Prospective Comparative Trial of Autologous Versus Allogeneic Bone Marrow Transplantation in Patients With Non-Hodgkin’s Lymphoma

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A prospective comparative trial of allogeneic versus autologous bone marrow transplant (BMT) was conducted. Sixty-six consecutive patients (median age, 41; range, 15 to 60; female:male ratio = 21:45) entered this clinical trial. Priority for autologous BMT was given to patients who were 55 or younger and had a major histocompatibility complex-matched or 1-antigen-disparate sibling donor. Autologous BMT was offered to all other patients whose age was 60 or younger. Patients who had no sibling donor and who had BM involvement at the time of evaluation were not eligible. Thirty-one patients received an allograft, and 35 patients received an autograft. Thirteen patients received a BM graft purged with 4-hydroperoxycyclophosphamide because of previous BM involvement. Patients who had previous radiation to the thoracic and/or abdominal areas of more than 20 Gy received a preparative regimen consisting of cyclophosphamide (1,800 mg/m²/d for 4 days), VP-16 (200 mg/m² every 12 hours for 8 doses), and 1,3-bis(2-chloroethyl)-1-nitrosourea (600 mg/m² as 1 dose). Other patients received cyclophosphamide 1,800 mg/m²/d for 4 days followed by total body irradiation of 12 Gy administered as a single daily fraction over 4 days. With a median follow-up of 14 months, the progression-free survival (PFS) for autograft and allograft recipients was 24% ± 8% (±SE) and 47% ± 9%, respectively, (P = .21). However, the probability of disease progression was significantly higher in the autologous group (89% ± 9%) than in the allogeneic group (20% ± 10%; P = .001). Other confounding prognostic factors were adjusted in the multivariate analysis, chemo-sensitive disease and allograft were found to have a significant favorable influence on probability of disease progression (P = .03 and .003), but only chemosensitive disease had a significant influence on the PFS (P < .002). Our results suggest the existence of graft-versus-lymphoma effect and also support the rationale of using immunotherapy after autologous BMT. Allogeneic BMT should be preferable to autologous BMT in younger patients with lymphoma.

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Although graft-versus-leukemia effect has been well-established for more than a decade, the existence of a graft-versus-lymphoma effect remains elusive.1 Because of the initial success of autologous bone marrow transplantation (BMT) in curing patients with relapsed or refractory high-grade lymphoma, most BMT centers are inclined to use autologous BMT over allogeneic BMT.2-5 The other reasons for the infrequent use of allogeneic BMT in lymphoma are the frequent lack of a matched sibling donor, the higher cost of care, and its immunologic complications. To date, only three retrospective studies that attempt to assess the relative merits of autologous and allogeneic BMT have been published, the results of which are conflicting. The probabilities of disease progression were not different between the allograft and autograft recipients in the Seattle series,6 were lower in the allograft recipients in the Johns Hopkins series,7 and were lower only in allograft recipients with lymphoblastic lymphoma in the European Bone Marrow Transplant Group Registry (EBMT) series.8 Thus, the issue of graft-versus-lymphoma is still unsettled, but its existence may have a profound therapeutic significance.

In this prospective study, we compared the rate of disease progression and progression-free survival (PFS) in patients with lymphoma who received either autologous or allogeneic BMT. The study is aimed at determining whether a graft-versus-lymphoma effect exists and to evaluate its clinical implications.

MATERIALS AND METHODS

Patient Selection and Eligibility

Between September 1987 and July 1992, consecutive patients with relapsed or refractory non-Hodgkin’s lymphoma (NHL) who fulfilled the eligibility criteria were enrolled in this study. After an interim analysis in December of 1989, the patients who were eligible for the autologous arm from December 1989 to January 1992 were entered into another study using interferon-α2a to enhance cyclosporine-induced autologous graft-versus-host disease (GVHD). The results of this study will be reported separately.9 After January 1992, 3 additional patients were accrued in the autologous arm. The allogeneic arm continued to accrue patients throughout the entire study period.

