Biology of Blood and Marrow Transplantation 6:434-440 (2000)

Comparison of Granulocyte Colony-Stimulating Factor (G-CSF)–Mobilized Peripheral Blood Progenitor Cells and G-CSF–Stimulated Bone Marrow as a Source of Stem Cells in HLA-Matched Sibling Transplantation

Jonathan S. Serody,[1,4] Scotty D. Sparks,[3] Yuhua Lin,[4] Eileen J. Capel,[1] Sharon H. Bigelow,[1] Suzanne L. Kirby,[1,4] Don A. Gabriel,[1] Joseph M. Wiley,[1] Mark E. Brecher,[3] Michael J. Schell,[4] James Folds,[3] Thomas C. Shea[1,4]

[1]Departments of Medicine, [2]Pediatrics, and [3]Laboratory Medicine and Pathology, University of North Carolina at Chapel Hill, the Lineberger Comprehensive Cancer Center, and the [4]University of North Carolina Hospitals, Chapel Hill, North Carolina

Correspondence and reprint requests: Jonathan S. Serody, Campus Box #7295, Lineberger Comprehensive Cancer Center, University of North Carolina School of Medicine, Chapel Hill, NC 27599 (e-mail: serody@med.unc.edu)

(Received December 20, 1999; accepted February 10, 2000)

ABSTRACT

HLA-identical bone marrow or stem cell transplantation from a sibling is the preferred treatment for patients with chronic myelogenous leukemia, bone marrow failure syndromes, relapsed acute leukemia, and specific inborn errors of metabolism. Several groups have shown that granulocyte colony-stimulating factor (G-CSF)–mobilized peripheral blood progenitor cells (PBPCs) obtained from HLA-matched siblings are effective in reconstitution of marrow function after marrow ablative conditioning therapy. To evaluate whether G-CSF treatment before bone marrow harvest leads to enhanced recovery of PBPC counts and recovery from limited graft-versus-host disease (GVHD), we assessed the outcome of a sequential cohort of patients treated identically and then given either G-CSF–mobilized PBPCs or G-CSF–stimulated bone marrow from HLA-identical siblings. We show that the time to neutrophil engraftment is identical in the 2 cohorts, whereas platelet engraftment is earlier with the use of PBPCs. The incidence of acute GVHD was decreased, and that of chronic GVHD significantly decreased, in the group receiving bone marrow. Overall survival was not different between the 2 groups. Thus, G-CSF–stimulated bone marrow offers a source of stem cells that allows for early neutrophil engraftment with a decreased risk of GVHD.

KEY WORDS

G-CSF • Allogeneic transplantation • Graft-versus-host disease

INTRODUCTION

Over the past several years, a number of groups have reported on the outcome of patients who received granulocyte colony-stimulating factor (G-CSF)–mobilized peripheral blood progenitor cells (PBPCs) during HLA-matched allogeneic transplantation [1-5]. These results have consistently shown that patients engraft earlier with this product than historically treated patients receiving bone marrow and that, whereas engraftment is markedly improved after administration of growth factors, it is not dependent on this. Patients receiving PBPCs have an incidence of acute graft-versus-host disease (aGVHD) of 14% to 70%, which is surprising given the large number of T cells found in the product. The incidence of chronic GVHD (cGVHD) after PBPC infusion is approximately 70% to 80% compared with 40% to 50% with conventional bone marrow [6,7]. This increased incidence of cGVHD after PBPC infusion is a substantial barrier to using this product.

Several large ongoing clinical trials are underway to compare the outcome of patients treated with either G-CSF–mobilized PBPCs or unmanipulated bone marrow in allogeneic transplantation. Only 2 small published reports and 1 recent presentation, however, describe the outcome of patients who received G-CSF–stimulated bone marrow [1,8,9]. Previous reports in the autologous setting have shown that engraftment of neutrophils is similar in patients receiv-

ing G-CSF–stimulated bone marrow or G-CSF–mobilized PBPCs [10]. Furthermore, previous investigators have shown that G-CSF has an impact on the function of alloreactive T cells by several mechanisms, which include T helper 2 (Th2) polarization, alterations in cytokine production by antigen-presenting cells, and changes in CD28/CD80 signaling [11-14]. Thus, we have undertaken a series of studies to investigate the time to engraftment, incidence of GVHD, and overall survival in 2 cohorts of patients undergoing allogeneic transplantation. The 2 cohorts were treated identically except for the receipt of either G-CSF–mobilized PBPCs or G-CSF–stimulated bone marrow.

MATERIALS AND METHODS

Study Protocol

Patients ages 12 to 55 years were eligible to participate in the 2 trials. Eligible patients were enrolled from patients referred to the Bone Marrow Transplantation Clinic at the University of North Carolina Hospitals. All patients were enrolled after discussion of the potential risks and benefits of the procedures. The studies were approved by the Committee on the Protection of the Rights of Human Subjects at the University of North Carolina. Patient accrual was initiated in February 1994 and terminated in December 1998.

