Treatment for Acute Myelocytic Leukemia With Allogeneic Bone Marrow Transplantation Following Preparation With BuCy2

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One hundred twenty-seven patients with acute myelocytic leukemia (AML) were given busulfan 4 mg/kg on each of 4 days and cyclophosphamide 60 mg/kg on each of 2 days (BuCy2) followed by allogeneic bone marrow transplantation from an HLA-identical or one antigen disparate sibling. For 71 patients in first complete remission, 23 in second complete remission or initial relapse, and 33 patients with primary refractory disease, second or subsequent relapse, or a preceding hematologic disorder, the 3-year leukemia-free survival (LFS) is 63.1%, 32.6%, and 24.2% respectively. The actuarial probability of relapse for each group is 14.1%, 40.6%, and 61.0%. In multivariate analyses, relapse and decreased LFS were associated with advanced disease phase and with M4/M5 French-American-British classification. The LFS of first remission patients was adversely associated with a short time interval from diagnosis to transplantation. This study indicates that BuCy2 is an attractive preparative regimen for marrow transplantation in patients with AML and that prognostic factors for relapse and LFS are similar to those described for regimens containing total body irradiation.

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ALLOGENEIC BONE marrow transplantation (BMT) following high-dose chemotherapy and total-body irradiation (TBI) cures many patients with acute myelocytic leukemia (AML). Large single and multi-institutional studies demonstrate that long-term leukemia-free survival (LFS) is achieved in 45% to 50% of individuals under 50 years of age when transplantation is performed in first complete remission, and in a lower percentage of patients who have more advanced disease. Failure of marrow transplantation to achieve long-term survival occurs due to treatment-related mortality or leukemic relapse.

The combination of cyclophosphamide and TBI is the standard preparative regimen for allogeneic marrow transplantation for AML; however, alternate conditioning schemes with less severe toxicity and/or greater antileukemic effectiveness would be desirable. Santos et al initially reported that a radiation-free regimen consisting of 16 mg/kg of oral busulfan (Bu) and 200 mg/kg of intravenous cyclophosphamide (Cy) (over 4 days) resulted in an extremely low relapse rate, but was associated with a high incidence of early transplant-related mortality. A pilot study suggested that a reduction of the Cy dose to 120 mg/kg administered over 2 days (BuCy2) might be associated with a lower incidence of lethal treatment-related complications. We present here results for 127 individuals with AML who underwent allogeneic marrow transplantation at five transplantation centers after BuCy2.

MATERIALS AND METHODS

Patient characteristics. The study group consisted of 127 patients with AML aged 7 to 55 (median 30) who were treated from March 1, 1984 through June 30, 1989 at five separate centers with allogeneic bone marrow transplantation (BMT) following preparative regimen with Bu and Cy. Fifty-five individuals underwent transplantation at The Ohio State University Hospitals (OSU; Columbus), 23 at Wilford Hall at Lackland Air Force Base (San Antonio, TX), 22 at Hahnemann University (Philadelphia, PA), 17 at St Vincent’s Hospital (Sydney, Australia), and 10 at the Alfred Hospital (Melbourne, Australia). This study was designed in 1984 as a single institution (OSU) study and was modified in 1987 to include all institutions known to be using this preparative regimen in all patients with AML. All patients who underwent marrow transplantation for AML using this preparative regimen at the participating institutions are reported. Informed consent was obtained for each patient as required by the Institutional Review Boards of the participating centers. According to the study design, patients were categorized into three groups, Group I consisted of 71 patients with de novo AML in first complete remission. Group II included 23 patients with de novo AML who underwent BMT during first relapse or second remission. Group III included 33 patients who had never achieved complete remission, were in second or subsequent relapse, or who had a history of chemotherapy, radiation exposure, or a myelodysplastic syndrome preceding the development of AML. French-American-British (FAB) classifications were made by referring physicians using standard morphologic criteria with confirmation at the transplantation center. Remission induction and consolidation regimens varied among institutions. Among the 71 Group I patients, 18 required more than one induction course to achieve complete remission and 25 received one or more courses of consolidation. The clinical characteristics of the patients in each group are described in Table 1. Preliminary information for 45 of these 127 patients has been published.

