endpoints and statistical methods

The primary endpoint was overall survival (OS), defined as the time from the SCT to death from any cause or to the study closure date of November 1st, 2000. Probabilities of TRM and relapse were also considered. Time to transplant-related death was measured from the date of SCT to the date of death in CR, censoring relapses and two cases of death in CR caused by a second tumor and a cardiac attack occurring 8.5 and 3.3 years after SCT, respectively. Time to relapse was measured from the date of SCT to the date of relapse, censoring deaths in CR. Baseline patient characteristics were compared using the x² test for categorical variables and the Mann-Whitney test for continuous ones. The actuarial probabilities of survival, relapse and TRM were estimated by the method of Kaplan and Meier.[18] Comparison of survival curves for selected subgroups was performed by the log-rank (Mantel-Cox) test.[19] The following features were studied: type of acute leukemia;
leukemia (AML vs ALL), patients’ sex (male vs female), patients’ age (< vs > median age), period of SCT (< vs > median date), disease phase at time of transplant (intermediate vs advanced), duration of 1st CR (< vs > median time), conditioning regimen (standard vs alternative), GVHD prophylaxis (CsA±PDN vs CsA+MTX±PDN), sex combination (donor female/recipient male vs other), acute GVHD (0 vs 1-2 vs 3-4), chronic GVHD (no vs yes). A p value < 0.05 was considered statistically significant. A Cox proportional hazards regression single step model was set up. The relative risks of death, relapse and TRM using standard conditioning regimens as the reference group were calculated after adjustment for diagnosis, age, period, leukemia phase at time of SCT, duration of 1st CR, GVHD prophylaxis and donor-recipient sex combination. Ninety-five percent confidence intervals (CI) are reported for the main summary statistics.

**Results**

**Patients’ characteristics**

The characteristics of the patients and their diseases are summarized in Table 1. The only significant difference between the two groups consisted of a progressively more frequent use of alternative conditioning regimens over the years.

**Outcomes**

Table 2 details the patients’ outcome. No difference in terms of acute and chronic GVHD incidence and severity was observed between the two treatment groups, but a significantly lower proportion of patients prepared with an alternative regimen was evaluable for chronic GVHD (64% vs 84%) (p = 0.026). This difference was mainly due to the higher number of early relapses and regimen-related deaths occurring in patients conditioned with an alternative regimen. Thirty-nine relapses occurred at a median of 0.4 years after SCT (range, 0.1-5 years) of which 13 were observed within 100 days; it is noteworthy that only 2 of these 13 patients (15%)...
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Developed a grade 2 acute GVHD. Only 1 patient with AML is alive in CR at 5.8 years from relapse occurring 317 days after SCT and successfully treated with chemotherapy and donor lymphocyte infusion. Sixty-six patients died. The causes of death were: leukemia relapse (n = 38), acute GVHD (n = 9), cerebral hemorrhage (n = 4), chronic GVHD (n = 3), infections (n = 3), multi-organ failure (n = 3), respiratory distress syndrome (n = 3), veno-occlusive disease (n = 1), second tumor (n = 1) and cardiac attack (n = 1). Twenty-six transplant-related deaths occurred at a median of 71 days from SCT (range, 3-2170 days); only 2 of them were observed beyond the 1st year from SCT, at 524 and 2170 days, due to fungal infection and chronic GVHD, respectively. For the 38 survivors, the follow-up ranges from 0.7 to 13.8 years (median of 7.1 years) and the 7-year actuarial probability of OS, relapse and TRM is 36% (95% CI, 26-45%), 48% (95% CI, 37-59%) and 30% (95% CI, 20-40%), respectively.

Univariate analysis

In univariate analysis, the overall survival (OS) was significantly affected by the leukemia phase at time of SCT (p = 0.0133), duration of 1st CR (p = 0.0294), chronic GVHD (p = 0.0157) and conditioning regimen (p = 0.0163) (Table 3). Comparing standard versus alternative regimens, the 7-year probability of OS was 52% vs 25% (95% CI, 36-68% vs 13-37%; p = 0.0163) (Figure 1), that of relapse was 34% vs 58% (95% CI, 18-51% vs 43-73%; p = 0.0377) (Figure 2) and that of TRM was 25% vs 32% (95% CI, 9-41% vs 19-44%; p = ns) (Figure 3). Similar results were obtained in the 87 patients with intermediate phase of acute leukemia at the time of transplantation. For this subgroup of patients the 7-year probability of OS was 52% vs 28% (95% CI, 42-74% vs 14-42%; p = 0.0221), that of relapse was 25% vs 56% (95% CI, 9-41% vs 40-72%; p = 0.017) and of TRM was 25% vs 26% (95% CI, 10-40% vs 14-38%; p = ns).