The study was performed as 2 sequential nonrandomized studies. Patients with an HLA-identical sibling and acute lymphoblastic leukemia (ALL) or acute myelogenous leukemia (AML) in second remission or beyond or having high-risk features in first complete remission (CR1), multiple myeloma, recurrent or refractory non-Hodgkin's lymphoma (NHL), Hodgkin's disease, or chronic myelogenous leukemia (CML) were eligible. Patients were excluded from the trials if the creatinine clearance was <60 mL/min, ejection fraction <50% using resting ventriculogram or echocardiogram, or carbon monoxide diffusion capacity (DLCO) <50%. All patients who entered the trial were negative for hepatitis surface antigen and antibodies to human immunodeficiency virus (HIV). HLA typing for class I alleles was performed using serological methods at the University of North Carolina Hospitals. Class II typing was performed using DNA analysis by sequence-specific oligonucleotide primers.

Donor Treatment

All donors received 10 μg/kg G-CSF for 4 days. On the fifth day, donors underwent either leukapheresis using a Cobe Spectra or Fresenious AS104 machine or bone marrow harvest. Ten to 15 L blood was processed, and the leukapheresis was continued until at least 5×10^6 $CD34^+$ cells were obtained. The median number of collections performed was 2 (range, 1-4). Bone marrow was collected to a target volume of 18 mL/kg recipient body weight, with approximately 5 mL bone marrow aspirated from each site. PBPC products were stored frozen in liquid nitrogen until the day of infusion.

Treatment and Supportive Care

All patients were treated prophylactically with trimethoprim/sulfamethoxazole, fluconazole, and ciprofloxacin as described [15]. Intravenous antibiotics were administered for fevers >38.3°C. Patients seropositive for herpes simplex virus (HSV) received 200 mg acyclovir 3 times a day. All cytomegalovirus (CMV)-seronegative recipients who received seropositive grafts were treated with ganciclovir starting at day 21 until approximately day 100. All other patients were screened by CMV antigen tests and treated if positive. Patients with grade II or higher aGVHD were treated with 2 mg/kg intravenous 6-methylpredisolone and were routinely given ganciclovir as prophylaxis. Packed red blood cells were transfused if the morning hematocrit was <25%. Platelets were administered for a platelet count of ≤10,000/mm^3.

All nonmyeloma patients were conditioned with busulfan 16 mg/kg and cyclophosphamide 120 mg/kg as reported [16]. Patients with multiple myeloma received busulfan 14 mg/kg in addition to the cyclophosphamide [17]. GVHD prophylaxis used short-course methotrexate (15 mg/m^2 on day 1 and 10 mg/m^2 on days 3 and 6) and cyclosporine (administered to maintain trough levels between 250 and 350 ng/mL). Acute GVHD was graded as reported [18]. G-CSF (300 μg subcutaneously) was administered to 2 recipients of G-CSF–stimulated bone marrow, 1 at day 9 and 1 at day 17 after marrow infusion.

Outcome

Engraftment of neutrophils was defined to be the first of 3 consecutive days with a neutrophil count $>500 \times 10^9$/L. Platelet engraftment was defined as the first of 10 consecutive days of platelets $>20{,}000 \times 10^9$/L without transfusion. Patients with AML or ALL in first remission, NHL responsive to salvage chemotherapy, and CML in chronic phase were considered standard risk. All other patients were in the high-risk cohort.

Flow Cytometry

Cell counts were adjusted to 1×10^7 cells/mL in phosphate-buffered saline. All cells were blocked for 10 minutes using 5% human AB serum. Cells were stained for 15 minutes with the following monoclonal antibodies (Becton Dickinson, San Jose, CA): fluorescein isothiocyanate (FITC)-conjugated anti-CD3, anti-CD34, and immunoglobulin (Ig) G1 control; phycoerythrin (PE)-conjugated anti-CD4, anti-CD8α, anti-CD14, anti-CD16, anti-CD19, anti-CD33, anti-CD38, and IgG1 control; and Texas red–conjugated anti-CD45. Three-color flow cytometry was performed, and 25,000 to 100,000 events were analyzed using a FACScan flow cytometer and CellQuest Software (Becton Dickinson). The total number of cells in each lineage was determined by multiplying the total number of cells by the percentage derived from the flow histograms for the specific antibody used.