Marrow donors. All donors were siblings of recipients and gave informed consent before marrow procurement. One hundred twenty-four donor-recipient pairs were HLA-A, -B, and -DR identical as determined by standard serotyping. Their cells were mutually nonreactive in mixed lymphocyte cultures. Three pairs were mismatched at a single HLA-A, -B, or -DR site. The
Table 1. Clinical Characteristics of 127 Patients Undergoing Marrow  
Transplantation After BuCy2

<table>
<thead>
<tr>
<th>Total no. of patients</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males/females</td>
<td>38/33</td>
<td>22/15</td>
<td>20/15</td>
<td>98/58</td>
</tr>
<tr>
<td>Median age, years</td>
<td>29</td>
<td>36</td>
<td>38</td>
<td>33.5</td>
</tr>
<tr>
<td>(range)</td>
<td>(13-50)</td>
<td>(21-55)</td>
<td>(7-48)</td>
<td>(7-55)</td>
</tr>
</tbody>
</table>
| Time from diagnosis to 
transplantation: Median, months | 4.5 | 8.5 | 6.5 | 6 |
| (range)               | (1-15)  | (4-64)  | (8-30)  | (8-64) |
| FAB class             |         |         |         |       |
| M1                    | 8       | 0       | 2       | 10    |
| M2                    | 20      | 8       | 3       | 31    |
| M3                    | 16      | 5       | 1       | 22    |
| M4                    | 18      | 6       | 19      | 43    |
| M5                    | 4       | 1       | 2       | 7     |
| M6                    | 3       | 0       | 3       | 6     |
| M7                    | 0       | 0       | 1       | 1     |
| FAB unclassified      | 2       | 3       | 2       | 7     |

Table 1: Clinical Characteristics of 127 Patients Undergoing Marrow Transplantation After BuCy2

nucleated cell dose ranged from 0.20 to 9.5 × 10^8 (median 3.7 × 10^8) cells/kg recipient.

Preparation for transplant. All patients at three hospitals received intrathecal methotrexate (≤ 12 mg per injection) before initiating systemic therapy. At Wilford Hall only patients whose leukemia was categorized as FAB subclass M4 or M5 routinely received intrathecal methotrexate. Prophylactic intrathecal therapy was not administered at the Alfred Hospital (AH). A total dose of 16 mg/kg Bu was administered orally in four divided daily doses over 4 days. Cy 60 mg/kg was administered intravenously on each of the next 2 days. Most patients received phenytoin during the period of Bu administration to prevent seizures. Marrow was infused 48 hours after the second Cy dose.

Graft-versus-host disease (GVHD). One hundred patients (61 in group I, 46 in group II, 23 in group III) at three institutions received cyclosporine plus methylprednisolone as prophylaxis for GVHD as previously described or according to a slightly modified version. Eighteen individuals (10 in group I, 3 in group II, 5 in group III) at two institutions received methotrexate and cyclosporine. The median duration of neutropenia was 9 days (range 4 to 21 days) and 13 days (range 8 to 23 days), respectively, in these groups. During the period of marrow aplasia, patients received a median of 4 U of red blood cells (RBCs) (range 0 to 21 units) and five transfusions (range 0 to 30 transfusions) of single-donor or random-donor platelets. Patients were discharged from the hospital a median of 23 days (range 16 to 79 days) after transplantation. No significant difference in transfusion requirements or time to discharge was detected between the groups analyzed according to GVHD prophylactic regimen.

Statistical methods. Analysis was performed on all data collected as of October 1, 1990. The time on study was defined as the shortest of the times from transplantation to relapse, death, or date of most recent follow-up. For LFS the event of interest was death or relapse. For estimation of relapse rates those patients who died before relapse were treated as censored. Estimates of the LFS and relapse rates were obtained using the Kaplan-Meier estimator.

In order to estimate the effects of the various potential risk factors a Cox regression analysis was performed. Fixed time effects used in this multivariate analysis were sex (≤ 30 or ≥ 30); acute GVHD grade (1); treatment regimen (methotrexate + cyclosporine, yes or no); transplant hospital (four indicator variables); FAB subclass (M4 or M5, yes or no); interval from diagnosis to transplantation; group by clinical phase; and number of days that the patient received intravenous (IV) antibiotics. Two-time-dependent covariates were used. These included an indicator for prolonged neutrophil recovery (coded as 1 if at time t the patient had a granulocyte level ≥ 0.5 × 10^9/L, and 0 otherwise) and time to platelet recovery (coded 1 if at time t the platelet count was ≥ 40 × 10^9/L, 0 otherwise). Tests for covariate effects were analyzed using the fitted multivariate model using Wald statistics.

