Cyclosporine v Methotrexate for Graft-v-Host Disease Prevention in Patients Given Marrow Grafts for Leukemia: Long-Term Follow-up of Three Controlled Trials

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One hundred seventy-nine patients with acute nonlymphoblastic leukemia in first remission (n = 75), chronic myelocytic leukemia in chronic or accelerated phase (n = 48) or leukemia in advanced stage (n = 56) were given HLA-identical marrow grafts and randomized to receive methotrexate or cyclosporine for prevention of graft-v-host disease (GVHD). The current report updates the three prospective trials with follow-ups ranging from 3.2 to 6.2 years after marrow grafting. Results were analyzed separately for each individual study and for all three studies combined. Overall, 40% of patients given cyclosporine and 55% of those given methotrexate developed acute GVHD (P = .13); the incidence of chronic GVHD was 42% and 48%, respectively (P = .67). Twenty-two percent of cyclosporine-treated patients and 30% of methotrexate-treated patients developed interstitial pneumonia of any etiology (P = .25), and the figures for cytomegalovirus pneumonia were 18% and 20%, respectively (P = .41). The overall incidence of leukemic relapse was 31% in cyclosporine-treated patients and 36% in methotrexate-treated patients (P = .75). The probabilities of survival for cyclosporine-v methotrexate-treated patients were comparable for all three study groups: 52% v 48% in patients with acute nonlymphoblastic leukemia (P = .42), 55% v 60% for those with chronic myelocytic leukemia (P = .61), 12% and 12% for those with advanced leukemia (P = .93), and 39% v 38% overall (P = .72). We conclude that cyclosporine and methotrexate are comparable regarding the likelihood of acute/chronic GVHD, interstitial pneumonia, leukemic relapse, and long-term survival.

MATERIALS AND METHODS

From September 1, 1980, to December 31, 1983, 179 patients with leukemia were entered into the three controlled trials. Patient characteristics are shown in Table 1. Study 1 included 75 patients with ANL who received a graft in first remission,1 study 2 involved 48 patients with CML,1 and study 3 consisted of 56 patients above the age of 30 years with advanced leukemia.2 Details on the pretransplantation and early posttransplantation courses of these patients have been published previously.3,4 Briefly, all patients were given intravenous (IV) cyclophosphamide, 60 mg/kg, on each of two consecutive days followed by total-body irradiation (TBI) delivered from two opposing cobalt 60 sources at an exposure rate of 6.5 cGy/min. Patients in study 1 were given 2 Gy of TBI per day on each of six consecutive days as were 46 patients in study 2. All patients in study 3 and one patient in study 2 received 2.25 Gy TBI on each of seven days. One patient in study 2 was given 2.25 Gy on each of six days. Within four hours of the last dose of TBI, donor marrow was infused intravenously. The day of marrow infusion was designated day 0. Informed consent was obtained from all patients or responsible family members according to the guidelines of the Institutional Review Board of the Fred Hutchinson Cancer Research Center.

Immunosuppressive GVHD prophylaxis postgrafting was assigned by random permutation of a set of numbers known only to the protocol registrar. Assignment was stratified for age (by decade in years). Patients randomized to receive methotrexate (n = 92) were given 15 mg/m² IV on day 1, 10 mg/m² on days 3, 6, and 11, and then once weekly until day 102. Cyclosporine administration was begun for 87 patients on day −1 and was given either orally at a dose of 6.25 mg/kg every 12 hours or IV at a dose of 1.5 mg/kg every 12 hours until recovery from chemotherapy-induced gastrointestinal toxicity. The full dose of cyclosporine was given until day 50 unless renal toxicity developed; the dose was reduced by 50% if the serum creatinine doubled above baseline values and was withheld if the creatinine level exceeded 2.0 mg/dl. After day 50, the cyclosporine dose was decreased by 5% per week and prophylaxis stopped 6 months after grafting.

