Peripheral blood stem cells (PBSCs) have been used rarely for allogeneic transplantation because of concerns regarding graft failure and graft-versus-host disease (GVHD). We evaluated the results of allogeneic PBSC transplantation (allo-PBSCT) in 9 patients with refractory leukemia or lymphoma receiving myeloablative therapy followed by allo-PBSCT from an HLA-identical sibling donor. Three patients had relapsed 11 to 21 months after allogeneic bone marrow transplantation (allo-BMT) and underwent allo-PBSCT using the same donor. Six patients received PBSCs as their initial allogeneic transplant. Filgrastim-mobilized PBSCs were collected from the donors in 3 to 4 aphereses and cryopreserved. The apheresis collections contained a median nucleated cell count of 16.5 x 10^6/kg (range, 10.8 to 28.7 x 10^6), 10.7 x 10^3 CD34+ cells/kg (range, 7.5 to 22.5 x 10^3), and 300.0 x 10^3 CD3+ cells/kg (range, 127.8 to 1,523.2 x 10^3). The median recovery of CD34+ progenitor cells after freezing, thawing, and washing was 106.4% (range 36.7% to 132.9%). All patients received filgrastim posttransplant through engraftment, and cyclosporine and methylprednisolone were used for GVHD prophylaxis. Neutrophil recovery to greater than 0.5 x 10^9/L and greater than 1.0 x 10^9/L occurred at a median of 9 days (range, 6 to 10) and 9 days (range, 8 to 11) posttransplant, respectively, which was similar to historical controls after allo-BMT and granulocyte-colony-stimulating factor therapy. Platelets recovered to greater than 20 x 10^11/L and greater than 50 x 10^11/L at a median of 12 days (range, 8 to 25) and 15 days (range, 11 to 59), respectively, which was significantly more rapid than for the controls (P<.01). Donor cell engraftment was documented by cytogenetics, fluorescence in situ hybridization, and/or restriction fragment length polymorphisms with longest follow-up of 283+ days. Three patients developed grade 2 acute GVHD involving the skin. Three of five evaluable patients show limited chronic GVHD. Cryopreserved, filgrastim-stimulated allogeneic PBSCs may be a suitable alternative to allogeneic marrow for transplantation with the advantage of more rapid platelet recovery. Acute GVHD was minimal despite the infusion of 1 log more CD3 cells than with marrow allografts. Further studies are required to assess long-term risks of chronic GVHD.

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The patients were hospitalized in laminar airflow rooms. Infection prophylaxis during the peritransplant period consisted of nonabsorbable antibiotics orally, 1 g vancomycin IV daily, 200 mg fluconazole IV twice daily, and 5 mg/kg acyclovir IV every 8 or 12 hours. All patients received broad spectrum antibiotics for neutropenic fever, hyperalimentation when needed, and irradiated blood products per standard routine. IV Ig (500 mg/kg) was administered weekly through day 100 and monthly thereafter through 1 year. Once engrafted, the patients also received twice-weekly trimethoprim/sulfamethoxazole orally or pentamidine by inhalation every 3 weeks; cytomegalovirus (CMV)-seropositive patients received prophylactic ganciclovir 5 times per week.

