Patients with acute nonlymphoblastic leukemia (ANL) in first remission (n = 38) or chronic myelocytic leukemia (CML) (n = 55) were given cyclophosphamide and total body irradiation, followed by marrow infusion from HLA-identical siblings. To evaluate postgrafting prophylaxis for acute graft-versus-host disease (GVHD), the patients were randomized to receive either methotrexate and cyclosporine (n = 43) or cyclosporine alone (n = 50). Methotrexate/cyclosporine significantly reduced the incidence and severity of acute GVHD, and improved early survival. This report updates the results with a 3.0 to 4.5 year follow-up. Methotrexate/cyclosporine did not interfere with sustained hematopoietic engraftment, although granulocyte recovery to 1,000/µL was delayed by five days on the average. The incidence of chronic GVHD was identical in the two groups (26% v 24%). Disease-free 3-year survival was slightly better in the methotrexate/cyclosporine group (65% v 54%), but this benefit was restricted to patients with CML (73% v 54%), while no improvement was seen in patients with ANL (41% v 41%). In contrast to patients with CML (relapse rates 8% v 9%), the early survival benefit among patients with ANL given methotrexate/cyclosporine was offset by an increase in leukemic relapses (25% v 16%).

Materials and Methods

From August 9, 1983 to April 10, 1985, 93 patients with leukemia were entered into the randomized study. Details on these patients have been reported previously. Table 1 summarizes the important patient characteristics. Patients were entered on this study after detailed consultation with their physicians and after outpatient and inpatient conferences that fully explained the advantages and disadvantages of the transplantation procedure. Protocols and consent forms were approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center.

Before marrow transplantation, patients in the randomized study received intravenous (IV) cyclophosphamide (60 mg/kg body weight) on each of two successive days. In 88 patients, this was followed by 2 Gy of total body irradiation (TBI) per day on each of six successive days delivered from two opposing Cobalt 60 sources at a rate of 6.5 cGy/min. Four patients were given 2.25 Gy of TBI on each of seven successive days, and for one patient, because of special donor considerations, a single dose of 10 Gy of TBI was administered. The donor marrow was infused IV within four hours of the last dose of TBI. The day of marrow infusion is designated day 0.

Postgrafting immunosuppressive treatment was assigned by the protocol registrar with the use of the variance method of Freedman and White. This method achieves approximately equal numbers of patients assigned to each of the treatments within each category of age (<18, 18 to 29, and 30 to 50 years), underlying disease (ANL v CML), patient/donor sex combination (male to male, female to male, male to female, female to female), and the availability of a laminar airflow isolation room. Patients randomly assigned to receive both methotrexate and cyclosporine were given 15 mg of methotrexate per square meter of body surface area IV on day 1 and 10 mg/m² on days 3, 6, and 11 after transplantation. IV cyclosporine was begun on the day before transplantation at a dose of 1.5 mg/kg every 12 hours until the patient was able to receive oral cyclosporine at a dose of 6.25 mg/kg every 12 hours until day 50 unless nephrotoxicity developed. After day 50 the cyclosporine dose was decreased by 5% per week and discontinued at 6 months after transplantation. Patients assigned to receive cyclosporine alone were given the drug on the same schedule.

Donors and recipients were genotypically HLA-identical siblings as determined by serological histocompatibility typing and the results of mixed leukocyte culture. Assessment, grading, and treatment of acute and chronic GVHD were performed as previously reported. Documentation of hematopoietic engraftment was based

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on the recovery of normal blood counts and routine marrow aspirates. Cytogenetic studies to determine the origin of the marrow cells were performed at frequent intervals after grafting; in those cases where cytogenetic markers were absent, analysis of DNA restriction fragment length polymorphism was carried out.

The summary analyses of the trial used the Cox Relative Risk Regression Model. The primary factor analyzed in the previous report was the disease-free survival curves for the two treatment groups were calculated and compared using the log rank statistic. Cumulative incidence curves for acute and chronic GVHD, interstitial pneumonia, and leukemic relapse were also calculated. Cumulative incidence curves adjust only for differing times since transplantation but not for deaths due to other causes or for other competing events. Comparisons were based on the Pearson chi-square statistic since follow-up was similar in the two treatment groups. The results of the study presented here include our findings as of March 1988.