Statistics

The study was designed to show a 2-day earlier engraftment of neutrophils in patients receiving G-CSF–mobilized PBPCs compared with G-CSF–stimulated bone marrow. With an estimated SD of 2.25 days for the median time to neutrophil engraftment for the 2 groups, the inclusion of 21 patients in each group allowed for an 80% power to detect this difference with an α error of ≤0.05. The group receiving bone marrow was increased to 26 patients, which gave the study a power of 85% to detect this difference. Data were censored at the time of death or last follow-up, which was July 15, 1999. Survival curves were determined using the method of Kaplan and Meier [19]. Group compar-

Table 1. *Patient Characteristics**

	PBPCs	Bone Marrow
n	20	26
Age, y†	37 (13-52)	44 (29-55)
Sex‡		
Male	8	18
Female	12	8
Race		
Caucasian	13	20
African American	7	6
Diagnosis		
CML CP	9	9
CML CP2	0	1
AML	4	5
ALL	0	2
NHL	1	5
Myeloma	5	4
HD	1	0
Risk		
Standard	11	15
High	9	11
Female/Male Donor/Recipient	1/20	5/26
Age§	37 (range 15-55)	45 (range 32-54)

*Data are mean (range) or n. PBPCs indicates peripheral blood progenitor cells; CML, chronic myelogenous leukemia; CP, chronic phase; AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; NHL, non-Hodgkin's lymphoma; HD, Hodgkin's disease.
†$P = .02$.
‡$P = .006$.
§$P = .008$.

isons were performed using either Mann Whitney log rank test or Student *t* test. Analysis of differences in the cause-of-death was performed using Fisher exact test. All tests were 2-tailed. *P* values ≤ .05 were considered significant.

RESULTS

Patient Characteristics

The characteristics of the patients receiving PBPC transplant and the patients receiving bone marrow transplant are given in Table 1. Both groups were equally balanced for type of disease and the number of patients in each risk group. There was a significant difference in the mean age of the recipients ($P = .02$) and donors ($P = .008$), which favored a decreased risk for grades II to IV GVHD in the group receiving PBPCs (mean age for recipients and donors 37) compared with bone marrow (mean age for recipients 44 and for donors 45). A significant difference was also found in the ratio of males and females between groups ($P = .006$), with a greater number of males in the cohort that received G-CSF–stimulated bone marrow. The median follow-up for the group that received PBPCs was 887 days, and 367 days for the group receiving G-CSF–stimulated bone marrow. The difference in follow-up, which was due to the sequential nature of the treatments, was statistically significant ($P = .008$). Complications in the donors were strictly limited to bone pain and headache, which required therapy with either acetaminophen or oxycodone in the majority of donors. Platelet counts decreased in the majority of donors giving PBPCs but did not decrease below 20,000/mm^3 and the decrease was not associated with clinical bleeding or the need for transfusion [20]. Donors underwent a median of 2 leukapheresis procedures.

Engraftment

The data for engraftment of white blood cells, platelets, and transfusion support are presented in Table 2. The median time to a neutrophil recovery >500 × 10^9/L was 17 days (range, 12-28 days) for patients receiving PBPCs and 16 days (range, 12-27 days) for patients receiving bone marrow. The median time to platelet recovery >20,000 × 10^9/L without transfusion was 13 days (range, 0-65 days) for the patients receiving PBPCs and 16 days (range, 9-68 days) for the patients receiving bone marrow. There was no significant difference in the time to neutrophil engraftment ($P = .90$); however, there was a strong trend toward earlier platelet engraftment in the cohort receiving PBPCs ($P = .06$). There were no differences in the mean number of red blood cell or platelet transfusions during admission for the PBPC cohort (7.7 ± 1.1 red blood cell units, $P = .30$; 5.7 ± 1.3 platelet units, $P = .80$) compared with the bone marrow cohort (6.3 ± 0.9 red blood cell units; 5.8 ± 1.2 platelet units).

Two recipients who received G-CSF–stimulated bone marrow were treated with G-CSF after marrow infusion. One patient, treated on day 9, received G-CSF because of significant renal impairment from veno-occlusive disease complicated by treatment with amphotericin B because of neutropenic fever. Our protocol stipulated that patients with a neutrophil count <500/mm^3 receive G-CSF starting on day 17 after marrow infusion. The second patient received G-CSF on day 17 because of a neutrophil count of 200/mm^3 on that day. The time to engraftment did not differ when the group that received G-CSF–stimulated marrow was evaluated without these 2 patients (median day 16 with or without the 2 patients).

Phenotypic Analysis of PBPC and Bone Marrow Products

Using flow cytometry, we analyzed the number of T lymphocytes and CD34$^+$ progenitor cells infused in the PBPC and bone marrow fractions. These data are shown in Table 2. The mean number of T cells infused in the

Table 2. *Engraftment Characteristics**

	PBPCs	Bone Marrow
Absolute neutrophil count >500/mm^3, d	17 (12-28)	16 (12-27)
Platelets >20,000/mm^3, d	13 (0-65)†	16 (9-68)
Red blood cells		
Transfusions	7.7 ± 1.1	6.3 ± 0.9
Platelet transfusions	5.7 ± 1.3	5.8 ± 1.2
CD3, × 10^6 cells/kg	54 ± 18 (15-85)‡	4.0 ± 5.1 (0.08-25)
CD34, × 10^6 cells/kg	6.6 ± 0.8 (5.2-8.3)‡	1.6 ± 0.6 (0.6-2.8)

*Data are mean (range), mean ± SD, or mean ± SD (range). PBPCs indicates peripheral blood progenitor cells.
†$P = .06$.
‡$P < .001$.