Marrow donors and recipients were all genotypically HLA identical as determined by serological histocompatibility testing and mixed leukocyte cell culture results. Documentation of hematopoietic

Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Disease</th>
<th>Characteristic</th>
<th>Cyclosporine</th>
<th>Methotrexate</th>
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<tr>
<td>ANL in 1st remission (study 1)</td>
<td>(n)</td>
<td>36</td>
<td>39</td>
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<td></td>
<td>Median age (range) in yr</td>
<td>25 (13-49)</td>
<td>28 (14-47)</td>
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<td></td>
<td>M/F (n)</td>
<td>21/15</td>
<td>19/20</td>
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<tr>
<td></td>
<td>Median (range) mo from diagnosis to marrow graft</td>
<td>4 (2-8)</td>
<td>4 (2-8)</td>
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<tr>
<td>CML (study 2)</td>
<td>(n)</td>
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<td>23</td>
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<td></td>
<td>Accelerated/chronic phase of CML (n)</td>
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<td>5/18</td>
</tr>
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<td>Median age (range) in yr</td>
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<td>27 (11-47)</td>
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<td></td>
<td>M/F (n)</td>
<td>18/7</td>
<td>17/6</td>
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<td></td>
<td>Median (range) mo from diagnosis to marrow graft</td>
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<td>21 (6-80)</td>
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<td>Advanced leukemia (study 3)</td>
<td>(n)</td>
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<td>30</td>
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<tr>
<td></td>
<td>Median age (range) in yr</td>
<td>37 (30-47)</td>
<td>35 (30-45)</td>
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<td>M/F (n)</td>
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<tr>
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Abbreviations: ALL, acute lymphoblastic leukemia; BC, blast crisis; AP, accelerated phase; Rem, remission; Rel, relapse; Ref, refractory to chemotherapy.

RESULTS

Acute GVHD. Figure 1 compares the probabilities of developing grades II to IV acute GVHD among methotrexate- and cyclosporine-treated patients. Overall, 39% of patients given cyclosporine and 55% of those given methotrexate developed grades II to IV acute GVHD between days 10 and 67 after marrow transplantation (Fig 1A). This difference was not statistically significant \( P = .13 \). When results for each of the three study groups were analyzed separately, slight to moderate but not statistically significant increases in the incidence of GVHD were seen in methotrexate-treated patients who received grafts for ANL in first remission \( P = .10 \), or CML \( P = .70 \), or leukemia in relapse \( P = .46 \); the \( P \)-value of the latter differs from the one previously reported\(^3\) in part because of an error in statistics and in part because of a subsequent reassignment of GVHD codes for some of the patients. Analysis of data in patients older than 30 years also failed to show significant differences between methotrexate- and cyclosporine-treated patients \( P = .27 \).

Interstitial pneumonitis. Slight but not statistically significant increases in the incidence of cytomegalovirus inter-
stitial pneumonia (Fig 2B) and interstitial pneumonia of any kind (Fig 2A) were found for methotrexate-treated patients. These findings were made both when data for all three study groups were combined and when each individual study group was analyzed separately. Overall, 22% of cyclosporine-treated patients and 30% of methotrexate-treated patients developed interstitial pneumonia sometime within the first year after transplantation ($P = .25$). The likelihood of developing cytomegalovirus interstitial pneumonia was 18% and 20%, respectively ($P = .41$). No interstitial pneumonias were observed beyond 1 year.

**Leukemic relapse.** Figure 3 shows the results. The probability of relapse for patients with ANL who received grafts in first remission was quite similar for cyclosporine-treated (20%) and methotrexate-treated patients (23%), with the latest relapse seen at 2.75 years ($P = .80$). Among patients with CML there was a moderately but not significantly higher incidence of relapse in methotrexate-treated patients (55%) compared with cyclosporine-treated patients (17%) ($P = .16$). Relapse figures included both hematologic and cytogenetic relapse. Cyclosporine-treated patients with leukemia who received grafts in relapse showed a probability of relapse of 75% compared with 30% in methotrexate-treated patients ($P = .06$). This difference also did not reach statistical significance. When data in all three study groups were combined, the probabilities of relapse among the methotrexate- and cyclosporine-treated patients were identical ($P = .69$). As far as could be determined by blood genetic markers, leukemic relapse occurred in cells of host type.

To date, none of the patients in either of the treatment groups developed evidence of secondary malignancies including B cell lymphomas.

**Survival.** Figure 4 summarizes the probabilities of survival for each of the three study groups and for all three study groups combined. No significant differences were found, with observation periods ranging from 3.2 to 6.2 years after transplantation. Five of the surviving patients, all with CML, are alive after relapse of their disease. In one of these, relapse consisted of transient reemergence of cells with the Philadelphia chromosome; subsequently, Philadelphia chromosome-positive cells have disappeared. Two patients have shown evidence of cytogenetic relapse in marrow cells that has persisted. Two patients have been treated by a second marrow graft from the same donor after a combination of busulfan and cyclophosphamide; they are alive and in remission 0.5 and 2 years after the second graft, respectively.