### Table 1. Patient Characteristics, Engraftment, and Chimerism

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Diagnosis</th>
<th>Patient Age/Gender</th>
<th>Donor Age/Gender</th>
<th>Preparative Regimen</th>
<th>Days to Neutrophils</th>
<th>Days to Platelets</th>
<th>Latest Chimerism/MRD Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AML</td>
<td>24/F</td>
<td>28/M</td>
<td>CY/TBI</td>
<td>&gt;0.5 x 10^9/L</td>
<td>&gt;0.5 x 10^9/L</td>
<td>20/20 46,XX (283) Donor (172)</td>
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<tr>
<td>2</td>
<td>AML</td>
<td>46/M</td>
<td>48/M</td>
<td>TBI</td>
<td>&gt;0.5 x 10^9/L</td>
<td>&gt;0.5 x 10^9/L</td>
<td>20/20 46,XX (132) Donor (132)</td>
</tr>
<tr>
<td>3</td>
<td>Hodgkin’s</td>
<td>24/M</td>
<td>22/F</td>
<td>CBV</td>
<td>9</td>
<td>9</td>
<td>20/20 46,XX (102) Donor (90)</td>
</tr>
<tr>
<td>4</td>
<td>Lymphoma</td>
<td>36/F</td>
<td>29/M</td>
<td>TBC</td>
<td>8</td>
<td>8</td>
<td>20/20 46,XX (83) Donor (84)</td>
</tr>
<tr>
<td>5</td>
<td>CML</td>
<td>51/M</td>
<td>46/M</td>
<td>CY/TBI</td>
<td>9</td>
<td>9</td>
<td>21/21 46,XX (281) Donor (28)</td>
</tr>
<tr>
<td>6</td>
<td>Lymphoma</td>
<td>38/F</td>
<td>29/F</td>
<td>TBI</td>
<td>9</td>
<td>9</td>
<td>21/21 46,XX (281) Donor (28)</td>
</tr>
<tr>
<td>7</td>
<td>Lymphoma</td>
<td>39/M</td>
<td>41/M</td>
<td>TBI</td>
<td>9</td>
<td>9</td>
<td>21/21 46,XX (281) Donor (28)</td>
</tr>
<tr>
<td>8</td>
<td>AML</td>
<td>36/M</td>
<td>40/M</td>
<td>CY/TBI</td>
<td>10</td>
<td>10</td>
<td>21/21 46,XX (281) Donor (28)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Control group</th>
<th>(8-16)</th>
<th>(8-19)</th>
<th>(11-100+)</th>
<th>(13-100+)</th>
</tr>
</thead>
</table>
| Patients no. 1, 2, and 5 had clonal cytogenetic abnormalities while in relapse. Numbers in brackets refer to transplant day of latest study.

Abbreviations: CY/TBI, cyclophosphamide, total body irradiation; TBC, Thiotepa, busulfan; CY; CBV, CY, BCNU, etoposide; NS, not significant.

### RESULTS

**Immunophenotypic characterization of the apheresis collections.** The total apheresis collections had a mean num-

...
Patients had complete donor chimerism in marrow samples as determined by cytogenetics and/or RFLP (Table 1). Patients no. 1 also had 85 of 85 interphase cells positive for X and Y chromosomes by FISH at day 60.

In Patient no. 1, leukemia cell metaphases identified by the t(16;17) were not found posttransplant (Table 1). However, in Patient no. 2, despite a marrow in morphologic remission, cytogenetic abnormalities consistent with the original leukemia were detected in a small number of cells posttransplant. During first remission, the karyotype was 46,XY. At the time of initial relapse, the karyotype was 45,XY,inv(3),−7, but no −7 was present after reinduction was attempted with conventional chemotherapy pretransplant. At 3 weeks posttransplant, 1 of 30 metaphases had the inv(3). At 2, 4, 8, and 19 weeks posttransplant, 3 of 500 (0.6%), 5 of 500 (1.0%), 4 of 500 (0.8%), and 4 of 500 (0.8%) interphase cells, respectively, had a single chromosome 7 by FISH. These levels are within background range for this probe (mean, 1.17% ± 0.67% with −7 for normal peripheral blood buffy coat cells). Patient no. 5 had no evidence of the Philadelphia chromosome as late as day 83 posttransplant, and the bcr gene was germline by Southern blot analysis.

GVHD. Three of the nine patients developed isolated stage 3 acute GVHD of the skin (Table 3); two responded rapidly to treatment with high-dose methylprednisolone and one resolved with topical therapy. Patient no. 1 had no acute GVHD after either the first or second transplant, patient no. 5 had grade 2 cutaneous and visceral GVHD after his first transplant and only skin involvement after the second transplant, and patient no. 9 had skin involvement after the first transplant and no GVHD after the second transplant to date. None of the allo-PBSCT recipients required ATG. For the control group, the rate of grades 2 to 4 acute GVHD was 70%, and 94% of the patients who developed acute GVHD did so within 1 month posttransplant.