Multivariate analysis

After adjusting for diagnosis, age, period, leukemia phase, duration of 1st CR, GVHD prophylaxis and donor-recipient sex combination, the use of alternative regimens significantly affected the OS (p = 0.0071) and relapse risk (p = 0.0187) but had no effect on TRM (p = 0.13), even though the risk of TRM was higher in the group prepared with alternative regimens (Table 4).
Discussion

In order to reduce the RR after transplantation in patients with acute leukemia prepared with a standard regimen, a number of alternative myeloablative conditionings were adopted in the 1980s and early 1990s. Unfortunately, the patients’ outcome did not improve, as any reduction of the RR was usually associated with an increase of the TRM mainly due to the regimen-related toxicity. In particular in our study the patients with refractory leukemia or very advanced disease at the time of transplant were unlikely to become long-term survivors, with a 5-year probability of < 10%, while the year of transplant did not influence survival. Recurrent leukemia after SCT represented the main cause of failure and was not counteracted by the use of alternative regimens. Indeed, RR and TRM were lower among graft recipients prepared with standard regimens, which in multivariate analysis were found to be significantly associated with better survival. The distribution of patients in the two groups was similar in terms of diagnosis, age, disease phase at time of SCT, duration of 1st CR, GVHD prophylaxis, donor-recipient sex combination and incidence and severity of acute and chronic GVHD. Therefore, the increased likelihood of relapse in recipients of an alternative regimen, confirmed by the adjusted multivariate analysis, might be explained by the significantly lower proportion of these patients, compared to those prepared with a standard regimen, who survived long enough after transplant to be exposed to the risk of chronic GVHD and so harness its immune-therapeutic effect. The lower number of early relapses and regimen-related deaths occurring within 100 days after transplantation in recipients of standard regimens has meant that more patients have been able to take advantage of exposure to chronic GVHD. The importance of GVHD in preventing relapse is widely documented in the literature. Particularly, Copelan et al. reported that the development of acute and chronic GVHD is an important factor in reducing the post-transplant RR and increasing the long-term leukemia-free survival in patients transplanted in advanced disease. Therefore, it might be strategically useful to aim at decreasing regimen-related toxicity by using reduced intensity conditioning and, consequently, at increasing the proportion of patients who might benefit from the immune-therapeutic GVL effect of chronic GVHD. In this respect, a number of reports have recently been published focusing on the potential benefits of non-myeloablative SCT. However, larger cohorts of patients and longer follow-up periods are required to assess the efficacy of the reduced intensity conditioning in advanced acute leukemias, in which the curative potential of such an approach, especially for ALL, is

Table 4. Overall survival (OS), relapse and transplant-related mortality (TRM) analysis: significance of conditioning regimen adjusting for diagnosis, age, period, leukemia phase, duration of 1st CR, GVHD prophylaxis and donor-recipient sex combination.

<table>
<thead>
<tr>
<th>Hazard ratio</th>
<th>95% confidence interval</th>
<th>p</th>
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<tr>
<td>Death</td>
<td></td>
<td></td>
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<tr>
<td>Standard regimens</td>
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<td></td>
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<tr>
<td>Alternative regimens</td>
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<td>1.26-4.26</td>
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<tr>
<td>Relapse</td>
<td></td>
<td></td>
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<tr>
<td>Standard regimens</td>
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<td></td>
</tr>
<tr>
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<td>1.18-6.38</td>
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<tr>
<td>TRM</td>
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<tr>
<td>Standard regimens</td>
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<tr>
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limited by the spectrum of activity of the GVL reaction, less pronounced in this setting. Therefore, hopefully looking forward to more intensive cell-mediated immune-therapeutic approaches by adoptive transfer of specifically immune rather than naive lymphocytes, the search for more effective but less toxic pre-transplant regimens alternative to the standard ones remains an interesting field of investigation in SCT for advanced acute leukemias.

Contributions and Acknowledgments
AMe and WA contributed equally to this work and should be considered as the principal authors. API was responsible for transplant co-ordination and data collection. CG was responsible for the statistical analyses. AR and RC were responsible for the care of patients. CT, AMi, SF, LL and LDF performed all laboratory investigations. VD was responsible for the statistical analyses. AR and RC were responsible for the data collection. CG was responsible for the statistical analyses. VD was responsible for transplant co-ordination and data collection. All authors contributed in revising the manuscript. They are listed according to a criterion of decreasing individual contribution to the work, with the exception of the last author who had a major role as senior author in interpreting the data. We would like to thank Roberto Ricci and Lorenza Cerilli for their support in producing the figures and carrying out the statistical analyses.

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References
Potential implications for clinical practice
This report supports the use of standard conditioning regimens for patients with high-risk acute leukemia. It also highlights the importance of controlling GVHD with immunosuppression and its potential adverse effects. Further studies are needed to optimize the balance between GVHD prevention and leukemia control.

Jordi Sierra Gil, Deputy Editor