Comparisons of the incidence of acute and/or chronic GVHD between participating hospitals, clinical stages, and prophylactic regimens were made using Fisher's exact test. Comparison of other continuous variables were made using the Wilcoxon test.

RESULTS

Engraftment. Marrow engraftment occurred in 124 of the 127 patients. Two patients who underwent marrow transplantation for leukemia that was refractory to chemotherapy failed to engraft. Residual leukemia cells were subsequently detected in both. One patient died 2 days after transplantation without evidence of engraftment. The granulocyte count decreased to below 0.5 × 10^9/L at a median of 4 days (range 0 to 7 days) after transplantation with neutropenia lasting a median of 10 days (range 4 to 23 days). The median time of recovery to granulocyte levels ≥ 0.5 × 10^9/L for 2 consecutive days was 14 days (range 9 to 28 days) after transplantation. Thirty-three patients (26%) did not develop a fever or receive IV antibiotics during their neutropenic period. The median time to reach a platelet count ≥ 40 × 10^9/L for 2 consecutive days was 17 days (range 7 to 108 days). Patients receiving methylprednisolone and cyclosporine as GVHD prophylaxis experienced a more rapid return of the granulocytes to ≥ 0.5 × 10^9/L (P = .001), a shorter duration of neutropenia (P = .001), and a more rapid rate of return of platelets to ≥ 40 × 10^9/L (P = .008) than did patients receiving methotrexate and cyclosporine. The median duration of neutropenia was 9 days (range 4 to 21 days) and 13 days (range 8 to 23 days), respectively, in these groups. During the period of marrow aplasia, patients received a median of 4 U of red blood cells (RBCs) (range 0 to 21 units) and five transfusions (range 0 to 30 transfusions) of single-donor or random-donor platelets. Patients were discharged from the hospital a median of 23 days (range 16 to 79 days) after transplantation. No significant difference in transfusion requirements or time to discharge was detected between the groups analyzed according to GVHD prophylactic regimen.

Nonmortal transplant-related complications. Two patients, neither of whom received phenytoin prophylaxis, developed seizures during Bu administration. Moderate oral mucositis was common; severe mucositis requiring preventative intubation or resulting in aspiration pneumonia did not occur. Symptomatic hemorrhagic cystitis developed in 27 patients (21%), none of whom required surgical intervention.

GVHD. The Kaplan-Meier prediction of the incidence of grade 2 or greater GVHD was 16.5% (95% confidence interval [CI], 9.7% to 23.3%). The estimated incidence of chronic GVHD at 3 years was 48.8% (95% CI, 38.4% to 59.2%). The incidence of chronic GVHD was significantly different among the groups analyzed according to the clinical grouping at transplantation (P = .04). Patients in group III (72.6%; 95% CI, 50.6% to 94.6%) had a higher
actuarial incidence of chronic GVHD than did patients in group I (42.9%; 95% CI, 30.3% to 55.5%) or group II (43.8%; 95% CI, 16.4% to 71.2%). The incidence of acute GVHD was not significantly different among these groups, nor was the incidence of acute or chronic GVHD significantly different among patients analyzed according to GVHD prophylactic regimen ($P > .2$).

**Mortal transplant-related complications.** As shown in Table 2, 62 patients, 28 with relapsed leukemia, have died. One patient, transplanted for AML complicating treatment for Hodgkin's disease, died from recurrent Hodgkin's. Thirty-three of the 127 patients (26%) died of treatment-related complications, 14 (11%) within 100 days of transplantation. Sixteen of 71 (23%) first-remission patients died of treatment-related causes, seven (10%) within 100 days of transplantation. Six patients (5%) died directly as a result of extramedullary organ toxicity due to the preparative regimen. Hepatic veno-occlusive disease (VOD) contributed to the death of 5 (4% of total group) of 18 patients in whom it was identified. One patient developed left ventricular necrosis and died 2 days after BMTr. Lethal interstitial pneumonia (IP) developed in 10 patients (8%), eight of whom had cytomegalovirus and two adenovirus infections. The actuarial incidence of lethal IP was 10% (95% CI; 4% to 16%). Acute GVHD in six patients and chronic GVHD in seven patients were identified as important contributors to the deaths of those individuals.