Five patients have been observed for at least 100 days posttransplant and three have developed chronic GVHD (Table 3). Two have localized involvement and are on no treatment or topical therapy alone.

Outcome. One patient died of parainfluenza pneumonia and sepsis 82 days after allo-PBSCT. No evidence of
lymphoma was found at autopsy. The remainder of the patients are alive 24+ to 326+ days posttransplant. One patient with lymphoma achieved a partial remission, and the remaining patients are all in complete remission. For patient no. 1 the remission duration after allo-PBSCT has been 4 months longer than that after allo-BMT.

DISCUSSION

The long-term reconstitutive capability of blood-derived hematopoietic stem cells has not yet been proven after allogeneic transplantation in humans. Using sex-mismatched murine transplants and a molecular probe for Y-chromosome-specific DNA sequences, Moineux et al. showed

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**Table 3. GVHD and Outcome**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Maximum Acute GVHD</th>
<th>Chronic GVHD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Skin</td>
<td>Liver</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
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<td>7</td>
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<td>0</td>
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<tr>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviations: CR, complete remission; PR, partial remission.
that granulocyte colony-stimulating factor (G-CSF)–primed blood stem cell transplants are capable of durably reconstituting the hematopoiesis. Carbanel et al reported that DLA-mismatched canine blood stem cell transplantation resulted in long-term donor type engraftment for up to 3.2 years posttransplant. In this study, we report nine patients with successful lymphohematopoietic engraftment of allogeneic, HLA-identical PBSCs after myeloablative therapy. With a longest follow-up of 326+ days after allo-PBSCT, all three cell lineages of the hematopoietic system continue to function, indicating a sufficient self-renewal capacity of frozen/thawed, blood-derived stem cells. When exposed to stem cell suppressive drugs such as methotrexate or ganciclovir, the hematopoietic function after allo-PBSCT did not seem to be more susceptible than after allo-BMT.

Low levels of the CD34+CD38− subset, which includes putative reconstituting stem cells, were present and peaked in the early recovery phase after allo-PBSCT. This is consistent with a recent study demonstrating that G-CSF– and granulocyte-macrophage colony-stimulating factor (GM-CSF)–mobilized apheresis products contain CD34+ Thy-1+ and CD38− subsets.

Efficient blood stem cell mobilization in normal donors using filgrastim allows apheresis collections to match or exceed a single marrow harvest in progenitor content without exposing the donor to the risks of anesthesia and a marrow harvest. Sheridan et al reported a median 58-fold increase in colony-forming unit granulocyte-macrophage (CFU-GM) over pretreatment values with filgrastim at 12 μg/kg/d. The optimal cell dose and number of CD34+ cells for allogeneic PBSCT is unknown. Matsunaga et al suggested that an engrafting dose of PBSCs could be collected in as little as one apheresis from donors receiving filgrastim at a dose of 2.5 μg/kg for 6 successive days followed by 5 μg/kg for the following 4 days. Fritsch et al also reported that, by a single leukapheresis, sufficient PBSCs can be collected from a cytokine-stimulated normal donor to ensure hematopoietic engraftment in an adult recipient. Our data show that, using filgrastim at 6 μg/kg SC twice daily, a target engraftment dose of 4 × 106 CD34+ cells/kg could also be collected with 2 or less aphereses. But, even in normal donors, some variability in cytokine-mobilized and apheresis-derived stem cell yield has to be taken into consideration.

Neutrophil recovery was rapid in our patients after allogeneic PBSCT, but it did not occur any earlier than for the historical control group that did not receive methotrexate for GVHD prophylaxis after allo-BMT. In contrast, platelet recovery was more rapid in our patients after allo-PBSCT than for the allo-BMT historical control group, similar to the experience with autologous PBSCT. Filgrastim itself does not affect platelet recovery after allogeneic or autologous BMT. However, filgrastim does increase numbers of circulating megakaryocyte progenitors in PBSC donors, and this has been associated with an accelerated platelet recovery in recipients of autologous filgrastim-stimulated PBSCs.