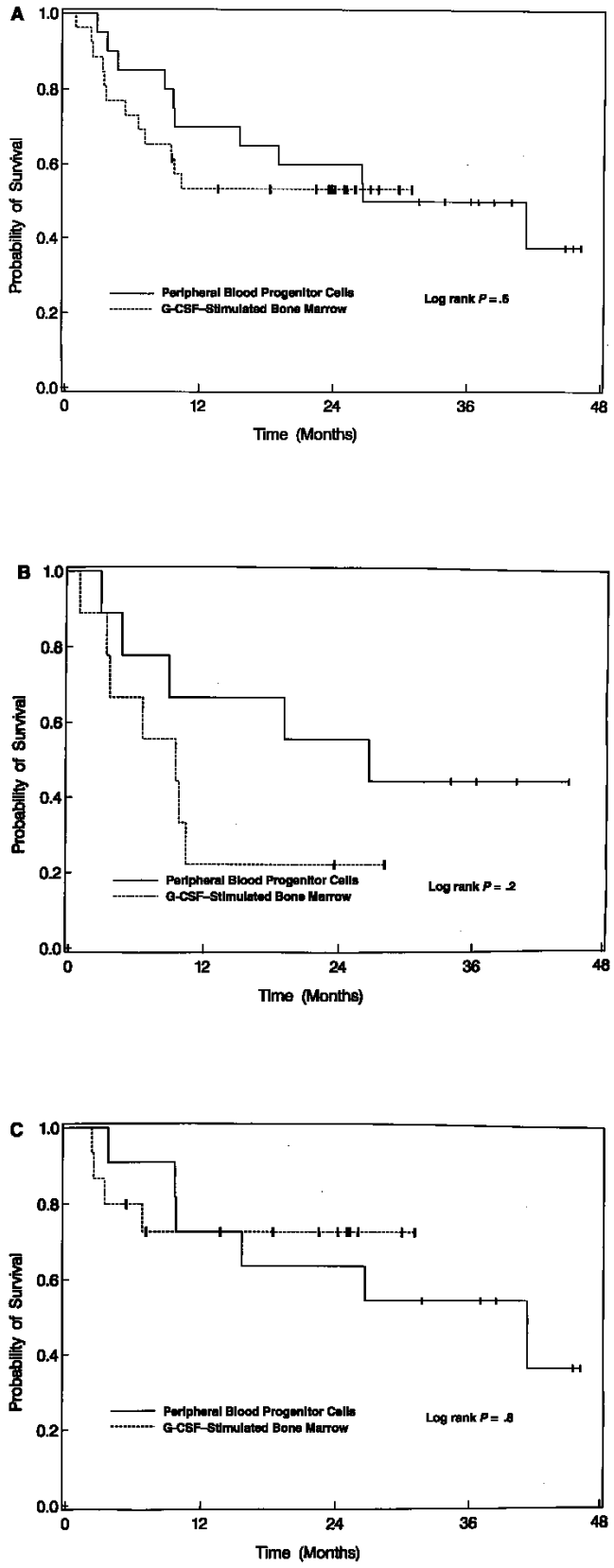

Figure 1. Actuarial overall survival of patients receiving either peripheral blood progenitor cells or bone marrow as a source of stem cells. A. All patients. B. High-risk patients. C. Standard-risk patients. G-CSF indicates granulocyte colony-stimulating factor.

patients receiving PBPCs was 5.4×10^7 cells/kg; by contrast, the mean number of T cells infused in patients receiving bone marrow was 4×10^6/kg ($P < .001$). The mean number of $CD34^+$ cells infused was 6.6×10^6/kg for the patients receiving PBPCs and 1.6×10^6/kg for the patients receiving bone marrow ($P < .001$).

Overall and Relapse-Free Survival

The differences in survival as a function of the product received are shown in Figure 1. There was a significant trend for an improved survival at day 100 posttransplantation for the group receiving PBPCs (100%) compared with the group receiving bone marrow (81%) ($P = .059$). Actuarial survival for the 2 groups at 24 months posttransplantation was 60% for patients receiving PBPCs compared with 54% for patients receiving bone marrow. This difference was not statistically significant ($P = .90$). When we separated patients into standard and high-risk groups, we identified trends in the outcome of the high-risk group dependent on the type of transplant. Patients in the high-risk PBPC group (Figure 1B) had a slight trend toward improved survival (47%) compared with the high-risk bone marrow group (22%; $P = .20$). However, the small number of patients in each group and the disparate follow-up limited the ability to draw firm conclusions from this analysis. At this time, we have not observed a significant difference in the outcome in the standard-risk group as a function of the product infused ($P = .80$) (Figure 1C).

The causes of death in the patients receiving PBPCs or bone marrow are presented in Table 3. For the group of patients receiving PBSCs, infections were due to group A streptococci (1), *Streptococcus pneumoniae* (2), *Pseudomonas aeruginosa* (1), *Escherichia coli* (1), and unknown (1).