**Relapse.** Thirty-one patients have relapsed, all within 25 months of transplantation. Only three of these patients survive. The estimated relapse rate for group I was 14.1% (95% CI; 6.1% to 22.1%), for group II 40.6% (95% CI, 13.6% to 67.6%), and for group III 61.0% (95% CI, 41.8% to 80.2%) (Fig 1). The most significant prognostic factors for relapse were clinical group ($P = .0001$) and M4/M5 FAB classification ($P = .003$). Twenty-one of the 50 patients classified as FAB M4 or M5 relapsed (estimated relapse rate 49.7%, 95% CI; 33.7% to 65.7%) compared with 8 of 70 patients classified as FAB M1, M2, M3, M6, or M7 (14.8%, 95% CI, 5.0% to 24.6%). The risk of relapse for patients classified as M4 or M5 was 4.73 times that for patients in other FAB groups.

Elevated white blood cell (WBC) count ($P = .002$) and peripheral blast count ($P = .005$) at diagnosis were associated with relapse; however, they also were significantly correlated with FAB subtype. The median initial WBC count was $57 \times 10^9$/L in 45 patients with FAB subtypes M4 or M5 and $19 \times 10^9$/L among 62 patients with other FAB subtypes. The combination of FAB subtypes M4 and M5 correlated more closely with relapse than did WBC count or peripheral blast count in a multivariate analysis. No significant decrease in the risk of relapse was detected for patients who developed acute GVHD, chronic GVHD, or both ($P > .2$). Among first-remission patients the small number of relapses did not permit reasonable analysis of differences on the basis of FAB classification, interval from diagnosis to transplantation, number of chemotherapy courses required for remission induction, or administration of consolidation therapy.

**LFS.** Sixty-two patients survive free of leukemia at a median of 36 months (range 17 to 78 months) of follow-up. The projected 3-year LFS for the entire group is 47.4% (95% CI, 38.2% to 56.6%). Grouping based on phase of disease was predictive of LFS ($P = .007$). An LFS of 63.1% (95% CI, 51.1% to 75.1%) is estimated for the 71 patients transplanted while in first complete remission, 32.6% (95% CI, 12.2% to 53.0%) for 23 patients in first relapse or second remission, and 24.2% (95% CI, 9.2% to 39.2%) for the 33 patients with refractory/advanced disease or myelodysplastic syndromes preceding AML (Fig 2). In group II, 4 of 11 patients in second remission and 4 of 12 patients in first relapse at the time of marrow transplantation are leukemia-free survivors. Among patients in group III, one of nine with primary refractory disease and 4 of 16 who were in second or subsequent relapse are leukemia-free survivors. Two of four patients with secondary AML and one of four with AML following a myelodysplastic syndrome who underwent marrow transplantation without prior chemotherapy (four patients, two alive), while in first complete remission (three patients, one alive) or with refractory disease (one patient, none alive) are leukemia-free survivors.

Within group I, a short time interval between diagnosis and transplantation predicted poor LFS ($P = .04$). Time interval was not associated with M4/M5 classification. Patients who received one or more courses of consolidation had longer time intervals from diagnosis to transplantation than those who did not receive consolidation ($P = .01$). However, a long time interval was associated more strongly with LFS than was the administration of consolidation therapy ($P = .1$). Neither number of induction courses, patient age, development of grade $\geq II$ acute or chronic GVHD, GVHD prophylactic regimen, prolonged neutropil recovery, nor the hospital where transplantation was performed were found to be statistically significant risk factors for prediction of LFS ($P > .2$). FAB subclasses M4 and M5 were associated with poorer LFS ($P = .07$). Delayed platelet recovery after transplantation predicted for poor LFS. (Relative risk for a patient with a platelet count $<40 \times 10^9$/L vs a patient with a count of $\geq 40 \times 10^9$/L at a given time was 4.32 [$P = .0003$] for the entire group, and 18.65 [$P = .0001$] for group I.)