RESULTS

The posttransplantation results among the randomized patients are summarized in Figs 1 through 4, the bottom portion of Table 1, and Table 2.

Engraftment and early toxicity. Ninety-two patients had sustained hematopoietic engraftment and one patient died on day 14 from sepsis, too early to evaluate engraftment. The median time to recovery to >1,000 granulocytes/μL was 24 days in patients given methotrexate plus cyclosporine compared with 19 days in patients given cyclosporine alone (P = .003 by two-sided Wilcoxon rank sum test). There were no significant differences, however, between the two groups in the time to recovery of platelet counts or RBC counts.

Acute GVHD. Results are presented in Fig 1A and the bottom half of Table 1. Patients who received cyclosporine alone developed grades II through IV acute GVHD more frequently than those who received both methotrexate and cyclosporine. Results from a Cox regression analysis presented previously showed that patients receiving cyclosporine alone had a 2.06-fold higher risk of developing acute GVHD than those receiving methotrexate/cyclosporine.
Fig 1. Probability of developing grades II-IV acute GVHD (A) and chronic GVHD (B) in 93 patients with leukemia who were given marrow grafts from HLA-identical siblings with either methotrexate/cyclosporine (MTX + CSP) or cyclosporine (CSP) prophylaxis.

\(P = .014; 95\%\) confidence limits, 1.08 to 3.88). No patient who received both drugs had life-threatening grade IV acute GVHD, whereas this problem developed in six patients who received cyclosporine alone (Table 1). A reduction in the incidence of acute GVHD was seen both in methotrexate/cyclosporine treated patients with ANL and CML when these two groups were analyzed separately (data not shown).

Chronic GVHD. There was no statistically significant difference between the two groups with respect to the cumulative incidence of chronic GVHD (Fig 1B). This was true also when patients with ANL and CML were analyzed separately (data not shown; \(P = .44\) and \(P = .66\), respectively). Approximately a quarter of all patients in each treatment group developed this complication (26% in the combination group and 24% in the cyclosporine-only group, respectively) or, equivalently, about one third (31% and 33%, respectively) of patients who survived for longer than 100 days. Mean (and median) Karnofsky performance scores (as of March 1988) among the patients with chronic GVHD in the two groups were 95% (and 95%), and 90% (and 90%), respectively. Four of the 12 surviving patients with chronic GVHD in the group treated with both methotrexate and cyclosporine and four of the ten in the cyclosporine group are currently being treated with immunosuppressive therapy.

Interstitial pneumonia. Patients who received methotrexate/cyclosporine had a lower incidence (14%) of interstitial pneumonia of any kind than those who received cyclosporine alone (28%) but this difference was not statistically significant (\(P = .10\)). Figure 2 illustrates this finding. The difference included both idiopathic and cytomegalovirus-associated interstitial pneumonias. No interstitial pneumonias occurred beyond 1 year.

Leukemic relapse. Figure 3 shows the results. The probability of relapse for patients with ANL was lower for
cyclosporine-treated (16%) vs methotrexate/cyclosporine-treated patients (29%), with the latest relapse seen at 3.31 years, though the difference was not statistically significant ($P = .26$). All but one of the patients with relapsed ANL have died. Among patients with CML, the relapse rate was similar with two patients in each group experiencing sustained relapse 8% vs 9%, $P = .9$). In addition, three methotrexate/cyclosporine-treated patients had transient appearance of a minority population of Philadelphia chromosome-positive marrow cells at 2, 5, and 7 months after transplantation. These cells were not detected on repeated subsequent cytogenetic examinations. Hence, these patients were not considered as having relapsed. The two patients with sustained relapse in the cyclosporine group have since died. The two patients in the methotrexate/cyclosporine group have both had second transplants, which were initially successful, although cells with the Philadelphia chromosome have reappeared in both patients 3.7 and 4 years after the original transplant, respectively. In all cases, the leukemic recurrences were in cells of host type as determined by blood genetic markers and DNA restriction fragment length polymorphism studies.