All patients had histologic confirmation of NHL and were classified according to the International Working Formulation.3 Patients with Hodgkin’s disease (HD) were not included. To be eligible, a patient must have one of the following features: (1) intermediate or high-grade lymphoma in relapse or failure to achieve a complete remission after a frontline therapy; (2) low-grade lymphoma with demonstrated transformation to an intermediate or high-grade histology; or (3) presence of at least two poor prognostic features in first complete remission regardless of histologic grade. The poor prognostic features included (1) tumor ≥10 cm in diameter, (2) extensive BM involvement, (3) “B” symptoms (presence of drenching sweats, weight loss, or fever), (4) LDH ≥500 IU/L, and (5) ≥2 sites of extranodal involvement.10-11 Patients were not eligible if they had significant major organ dysfunction or had BM involvement but no sibling donor. The protocol was approved by the Wayne State University Human Investigation Committee (Detroit, MI). Signed informed consents were obtained from the patients or parents.

Study Design

Assignments of marrow grafts. Typing of HLA-A, HLA-B, and HLA-DR on patients and available siblings was obtained at the time of initial evaluation. Because of the lower frequency of potential
allogeneic recipients, preference was given to an allograft when patients were candidates for both types of grafts. Candidates for an allogeneic recipient were given preference to an allograft when an allograft could not be obtained. Candidates for an unmodified except for red blood cell (RBC) and/or plasma depletion were given preference when an allograft could not be obtained. Candidates for an unmodified except for red blood cell (RBC) and/or plasma depletion were given preference when an allograft could not be obtained.

BM grafts were offered to all other patients whose age was 60 or younger. These patients did not have BM involvement with lymphoma on bilateral iliac crest biopsies at the time of BM harvest. 4-hydroperoxycyclophosphamide (4-HC) was used for tumor-purging the BM grafts in patients who had previous BM involvement. The BM suspension was RBC-depleted in a Cobe 2991 (Cobe BCT, Inc. Lakewood, CO) blood processor and incubated with 4-HC at a concentration of 100 µg/mL for 30 minutes. A single cycle of 4-HC treatment was used. Programmed freezing was used, and the BM was stored in the liquid phase of liquid nitrogen.

Assignments of preparative regimens. The patients were assigned to either the CVB or CY-TBI regimen determined according to the dosage and sites of prior radiation received. Patients who had 20 Gy or less of radiation to the thoracic or abdominal areas received the 'CY-TBI' regimen. The 'CY-TBI' regimen consisted of cyclophosphamide 1,800 mg/m²/d for 4 days followed by total body irradiation (TBI) 3 Gy daily for 4 days. TBI was delivered from a single Co⁶⁰ source at the dose rate of 5 to 8 Gy/min. The lungs were shielded during the third dose of TBI. The preparative regimen CVB was administered to those patients who previously received more than 20 Gy to the thoracic or abdominal areas. The CVB regimen consisted of a 1-hour infusion of 1,800 mg/m² of cyclophosphamide and 200 mg/m² of VP-16 every 12 hours for each of the first 4 days followed by a single dose of 600 mg/m² of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) on day 5 of the regimen. All chemotherapeutic agents were administered through a running intravenous line. The BM was infused within 24 hours of the last dose of TBI or 72 to 80 hours after completion of the BCNU infusion.

Supportive Care
Most patients received oral prophylactic antimicrobials consisting of nofloxacine and fluconazole starting 1 week before admission and continuing until the absolute neutrophil count (ANC) recovered to ≥500/µL for 2 consecutive days. Only allograft recipients received sulfamethoxazole/trimethoprim prophylaxis for Pneumocystis carinii starting when their ANC reached 500/µL and continuing until 4 weeks after the cessation of immunosuppressive therapy. Other supportive care, such as treatment of infections, blood component transfusions, and total parenteral nutrition, was uniform during the entire period of the study. All patients who received prophylaxis were screened for CMV serology. The blood products were irradiated with 24 Gy before transfusion.

Study Monitoring and Evaluation
During the initial transplant hospitalization, complete blood counts, electrolyte studies, and renal function studies were performed daily; liver function studies were performed 3 times a week and as clinically indicated. All autograft recipients had a diagnostic BM aspiration and biopsy for morphology and cytogentic performed before BMT. The diagnostic BM examination was performed in all autograft recipients during the 2 weeks before their BM harvests.