**DISCUSSION**

The present multi-institutional study permits analysis of a large number of patients treated for AML with allogeneic BMT after a radiation-free conditioning regimen of 16 mg/kg Bu and 120 mg/kg Cy. BuCy2 is sufficiently immuno-suppressive to permit permanent engraftment for related histocompatible donors. The total neutropenic period is
Fig 1. Actuarial probability of relapse among patients transplanted for AML in first remission (bottom curve), second remission or first relapse (middle curve), or with more advanced disease or history of a preceding hematologic disorder (top curve). Tick marks depict the duration of survival of patients who did not relapse.

brief, in part due to the absence of methotrexate from the GVHD prophylactic regimen of the majority of study patients, and also due to the slow decrease in the neutrophil count following this preparative regimen. A substantial percentage (26%) of individuals did not develop fever during the neutropenic period. The relatively large proportion of individuals who did not develop fever may be related to the short duration of neutropenia. Additionally, less severe injury to the mucosa of the gastrointestinal tract may be caused by BuCy2 than by TBI-containing regimens. These factors are likely to have contributed to the early hospital discharge described in this study because discharge is generally limited by cytopenias, infection, or inability to eat adequately.

Six patients (5%), five with VOD and one with left ventricular necrosis, died as a direct result of organ toxicity attributable to the preparative regimen. Ten patients (8%) died from IP. Twenty-six percent of the present study group died of treatment-related complications, 11% within 100 days of transplantation. Twenty-one percent developed symptomatic hemorrhagic cystitis. A higher frequency of this complication after this preparative regimen than with Cy/TBI has been previously reported. A high incidence of chronic GVHD occurred in this study; however, the influence of the preparative regimen is unclear. The vast majority of patients received corticosteroids for GVHD prophylaxis. A significantly higher rate of chronic GVHD in patients who received prednisone for prevention of acute GVHD was recently demonstrated by Storb et al., who suggested that corticosteroids might interfere with induction of tolerance after transplantation.

The association of subclasses M4 and M5 with relapse after marrow transplantation has been reported following preparation with Cy/TBI. Because of the limited number of patients, we were unable to define the factors responsible for the poorer survival of patients who underwent transplantation at shorter intervals after diagnosis. However, as patients survive in remission for increasing intervals follow-
ing treatment, they are less likely to relapse with or without marrow transplantation. These results emphasize the importance of time-dependent censoring of patients undergoing marrow transplantation when comparisons with chemotherapy results are performed.3

Because of its limited follow-up, this investigation cannot adequately address the important issue of late complications of transplantation. Preclinical and clinical studies have suggested that radiation-free regimens are associated with a lower frequency of these complications18,20; however, this has not been adequately studied.

The development of sensitive, specific assays for Bu has shown that patterns of absorption and elimination vary widely between patients and are associated with the development of VOD13 and perhaps other transplant-related complications.21,22 Thus, dose adjustment based on plasma Bu levels might reduce the risk of transplant-related death. Increased drug exposure in patients with low Bu plasma levels might result in better antileukemic effectiveness. Ongoing studies of dose adjustment based on Bu levels may improve the results of marrow transplantation using BuCy. Other modifications of the BuCy2 preparative regimen under investigation include the addition of etoposide or cytarabine in individuals at high risk of relapse.

This report demonstrates that BuCy2 is an effective preparative regimen for marrow transplantation in AML. The relatively low incidence of transplant-related deaths reported is noteworthy in view of the median age (30) of the participants in this trial and the previously reported adverse effect of age on transplant-related death and LFS.13 At the same time the relatively high proportion of group I patients

with FAB M3 morphology and the exclusion of individuals with secondary leukemia or preceding myelodyplasia from group I are two differences with other studies that might contribute to the favorable results obtained.

If BuCy2 is proven to be at least as safe and effective as Cy/TBI, the case of administration of Bu compared with TBI would favor its use. The present study suggests that comparatively brief durations of neutropenia and hospitalization may be additional advantages of BuCy2. However, it is impossible to adequately compare the results achieved in this study group with that of the other series of patients who have undergone transplantation for AML. Prospective randomized studies are necessary to compare the toxicities and effectiveness of BuCy2 to Cy/TBI. Such a study has been initiated in AML by the European Group for Bone Marrow Transplantation.26 Future treatment strategies include the addition of other agents to BuCy in patients at high risk for relapse and dose adjustment of Bu based on plasma levels in individual patients. The development of better preparative regimens is crucial to improving the results of allogeneic marrow transplantation in AML and other disorders.

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REFERENCES


Dedics A: Preparative regimes for marrow transplantation containing busulphan are associated with haemorrhagic cystitis and hepatic veno-occlusive disease but a short duration of leukopenia and little oro-pharyngeal mucositis. Bone Marrow Transplant 2:285, 1987


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