It is uncertain whether infusion of larger numbers of lymphocytes present in the PBSC allograft will improve immune reconstitution posttransplant. For autologous recipients, Roberts et al reported that total T cells and CD4+ cell number increased more rapidly after PBSC than after BMT, whereas Henon et al found little difference in immunologic recovery between PBSC and marrow recipients. Prolonged use of cyclosporine may obviate any advantage of PBSC for immune reconstitution, but further studies are warranted to fully investigate this.

Whether there is a linear relationship between the number of T cells infused and the development of GVHD as postulated by Owens and Santos in murine studies or whether above a certain number of T cells a plateau is reached at which MHC disparity is the major GVHD inducing factor remains to be determined. Nonetheless, despite the large numbers of T cells administered with the allo-PBSC, GVHD was mild or nonexistent in our patients. However, the observed presence of GVHD in target organs such as skin is in agreement with data reported by Sanders et al, who noticed GVHD after second transplant even if not present after the first transplant. In our experience of two evaluable patients who underwent a second allo-PBSC, acute GVHD was less than after first allo-BMT or nonexistent. Eckardt et al have also noted that cryopreservation of allogeneic marrow reduced the risk of acute GVHD, possibly through selective deletion of or induction of anergy in GVHD-inducing cells. How cryopreservation affects the alloreactivity of PBSCs needs to be investigated further.

The PBSC collections also contained a high number of CD3+CD4+CD8− (data not shown) and CD3+CD56+ subsets that may include the natural suppressor cells thought to be responsible for inhibition of GVHD effect early posttransplant, but the numbers or ratios of the lymphocyte subsets for optimal activity has not been determined.

It is noteworthy in our study that, at the time of writing, the duration of post allo-PBSC remission exceeds the prior post allo-BMT remission in patient no. 1 by 4 months, leading to speculation regarding a possible additional graft–versus-leukemia (GVL) effect after allo-PBSC. T lymphocytes present in the allogeneic graft confer a GVL effect, as evidenced by the increased relapse rate after T-cell–depleted BMT and the induction of remission by infusion of donor leukocytes in patients relapsing posttransplant. With increased numbers of T lymphocytes and natural killer cells present in PBSC collections, it remains to be determined whether these cells are able to confer an enhanced GVL effect.

In conclusion, a number of considerations might favor the circulating blood as a stem cell source for allografting: (1) shorter duration of posttransplant aplasia, (2) faster hematopoietic and/or immune-reconstitution, and (3) a potentially more pronounced GVL effect. In addition, general anesthesia is not necessary for collection and the procedure results in less discomfort for the donor. This experience shows that allo-PBSC is feasible and warrants further clinical trials. With confirmation of the safety and efficacy of allo-PBSC, this new technology has the potential to become the basis for an unrelated blood stem cell donor program that from a logistical point of view might not be very different from current apheresis donations.
ACKNOWLEDGMENT

We are indebted to the BMT nurses, clinical nurse specialists, and laboratory personnel for their excellent patient care and technical assistance.

NOTE ADDED IN PROOF

As of December 15, 1994, the total number of evaluable patients with refractory leukemia, lymphoma, or MDS who received a PBSC transplant from their HLA-identical sibling donors increased to 16. The longest follow-up with proven donor chimerism (RFLP) is 463+ days, with a median follow-up of 121 days. The median time to ANC greater than 500/μL and ANC greater than 1,000/μL was 9 days (range, 8 to 10 days) and 9 days (range, 8 to 11 days), respectively, and 12 days (range, 8 to 87+ days) and 15 days (range, 8 to 87+ days) to platelets greater than 20,000/μL, and greater than 50,000/μL, respectively. Platelet recovery after allo-PBSCT was significantly faster than after allo-BMT (P < .01). The actuarial rate of grades 2-4 acute GVHD was 47%; 2% of 8 evaluable patients developed limited chronic GVHD and 3 of 8 evaluable patients developed clinically extensive chronic GVHD. Four patients underwent an allo-PBSCT after relapse from allo-BMT. In 3 patients, acute GVHD was less than after allo-BMT; in 1 patient, acute GVHD became more severe. In patient no. 1, the duration of post–allo-PBSCT remission exceeds the prior post–allo-BMT remission by 8 months.

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