Graft-Versus-Host Disease

The incidences of grades II to IV acute and chronic GVHD as a function of the product infused are shown in Figures 2 and 3 and Table 4. At 90 days posttransplantation, there was a strong trend toward an increased incidence of aGVHD in the cohort that received PBPCs (60%) compared with marrow (27%; $P = .07$). At 1 year posttransplantation, we found a significantly increased incidence of cGVHD in PBPC patients (68%) compared with bone marrow patients (37%; $P = .049$).

DISCUSSION

We have investigated the outcome for patients who were treated identically with the exception of receiving either G-CSF–mobilized PBPCs or G-CSF–stimulated bone marrow. We have found that patients who received G-CSF–stimulated bone marrow had a similar time to neutrophil engraftment and a similar use of red blood cell and platelet products after transplantation. We observed a slightly prolonged time to platelet engraftment in the cohort that received marrow compared with PBPCs. There was a statistically significant decreased incidence of cGVHD after the use of G-CSF–stimulated bone marrow compared with PBPC and a nonsignificant trend for a decreased incidence of aGVHD. The actuarial survival for recipients of PBPCs and bone marrow was similar at 24 months posttransplantation.

Table 3. *Reasons for Death**

	PBPCs		Bone Marrow	
	High-Risk	Standard-Risk	High-Risk	Standard-Risk
Relapse	3	0	5	0
Treatment-related mortality	1	0	0	2
Acute GVHD	0	0	0	1
Late infections/chronic GVHD	1	5	1	2
Other†	1	0	1	0
Death/total patients	6/9	5/11	7/11	5/15

*PBPCs indicates peripheral blood progenitor cells; GVHD, graft-versus-host-disease.

†One patient who received PBPCs died after a myocardial infarction that was thought to be secondary to previous radiation treatment. One patient who received bone marrow discontinued immunosuppressive medications without a physician's approval and presented with refractory GVHD that did not respond to treatment.

One of the initial positive attributes of the use of G-CSF–mobilized PBPCs was earlier neutrophil and platelet engraftment. Previous investigators have shown that patients who receive G-CSF–mobilized PBPCs have an earlier time to engraftment than those who receive nonstimulated bone marrow [2,3,5,6,21]. In several studies using nonmanipulated bone marrow, the median time to neutrophil engraftment in individuals receiving cyclosporine and short-course methotrexate was 21 to 24 days posttransplantation, and the median time to a platelet count of 20,000/mm^3 without transfusion was 24 to 27 days [22-26]. Thus, the use of G-CSF–stimulated bone marrow in this report resulted in earlier engraftment of both neutrophils and platelets compared with historical control patients given nonmanipulated bone marrow.

The time to engraftment of neutrophils in individuals receiving PBPCs in this study was slightly longer than that of previous cohorts receiving PBPCs. The median time to neutrophil engraftment in previous series has been 12 to 13 days postinfusion. There are several possibilities for the longer time to engraftment. We did not routinely administer growth factors to our patients, but other centers have administered growth factors after PBPC infusion to improve the time to neutrophil recovery [4]. The group in Seattle infused PBPCs without prior freezing [21]. As all of the PBPCs given in this study were frozen, the delay in engraftment of neutrophils could be due to the effect of freezing on progenitor cell number or function. Other groups have used either a different conditioning regimen or not used methotrexate as GVHD prophylaxis [2]. Both of these factors can influence the time to engraftment.

One recent report treated patients in an identical fashion as in this series [8]. Although we infused a greater number of CD34$^+$ cells, 6.6 $\times$ 10^6/kg versus 5.4 $\times$ 10^6/kg, the median time to neutrophil engraftment in our series was still longer than that reported by Ustun et al. [8]. Interestingly, the 13-day time to platelet engraftment after administration of PBPCs found here was similar to those in all other trials.

As has been shown previously, the number of T cells was much greater in the G-CSF–mobilized PBPCs than in the G-CSF–stimulated bone marrow. Previous reports using historical controls have shown that despite the increased number of T cells in the product, the incidence of grades II to IV aGVHD was often not greater in PBPC recipients compared with bone marrow recipients. We found that the incidence of grades II to IV aGVHD was increased in patients who received PBPCs (60%) compared with bone marrow (27%), and this difference approached statistical significance. The longer time to neutrophil engraftment seen in this study may be 1 cause for the increased incidence of GVHD compared with prior trials using PBPCs. In animal models, there is a strong relationship between the release of endotoxin and the magnitude of aGVHD, suggesting that earlier recovery of neutrophils may decrease infectious complications and the incidence of GVHD [27-29]. Additionally, the group at M.D. Anderson Cancer Center (Houston, TX) has recently reported on the outcome of 160 recipients of allogeneic PBPCs [30]. Using regression residual statistics, they found a sharp increased risk of GVHD after the administration of between 6.3 $\times$ 10^6 and 10.0 $\times$ 10^6 CD34$^+$ cells/kg. For recipients of G-CSF–stimulated PBPCs in this series, 17 of 20 (85%) received between 6.3 $\times$ 10^6 and 10.0 $\times$ 10^6 CD34$^+$ cells/kg. This may have contributed to the increased risk of GVHD for recipients of G-CSF–stimulated PBPC in this study. Very few of the group that received PBPCs in this report received more than 7.5 $\times$ 10^6 CD34$^+$ cells/kg. Thus, we were unable to verify data showing an increased incidence of aGVHD for recipients of more than 8.2 $\times$ 10^6 CD34$^+$ cells/kg [30].