**Causes of death.** Causes of death are listed in Table 2 and included predominantly interstitial pneumonias and bacterial or fungal infections associated with acute and chronic GVHD. This was most marked among patients given cyclosporine only. Veno-occlusive liver disease caused the death of three patients. One patient in the methotrexate/cyclosporine group developed a malignant tumor in his lung, which led to his death through respiratory failure 21 months after transplantation.

### Table 2. Causes of Death (No. of Patients)

<table>
<thead>
<tr>
<th>Disease</th>
<th>Causes of Death</th>
<th>Methotrexate + Cyclosporine</th>
<th>Cyclosporine</th>
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</thead>
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<tr>
<td></td>
<td></td>
<td>With Acute GVHD</td>
<td>Without Acute GVHD</td>
</tr>
<tr>
<td>ANL</td>
<td>CMV IP</td>
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</tr>
<tr>
<td></td>
<td>Idiopathic IP</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Veno-occlusive liver disease</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Other infections</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Recurrent leukemia</td>
<td>—</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Chronic GVHD + infection</td>
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<td>1</td>
</tr>
<tr>
<td></td>
<td>Total</td>
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<td>5</td>
</tr>
<tr>
<td>CML</td>
<td>CMV IP</td>
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</tr>
<tr>
<td></td>
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<td></td>
<td>Veno-occlusive liver disease</td>
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<tr>
<td></td>
<td>Other infections</td>
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<tr>
<td></td>
<td>Recurrent leukemia</td>
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<tr>
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<td>Secondary malignancy</td>
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<tr>
<td></td>
<td>Cardiac failure</td>
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<td>—</td>
</tr>
<tr>
<td></td>
<td>Chronic GVHD + infection</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Total</td>
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<td>3</td>
</tr>
<tr>
<td>Total of both groups</td>
<td>6</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

*One additional patient without acute GVHD relapsed but is now again in remission after a second transplant.
†Two additional patients are alive with recurrent CML.
after marrow grafting. The tumor was of an epithelial type and, by DNA restriction fragment length polymorphism study, proved to be of host origin without evidence of the Philadelphia chromosome. Table 2 also shows that relapse of leukemia was the second most frequent cause of death.

Survival. Projected 3-year survival for all patients is 65% in the methotrexate/cyclosporine group compared with 54% in the group administered cyclosporine alone (P = .08) (data not shown). When analyzed separately, patients with ANL who received methotrexate/cyclosporine had a 3-year survival of 41% compared with 48% in those who received cyclosporine alone (P = .45) (Fig 4B). The corresponding estimates for CML patients were 81% and 54%, respectively (P = .03) (Fig 4A). Projected 3-year disease-free survival was 41% for patients with ANL in both study groups (P = .43) (Fig 4D). The corresponding estimates for CML patients were 73% and 54%, respectively (P = .09) (Fig 4C).

DISCUSSION

Ideally, prevention of acute GVHD should decrease mortality from infections during the first 3 months postgrafting and reduce the incidence of chronic GVHD. At the same time, hematopoietic engraftment should not be compromised and leukemic relapse postgrafting should not become more frequent due to the impairment of a graft-v-leukemia effect. The net effect of effective acute GVHD prevention should be improved patient survival.

How did the results of the present trial measure up to these expectations? The answers to this question are mixed. Unequivocally, there was a significant reduction in incidence and severity of acute GVHD in patients given the combination. The reduction in acute GVHD is the most likely explanation for the decrease in infections in methotrexate/cyclosporine-treated patients and the improved early survival reported previously. The reduction in acute GVHD achieved with the combination of methotrexate and cyclosporine is in agreement with findings made in another randomized study in patients grafted for aplastic anemia.