Pre-BMT tumor measurements were obtained by physical examination or appropriate roentgenographic studies and were repeated when there was a clinical suspicion of disease progression. Therefore, the follow-up tumor assessments including BM examination were made at 2, 4, 6, and 12 months and then yearly after BMT.

Definitions
All patients had received chemotherapy within 4 to 8 weeks before transplant admission. The frontline or salvage regimens were chosen and administered at the discretion of the referring physicians. There was no attempt by the transplant team to control the salvage regimen for uniformity. The response to the last regimen of chemotherapy was used to determine the chemosensitivity of the tumor. Patients whose tumors were reduced by at least 50% of the product of the perpendicular diameters of the measurable tumors were considered to have chemosensitive disease; otherwise, they were considered to have chemoresistant disease.

Appropriate imaging studies were performed on each patient within 2 weeks before the transplant admission. The diameter of the largest tumor was measured and classified to be either ≥5 cm or greater than 5 cm. The term disease progression was used because the abnormality on the imaging studies may not truly indicate the presence of active disease. An appearance of a new lesion or a progressive increase in the product of the perpendicular diameters of any tumor greater than 50% of the pretransplant measurements were noted as disease progression.

Statistical Methods
The Kaplan-Meier method was used to estimate the rate of disease progression and PFS. The rate of disease progression was calculated from the day of BMT to the date of disease progression. The PFS was calculated from the date of BMT to the date of disease progression or death from any cause. Comparisons of the probability of disease progression and survival within each prognostic group were evaluated by the log rank test. Multivariate analyses were based on the proportional hazards regression model. The distributions of categorical data were compared by Fisher’s exact test or x² test with Yate’s correction. Differences of continuous variables between groups were evaluated by Student’s t test. All statistical analyses were performed using BMDP microcomputer-based software (University of California, Berkley, CA). All P values reported were two-sided. Data were updated and analyzed as of August 31, 1993.

RESULTS
Patient Demographics and Characteristics
Sixty-six patients were enrolled into this study. The median age of the entire group was 41, ranging from 15 to 60. With the exception of a higher frequency of previous BM involvement in the allograft recipients, there were no differences between the autologous and the allogeneic groups in the distribution of demographics and clinical characteristics (Table 1).

Disease Progression and Progression-Free Survival
Of the entire group of 66 patients, 26 patients had a documented disease progression. At 2 years, the actuarial probabilities of disease progression and PFS of the entire group (±SE) were 44% ± 7% and 39% ± 6%, respectively. Disease progression was noted in 22 of 35 patients in the autologous group and in 4 of 31 patients in the allogeneic group.
The probability of disease progression was 69% ± 9% in the autologous group and 20% ± 10% in the allogeneic group; the difference was statistically significant (P = .001; see Fig 1). The longest interval from BMT to disease progression was 20 months in the allogeneic group and 41 months in the autologous group. Despite the higher rate of disease progression in the autologous group, the PFS of the autologous group (24% ± 8%) and the allogeneic group (47% ± 9%) was not significantly different (P = .21; see Fig 2).

**GVHD**

Of 31 allograft recipients, 30 received BM from a major histocompatibility complex-matched sibling, and only 1 patient received a 1-antigen–mismatched BM. Of 31 allograft recipients, 16 (52%) developed acute GVHD, and 11 (35%) developed chronic GVHD. There was no difference in the probability of disease progression in patients who did (15% ± 10%) or did not (22% ± 14%) develop acute GVHD (P = .85). The PFS of patients with acute GVHD (38% ± 12%) was lower than that of the patients who had no acute GVHD (57% ± 14%), but the difference was not statistically significant (P = .24). Patients who developed chronic GVHD had a lower probability of disease progression (14% ± 13%) compared with that of the patients who did not develop chronic GVHD (23% ± 12%). The difference was not significant (P = .35). Comparing the PFS of patients who developed chronic GVHD with that of those who did not develop chronic GVHD showed a trend of improved PFS in the former group (62% ± 15% and 40% ± 11%, respectively; P = .11).