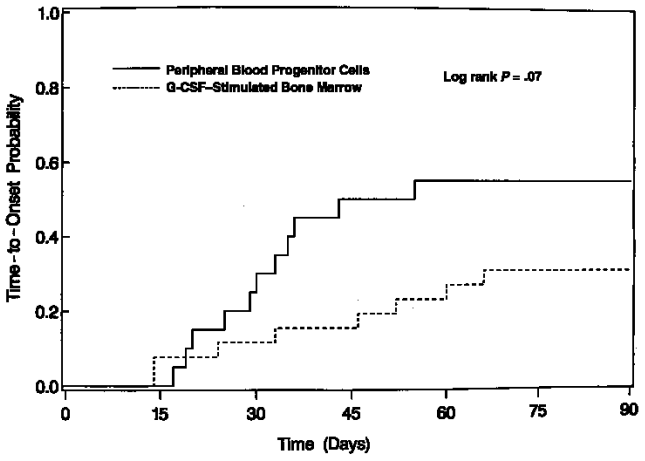

Figure 2. Incidence of grade II to IV acute graft-versus-host disease for patients receiving peripheral blood progenitor cells or bone marrow. G-CSF indicates granulocyte colony-stimulating factor.

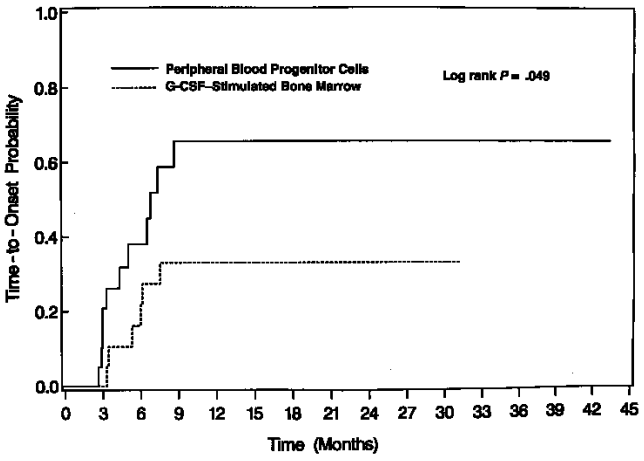

Figure 3. Incidence of chronic graft-versus-host disease for patients receiving peripheral blood progenitor cells or bone marrow. G-CSF indicates granulocyte colony-stimulating factor.

We found an increased incidence of cGVHD in the group that received PBPCs, which is consistent with previous observations [6,7]. Although the median follow-up for the group receiving bone marrow was more than 1 year posttransplantation, it was significantly shorter than that of the group receiving PBPCs. This difference may have influenced the decreased incidence of cGVHD in patients receiving bone marrow. The decreased incidence of cGVHD using G-CSF–stimulated bone marrow compared with G-CSF–mobilized PBPCs will need to be verified in randomized clinical trials and with longer follow-up.

We did not find a difference in overall survival of patients as a function of the type of product infused. The number of high-risk patients in the 2 series was small, and we did not detect differences in the relapse rate for this group as a function of the product received (33% PBPCs versus 50% bone marrow; $P = .65$), despite the significantly increased incidence of cGVHD in high-risk patients. We did observe a trend for late infectious deaths in the setting of cGVHD for recipients of PBPCs versus marrow. For standard-risk patients who survived past day 120, the incidence of late infectious deaths was 45% for recipients of PBPCs versus 15% for patients receiving bone marrow ($P = .18$). If this trend were to continue, there would be an improved outcome in patients in the standard-risk group given G-CSF–stimulated bone marrow due to the late infectious deaths in the cohort receiving PBPCs. In the standard-risk setting, where the risk of relapse is low, the greater incidence of infectious complications associated with cGVHD may outweigh the benefits of the graft-versus-tumor response. If this is true, it suggests an approach to management of transplant patients in which the risk of recurrent disease would help dictate the type of product infused.

Table 4. *Assessment of Graft-Versus-Host Disease**

Acute GVHD	PBPCs	Bone Marrow
Grades II-IV	12/20 (60%)	7/26 (27%)
Grades III-IV	4/20 (20%)	2/26 (8%)
Skin	10/12 (83%)	6/7 (86%)
Gastrointestinal Tract	4/12 (33%)	4/7 (57%)
Liver	0/12 (0%)	2/7 (29%)

*The percentages for involvement of the different organs with graft-versus-host disease (GVHD) are determined by dividing the number of patients with documented GVHD at that site by the total number with GVHD. PBPCs indicates peripheral blood progenitor cells.