Hematopoietic engraftment was not a problem in the present study despite the reduction in acute GVHD. While one patient died on day 14 from infection, too early to evaluate the fate of the graft, all others had prompt and sustained engraftment, and marrow and peripheral blood elements were of donor origin, as far as could be determined by blood genetic markers. Thus, absence of clinically detectable acute GVHD did not increase the risk of graft failure as seen in certain clinical trials with T-cell depleted marrow (reviewed in references 7 and 8). Whether it follows from the present findings that T cells help in establishing allogeneic marrow engraftment are distinct from those causing acute GVHD remains conjectural.

Despite the impressive decrease in acute GVHD among methotrexate/cyclosporine-treated patients, chronic GVHD was seen at a rate identical to that in cyclosporine-treated patients. This can be explained in part by the observation that those cyclosporine-treated patients who would have been at highest risk of developing chronic GVHD died earlier of complications from acute GVHD, whereas comparable methotrexate/cyclosporine-treated patients lived long enough to be at risk for chronic GVHD. Thus the fact that the observed rates are comparable may actually indicate a beneficial influence of methotrexate/cyclosporine on chronic GVHD also. In part, the mode of onset of GVHD seems to have been changed from an acute to a de novo chronic form with the help of the immunosuppression provided through methotrexate/cyclosporine. It is of interest that the steepest rise in the cumulative incidence curve for chronic GVHD is seen during the period of tapering of cyclosporine and during the 4 to 6 months following its discontinuation. These observations suggest two possible changes in the long-term immunosuppression after marrow grafting. One change would be to avoid the tapering of cyclosporine, for which there is no good theoretical nor experimental rationale. Alternatively, cyclosporine might be continued at doses that will sustain serum cyclosporine levels in the "therapeutic" range of 100 to 400 ng/dL without undue nephrotoxicity. A more extended period of immunosuppression might permit graft-host tolerance to develop, thereby avoiding morbidity and mortality from chronic GVHD. At least one report suggested a lessened incidence of chronic GVHD in a small number of patients administered cyclosporine for 1 year. Prolonged immunosuppression may have disadvantages including infectious complications and increased relapse rates. Measurement of overall benefit will probably require a prospective randomized trial.

Results from animal studies and retrospective analyses of clinical data have shown an inverse relation between recurrence of leukemia after marrow grafting and GVHD. Leukemic recurrence was seen less often in patients with GVHD than in those without it, which suggests a graft-v-leukemia effect. Results of certain clinical studies using T-cell--depleted marrow grafts seem to corroborate these findings. T-cell depletion has led to a dramatic reduction in the frequency of acute GVHD, but most trials have also shown increased graft failure and leukemic relapse. For example, the leukemic recurrence rate among patients with CML in chronic phase given T-depleted HLA-identical marrow grafts was reported to be more than 50% compared with only 9% among patients given unmanipulated marrow. The reduced incidence and severity of acute GVHD in the present study was not accompanied by an increase in the incidence of recurrent leukemia among patients with CML. Among patients with ANL grafted in first remission, however, there was an increase in the risk of leukemic recurrence, although this was not statistically significant with the number of patients studied. It is clear that for patients with ANL conditioning programs must be found that are more effective in eradicating residual leukemia. Only under those conditions will it be possible to derive the fullest benefit from effective prevention of acute GVHD.

We conclude that patients treated with a combination of methotrexate and cyclosporine had a significant decrease in the incidence and severity of acute GVHD and associated
infections along with an improvement in early survival compared with patients given CSP alone. The methotrexate/cyclosporine regimen did not interfere with consistent and sustained hematopoietic engraftment but failed to influence the incidence of chronic GVHD. There was a trend toward an improvement in overall disease-free survival, but this benefit was restricted to the subgroup of patients with CML.

Long-term survival was not improved in patients with ANL in first remission, since, in contrast to patients with CML, the early survival benefit may have been offset by an increase in the number of leukemic relapses.

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REFERENCES


Methotrexate and cyclosporine versus cyclosporine alone for prophylaxis of graft-versus-host disease in patients given HLA-identical marrow grafts for leukemia: long-term follow-up of a controlled trial

R Storb, HJ Deeg, M Pepe, F Appelbaum, C Anasetti, P Beatty, W Bensinger, R Berenson, CD Buckner and R Clift