**Chronic GVHD.** The cumulative incidence of chronic GVHD was approximately 40%, identical for methotrexate-and cyclosporine-treated patients (Fig 5). The one patient in the methotrexate group who developed chronic GVHD at 4.8 years did so after receiving a successful second transplant. Of the patients with chronic GVHD, 29% still require treatment with immunosuppressive drugs, either prednisone alone or prednisone combined with cyclosporine, and 71% have inactive chronic GVHD and are now receiving therapy. Seventy-one percent of the patients with chronic GVHD have Karnofsky performance scores of 100%, 18% have scores of 90%, and 11% have scores of 80% or less.

**Fig 2.** Probability of developing any interstitial pneumonia (A) or only cytomegalovirus (CMV) interstitial pneumonia (B) among 179 patients with hematologic malignancy who were given marrow grafts from HLA-identical siblings with either methotrexate or cyclosporine prophylaxis (Kaplan-Meier product limit estimates). $^{26}$ A) The results in 56 patients with leukemia in relapse; B) the results in 48 patients with CML; C) the results in 75 patients with ANL in first complete remission; and D) the results of all three study groups combined (179 patients).

**Fig 3.** Probability of leukemic relapse among patients with hematologic malignancies who were given marrow grafts from HLA-identical siblings with either methotrexate or cyclosporine prophylaxis (Kaplan-Meier product limit estimates). $^{26}$ A) The results in 56 patients with leukemia in relapse; B) the results in 48 patients with CML; C) the results in 75 patients with ANL in first complete remission; and D) the results of all three study groups combined (179 patients).

**Fig 4.** Probability of survival among patients with hematologic malignancies who were given marrow grafts from HLA-identical siblings with either methotrexate or cyclosporine prophylaxis (Kaplan-Meier product limit estimates). $^{26}$ A) The results in 56 patients with leukemia in relapse; B) the results in 48 patients with CML; C) the results in 75 patients with ANL in first complete remission; and D) the results of all three study groups combined (179 patients). Survival is shown as of January 1, 1987. Tick marks represent surviving patients.
LEGEND

Fig 6. Probability of development of chronic GVHD in 179 patients with hematologic malignancies who were given marrow grafts from HLA-identical siblings with either methotrexate or cyclosporine prophylaxis (Kaplan-Meier product limit estimates).29

DISCUSSION

The immunosuppressive drug methotrexate has been used for GVHD prevention since the late 1960s, and cyclosporine was introduced to clinical marrow transplantation in the late 1970s. The use of the two drugs in clinical trials has been supported by controlled studies in experimental animals. There has been controversy with regard to both beneficial and adverse effects of the two agents in clinical practice. Although there has been agreement that use of cyclosporine postgrafting was accompanied by less mucositis, faster rates of marrow engraftment (yet no difference in overall hospital stay), and an increased incidence of nephrotoxicity, hepatotoxicity, hypertension, and tremors, results of the various studies differed in regard to the influence of the two drugs on toxicity, hypertension, and tremors, results of the various studies differed in regard to the influence of the two drugs on acute and chronic GVHD, interstitial pneumonia, leukemic relapse, and survival.

The current report, analyzing data from three prospective, controlled trials involving 179 patients followed for 3.2 to 6.2 years after grafting, failed to show statistically significant differences between methotrexate- and cyclosporine-treated patients in regard to any of the five controversial parameters although there was a trend toward less acute GVHD with cyclosporine. This was true both when results from each individual study were analyzed separately and when data of the three studies were combined. These findings agree with those of two controlled trials comparing methotrexate and cyclosporine that were reported from Stockholm with 59 patients followed for 3 to 44 months23 and from Sydney with 36 patients followed for approximately 6 to 54 months.24 The City of Hope marrow transplant team has recently published preliminary results of a trial involving 107 patients with leukemia who were randomized to receive methotrexate plus methylprednisolone or cyclosporine plus methylprednisolone.25 Although the drug combinations in that study were not strictly comparable to the current study, results were in agreement with respect to interstitial pneumonia, leukemic recurrence, and survival, but there was a decrease in the incidence of grades II to IV acute GVHD from 47% among methotrexate-treated patients to 28% among patients given cyclosporine (P < .05). It is of interest that the reverse was true in the reports from Stockholm23 and Sydney24 where patients given methotrexate had a lesser incidence of GVHD than those given cyclosporine (22% v 40% and 19% v 45%, respectively), but these differences were not statistically significant. Given these two studies, it is inappropriate to emphasize the trend of the data in the present paper.