Two previous reports have described the outcome of small numbers of patients with limited follow-up given G-CSF–stimulated bone marrow in the allogeneic setting. Isola et al. [31] described the treatment of 10 patients who were treated with cyclophosphamide 120 mg/kg and (predominantly) 1500 cGy total body irradiation (TBI). The primary diagnosis was CML in first chronic phase (n = 4). GVHD prophylaxis was a combination of cyclosporine and methotrexate or cyclosporine and prednisone. Despite a smaller volume of bone marrow, the mean number of $CD34^+$ cells per kg was significantly higher in this cohort (9.4×10^6/kg) than in our patients (1.6×10^6/kg), which may be explained by the bone marrow having been collected earlier after the administration of G-CSF. Compared with patients described in this series, the median time to neutrophil engraftment was decreased by 2 days, but interestingly, the time to platelet engraftment was increased by 4 days. The incidence of grades II to IV GVHD was 0% in the 6 patients who lived past day 100.

Mavroudis et al. [9] had previously reported on the outcome of 12 patients who received T-cell–depleted G-CSF–stimulated bone marrow from HLA-identical donors. The conditioning regimen employed was cyclophosphamide (60 mg/kg per day for 2 days) and TBI (up to 1360 cGy). Approximately 1200 mL bone marrow was harvested and selected using a $CD34^-$ selection column. In that study, 4 patients developed pancytopenia after initial engraftment. The late incidence of graft failure observed with $CD34^-$-selected, G-CSF–stimulated bone marrow was not found in either our cohort of patients or that described by Isola et al. [31]. This difference may relate to the lack of T-cell depletion in our patients and those of Isola et al., as we infused a slightly decreased number of $CD34^+$ cells per kg (1.6×10^6 CD34 versus 1.8×10^6/kg) compared with Mavroudis et al. The 33% incidence of grades II to IV aGVHD described by Mavroudis et al. was similar to that found here (27%), suggesting little benefit from $CD34^-$ selection in the prevention of aGVHD after receipt of G-CSF–stimulated bone marrow.

In summary, we have found that G-CSF–stimulated bone marrow offers the early engraftment of neutrophils that is seen with PBPCs, with a decreased risk of both acute and chronic GVHD. These observations, however, are preliminary and are being verified in randomized clinical trials [1]. If these findings are verified, in the future, the type of product infused in the allogeneic setting may be stratified based on the risk of relapse. G-CSF–stimulated bone marrow may be preferable for patients with CML in chronic phase and patients with AML or ALL in first remission, where the lower incidence of GVHD would factor more favorably than the risk of recurrent disease.