**Causes of Death**

Thirty-eight patients have since died. Disease progression was the cause of death in 18 of the 22 deaths (82%) in the autologous group and in 4 of the 16 (25%) deaths in the allogeneic group. The remaining deaths in each group were from causes not related to lymphoma. The association between the type of grafts and cause of death was significant (P = .007). Of the 4 nonlymphoma deaths in autograft recipients, 2 died from sepsis, 1 died from acute myocardial infarction, and 1 patient died from acute myeloid leukemia (AML) at 36 months after BMT. Of the 12 nonlymphoma deaths among allograft recipients, 10 died from infections (5 of whom had either acute or chronic GVHD), 1 died from toxic epidermal necrolysis, and 1 died from severe acute GVHD.

**Analyses of Prognostic Factors**

In the univariate analysis, favorable prognostic factors influencing the probability of disease progression that were identified included allograft (P = .001) and chemosensitive disease (P = .94). The probability of disease progression in patients with chemosensitive disease was 60% ± 12% in the autologous group and 18% ± 12% in allogeneic group (P = .03). The probability of disease progression in patients with chemoresistant disease was 87% ± 12% in the autologous group and 19% ± 12% in the allogeneic group (P = .04).
Patients with chemosensitive disease had a significantly better PFS compared with that of patients with chemoresistant disease (43% ± 9% vs 23% ± 9%; *P* = .002). The PFS of the allogeneic and autologous groups did not differ depending on whether the disease was chemosensitive (60% ± 13% vs 32% ± 11%; *P* = .22) or chemoresistant (38% ± 12% vs 11% ± 9%; *P* = .31).

In the multivariate analysis, the hazard ratios of potential prognostic variables derived from Cox proportional hazards model were listed in Table 2. Both allograft and chemosensitive disease had a favorable influence on the probability of disease progression (*P* = .003 and *P* = .03). Allogeneic graft did not significantly influence the PFS (*P* = .23), but the patients with chemoresistant disease had a significantly poorer PFS (*P* < .002).

**DISCUSSION**

The question of the existence of a graft-versus-lymphoma effect is an important clinical issue in BMT. Most of the preparative regimens used in BMT for the treatment of malignancies have reached the limit of extramedullary toxicities. Yet, the recurrence of tumor remains a dominant cause of treatment failure. Graft-versus-lymphoma effect, if it exists, may play an important role in tumor eradication when tumor burden is at its minimum.

Several retrospective studies have attempted to determine the existence of graft-versus-lymphoma effect. The first study reported by Appelbaum et al included 100 patients with lymphoma and HD transplanted between 1970 and 1985. In this series, 74 patients had resistant disease, and 60 patients received an allogeneic BMT with methotrexate administered for GVH prophylaxis. The high peritransplant mortality of allogeneic BMT in the precyclosporine era, coupled with the inclusion of a high percentage of patients with chemoresistant disease, likely explained the lack of a detectable influence of the allograft on the rate of disease progression. The most recent report by the EBMT was a case-controlled study. The study groups were generated by com-
puter matching of the starting 101 allograft recipients with NHL to the same number of autograft recipients from the EBMT database. Half of the patients in this series were in first complete remission, and 57 patients had a high-grade lymphoma. The probability of disease progression was higher in the autograft recipients who had lymphoblastic lymphoma but was not higher in other histologies.

The existence of a graft-versus-lymphoma effect was first suggested by Jones et al. in a series of 118 consecutive patients with lymphoma and HD. In comparing 38 allograft recipients with 80 autograft recipients, the probability of relapse was significantly lower among the allograft recipients than among the autograft recipients. The PFS for the entire group did not differ between patients who received an allogeneic graft and those who received an autologous graft. Our findings in this study were similar to those of the Johns Hopkins series. In the univariate analysis, the probability of disease progression remained significantly lower among allograft recipients in both the univariate and multivariate analyses, which provides strong evidence for the existence of a graft-versus-lymphoma effect.