With six controlled, prospective trials including a total of 381 patients showing comparable results with methotrexate v cyclosporine, what is the controversy? Controversy has arisen from results of a number of uncontrolled clinical studies and from Bone Marrow Transplant Registry analyses, which were at variance, not only with data from the controlled trials, but often differed from each other with respect to acute and chronic GVHD, leukemic relapse, interstitial pneumonia, and survival. Most often, recent results in cyclosporine-treated patients were compared with historical data from methotrexate-treated patients.26,27 Earlier publications generally concluded that cyclosporine was superior to methotrexate in its ability to reduce the severity and incidence of acute GVHD along with the associated morbidity and mortality. The advantage of cyclosporine over methotrexate appeared especially striking in patients with aplastic anemia. Also, chronic GVHD was thought to be prevented by cyclosporine.4,5 More recent analyses from the International and European Marrow Transplant Registries have shown mixed results in regard to GVHD prevention. Although there still seemed to be a significant advantage of cyclosporine in patients with aplastic anemia,28 one report showed more GVHD among cyclosporine-treated patients with leukemia,13 and in a large analysis the International Registry reported the incidence of grades II to IV acute GVHD to be 44% and 46%, respectively, with the two drugs.22 Higher leukemic relapse rates in cyclosporine-treated marrow graft recipients with leukemia have been described by some13,14 but not all19-21 Marrow Transplant Registry reports. No differences in relapse rates were seen in the present study or in the three prospective trials from Sydney,24 Stockholm,25 and Duarte.26 Also, in contrast to the results of the six prospective trials, analyses of nonrandomized studies by the International Marrow Transplant Registry suggested lower incidences of both idiopathic and cytomegalovirus-associated interstitial pneumonias in cyclosporine-treated leukemic patients.30 Explanations for the differences between data from the prospective trials and from the Transplant Registry are purely conjectural. Registry data represent retrospective analyses of uncontrolled trials from multiple centers, some of which used methotrexate and some cyclosporine. The conditioning programs used to eradicate leukemia varied from center to center, with some regimens leading to higher leukemic recurrence rates than others. Less intensive conditioning regimens resulting in increased leukemic recurrence rates might also result in a decrease in the idiopathic and interstitial pneumonia rates.31,32 During the period when most transplant teams used methotrexate, single-dose TBI was the rule, whereas most centers now are using fractionated TBI. It has been shown that the incidence of idiopathic interstitial pneumonia has declined since the use of fractionated TBI.33 Also, the incidence of cytomegalovirus-associated interstitial pneumonia has decreased since the heavy use of granulocyte transfusions has been discontinued34 and certain patients have been supported with blood products from cytomegalovirus antibody-negative blood...
donors, thereby further decreasing the likelihood of cytomegalovirus infection. Because only 75% of the registry patients had biopsy or autopsy specimens available, the diagnosis of interstitial pneumonia was not always confirmed.

Finally, contrary to the results of the prospective, controlled trials, most but not all of the registry reports suggested better survival of cyclosporine- compared with methotrexate-treated patients. In part, this finding may be explained by the fact that follow-up was considerably longer with methotrexate-treated patients and, consequently, survival might be lower. Also, patient selection criteria have changed over the past 10 years, with many centers carrying out more and more "good-risk" transplants, eg, in patients in remission of their leukemia, whereas earlier transplants for the most part were done in "poor-risk," end-stage patients with advanced disease.

With the regimens used in the three current controlled prospective trials, we conclude that cyclosporine and methotrexate are comparable in regard to their ability to prevent chronic GVHD and probably acute GVHD. Also, the use of the two drugs is associated with similar probabilities of interstitial pneumonia, recurrent leukemia, and overall survival in patients with hematologic malignancies, findings that are supported by three additional independent prospective trials.

REFERENCES

Cyclosporine v methotrexate for graft-v-host disease prevention in patients given marrow grafts for leukemia: long-term follow-up of three controlled trials

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