REFERENCES

1. Durrant ST, Morton AJ. A randomised trial of filgrastim (G-CSF) stimulated donor marrow (BM) versus peripheral blood (PBPC) for allogeneic transplantation: increased extensive chronic graft-versus-host disease following PBPC transplantation [abstract]. *Blood.* 1999;94:608a.
2. Korbling M, Przepiorka D, Huh YO, et al. Allogeneic blood stem cell transplantation for refractory leukemia and lymphoma: potential advantage of blood over marrow allografts. *Blood.* 1995;85:1659-1665.
3. Mahmoud HK, Fahmy O, Kamil M, El-Haddad A, El-Kadi D. Peripheral blood vs bone marrow as a source for allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplant.* 1999;24:355-358.
4. Pavletic ZS, Bishop MR, Tarantolo SR, et al. Hematopoietic recovery after allogeneic blood stem-cell transplantation compared with bone marrow transplantation in patients with hematologic malignancies. *J Clin Oncol.* 1997;15:1608-1616.
5. Schmitz N, Dreger P, Suttorp M, et al. Primary transplantation of allogeneic peripheral blood progenitor cells mobilized by filgrastim granulocyte colony-stimulating-factor. *Blood.* 1995;85:1666-1672.
6. Brown RA, Adkins D, Khoury H, et al. Long-term follow-up of high-risk allogeneic peripheral-blood stem-cell transplant recipients: graft-versus-host disease and transplant-related mortality. *J Clin Oncol.* 1999;17:806-812.
7. Storek J, Gooley T, Siadak M, et al. Allogeneic peripheral blood stem cell transplantation may be associated with a high risk of chronic graft-versus-host disease. *Blood.* 1997;90:4705-4709.
8. Ustun C, Arslan O, Beksac M, et al. A retrospective comparison of allogeneic peripheral blood stem cell and bone marrow transplantation results from a single center: a focus on the incidence of graft-vs-host disease and relapse. *Biol Blood Marrow Transplant.* 1999;5:28-35.
9. Mavroudis DA, Read EJ, Molldrem J, et al. T cell-depleted granulocyte colony-stimulating factor (G-CSF) modified allogeneic bone marrow transplantation for hematological malignancy improves graft $CD34^+$ cell content but is associated with delayed pancytopenia. *Bone Marrow Transplant.* 1998;21:431-440.
10. Janssen WE, Smilee RC, Elfenbein GJ. A prospective randomized trial comparing blood- and marrow-derived stem cells for hematopoietic replacement following high-dose chemotherapy. *J Hematother.* 1995;4:139-140.
11. Tanaka J, Mielcarek M, Torok-Storb B. Impaired induction of the CD28-responsive complex in granulocyte colony-stimulating factor mobilized CD4 T cells. *Blood.* 1998;91:347-352.
12. Pan L, Delmonte J, Jalonen CK, Ferrara JL. Pretreatment of donors with granulocyte colony-stimulating factor polarizes donor T lymphocytes toward type 2 cytokine production and reduces severity of experimental graft-versus-host disease. *Blood.* 1995;86:4422-4429.
13. Pan L, Teshima T, Hill GR, et al. Granulocyte colony-stimulating factor-mobilized allogeneic stem cell transplantation maintains graft-versus-leukemia effects through a perforin-dependent pathway while preventing graft-versus-host disease. *Blood.* 1999;93:4071-4078.
14. Mielcarek M, Graf L, Johnson G, Torok-Storb B. Production of interleukin-10 by granulocyte colony-stimulating factor-mobilized blood products: a mechanism for monocyte-mediated suppression of T-cell proliferation. *Blood.* 1998;92:215-222.
15. Serody JS, Shea TC. Prevention of infections in bone marrow transplant recipients. *Infect Dis Clin North Am.* 1997;11:459-477.
16. Santos GW. Busulfan and cyclophosphamide for marrow transplantation. *Bone Marrow Transplant.* 1989;4(suppl):236-239.
17. Bensinger WI, Buckner CD, Clift RA, et al. Phase I study of busulfan and cyclophosphamide in preparation for allogeneic marrow transplant for patients with multiple myeloma. *J Clin Oncol.* 1992;10:1492-1497.
18. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HLA-matched sibling donors. *Transplantation.* 1974;18:295-304.
19. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc.* 1958;53:457-481.
20. Bandarenko N, Brecher ME, Owen H, Wiley J, Shea T, Serody J. Thrombocytopenia in allogeneic peripheral blood stem cell collections. *Transfusion.* 1996;36:668-669.
21. Bensinger WI, Clift R, Martin P, et al. Allogeneic peripheral blood stem cell transplantation in patients with advanced hematologic malignancies: a retrospective comparison with marrow transplantation. *Blood.* 1996;88:2794-2800.
22. Gondo H, Harada M, Taniguchi S, et al. Cyclosporine combined with methylprednisolone or methotrexate in prophylaxis of moderate to severe acute graft-versus-host disease. *Bone Marrow Transplant.* 1993;12:437-441.
23. von Bueltzingsloewen A, Belanger R, Perreault C, et al. Acute graft-versus-host disease prophylaxis with methotrexate and cyclosporine after busulfan and cyclophosphamide in patients with hematologic malignancies. *Blood.* 1993;81:849-855.
24. Ringden O, Horowitz MM, Sondel P, et al. Methotrexate, cyclosporine, or both to prevent graft-versus-host disease after HLA-identical sibling bone marrow transplants for early leukemia? *Blood.* 1993;81:1094-1101.
25. Storb R, Deeg HJ, Pepe M, et al. Graft-versus-host disease prevention by methotrexate combined with cyclosporine compared to methotrexate alone in patients given marrow grafts for severe aplastic anaemia: long-term follow-up of a controlled trial. *Br J Haematol.* 1989;72:567-572.
26. Storb R, Deeg HJ, Pepe M, et al. 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. *Blood.* 1989;73:1729-1734.
27. Cooke KR, Hill GR, Crawford JM, et al. Tumor necrosis factor-alpha production to lipopolysaccharide stimulation by donor cells predicts the severity of experimental acute graft-versus-host disease. *J Clin Invest.* 1998;102:1882-1891.
28. Hill GR, Cooke KR, Teshima T, et al. Interleukin-11 promotes T cell polarization and prevents acute graft-versus-host disease after allogeneic bone marrow transplantation. *J Clin Invest.* 1998;102:115-123.
29. Hill GR, Crawford JM, Cooke KR, Brinson YS, Pan L, Ferrara JL. Total body irradiation and acute graft-versus-host disease: the role of gastrointestinal damage and inflammatory cytokines. *Blood.* 1997;90:3204-3213.
30. Przepiorka D, Smith TL, Folloder J, et al. Risk factors for acute graft-versus-host disease after allogeneic blood stem cell transplantation. *Blood.* 1999;94:1465-1470.
31. Isola LM, Scigliano E, Skerrett D, et al. A pilot study of allogeneic bone marrow transplantation using related donors stimulated with G-CSF. *Bone Marrow Transplant.* 1997;20:1033-1037.