Other than the presence of graft-versus-lymphoma effect in the allogeneic BMT, the difference in the rate of disease progression may be caused by the reinfusion of tumor cells in the autologous BM grafts. To minimize the confounding element of tumor contamination in the autograft, it would be desirable to compare only the patients who received a tumor-free autologous BM graft (preferably defined by the absence of a specific gene rearrangement using polymerase chain reaction) with the patients who received an allogeneic BM graft. It is unlikely that this type of study can be feasibly accomplished in the near future. Furthermore, the failure of the preparative regimen to eradicate the tumor in the host appears to be the main reason for disease progression or relapse. In the case of autologous BMT, hope for improvement of the results may have to rely on the enhancement of the antitumor effect via immunologic means. Our data lend strong support to such approaches. In recent years, there has been a surge of interest in using cyclosporine to induce autologous GVHD and cytokines such as interleukin-2, interferon, and others to enhance cell-mediated cytotoxicity. These ventures reflect the sentiments of many investigators on the importance of immunotherapy in the BMT setting.

In the meantime, the allogeneic BMT may be underused in the treatment of lymphomas. To exploit the graft-versus-lymphoma effect, allogeneic BMT should be considered in younger patients who have a sibling donor. Allogeneic BMT in patients with lymphoma should be more frequently used, considering the continued improvement in the treatment and prevention of GVHD and its associated complications. The magnitude of tumor cell kill via graft-versus-lymphoma effect may be relatively small compared with what is achievable with the ablative therapy administered before BMT. But the antitumor activity mediated through a graft-versus-lymphoma effect may be critical for a prolonged immunosurveillance mechanism that leads to an eventual complete eradication of the tumor. Furthermore, the later occurrence of AML in 1 of our autologous recipients underscores the importance of the quality of stem cells used in BMT and the significance of previous exposure of the stem cells to leukemic cytotoxic therapy. The actuarial risk of myelodysplastic syndrome and AML after autologous BMT is approximately 10% to 16% according to two recent reports. In summary, this prospective comparative trial of autologous versus allogeneic BMT supports the existence of a graft-versus-lymphoma effect in the allograft recipients. This finding strengthens the rationale for the incorporation of immunotherapy into the autologous BMT. Further comparative study is needed to confirm these observations.

### Table 2. Influence of Prognostic Variables on Probability of Disease Progression and PFS

<table>
<thead>
<tr>
<th>Prognostic Variables</th>
<th>Disease Progression Hazard Ratio (95% CI)</th>
<th>PFS Hazard Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.02 (1.01-1.03)</td>
<td>1.02 (0.99-1.03)</td>
</tr>
<tr>
<td>Female sex</td>
<td>1.0 (1.0-1.03)</td>
<td>1.0 (1.0-1.03)</td>
</tr>
<tr>
<td>Duration of disease</td>
<td>0.87 (0.75-1.00)</td>
<td>0.90 (0.75-1.07)</td>
</tr>
<tr>
<td>Received CR</td>
<td>0.48 (0.25-1.33)</td>
<td>0.48 (0.25-1.33)</td>
</tr>
<tr>
<td>Intermediate grade histologies</td>
<td>0.63 (0.40-0.72)</td>
<td>0.63 (0.40-0.72)</td>
</tr>
<tr>
<td>Received &gt;1 prior &amp; CR</td>
<td>1.29 (0.82-1.30)</td>
<td>1.29 (0.82-1.30)</td>
</tr>
<tr>
<td>Chemoresistant disease</td>
<td>2.91 (1.28-7.82)</td>
<td>2.91 (1.28-7.82)</td>
</tr>
</tbody>
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caused by the small number of allograft recipients, which made a meaningful comparison impossible. On the other hand, the recent report from the International Bone Marrow Transplant Registry showed a significantly higher relapse rate of AML in the syngeneic recipients compared with that for the allograft recipients who did not develop clinical GVHD. Therefore, the antitumor activity mediated through allogeneic graft does not mandate the presence of clinical GVHD. In this study, the lower probability of disease progression among allograft recipients in both the univariate and multivariate analyses provides strong evidence for the existence of a graft-versus-lymphoma effect.
studies similar to this one will be needed to identify the prognostic indicators that help predict the benefits of one type of graft on each of the particular subsets of patients.

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