Peripheral Blood Progenitor Cell Transplantation in Lymphoma and Leukemia Using a Single Apheresis


Myeloablative treatment and peripheral blood progenitor cell (PBPC) transplantation are increasingly used for lymphomas and leukemias. We have sought to optimize conditions for priming, collection, and engraftment of the leukapheresis product. Fifty-four consecutive adult patients were eligible, 31 with high-grade non-Hodgkin's lymphoma, 12 with Hodgkin's disease in chemosensitive relapse, and 11 with poor prognosis acute lymphoblastic leukemia. Filgrastim was administered after routine chemotherapy with VAC-ECB or MICCOM to mobilize PBPC. A rapidly increasing white blood cell count was used to predict the time of peak PBPC release and plan leukapheresis. Fortye-five patients underwent leukapheresis. A median of 14 L of blood was processed at a single apheresis. A median of 2.4 x 10^9/kg mononuclear cells (MNCs), 1.04 x 10^9/kg granulocyte-macrophage colony-forming cells (GM-CFCs), and 10.6 x 10^9/kg CD34+ cells were obtained. Slightly fewer MNCs were obtained from the heavily pretreated Hodgkin's disease group. There were no other significant differences in the size or composition of the leukapheresis harvest in the three patient groups. Forty patients underwent high-dose therapy and PBPC transplantation. Filgrastim was administered by daily subcutaneous injection until the absolute neutrophil count was >1 x 10^9/L for 2 consecutive days. Rapid and sustained hematopoietic engraftment occurred in all patients. The median time to achieve a neutrophil count ≥ 0.5 x 10^9/L was 9 days (range, 8 to 16 days); to achieve a platelet count ≥ 20 x 10^9/L was 10 days (range, 6 to 88 days); and to achieve a platelet count ≥ 50 x 10^9/L was 15.5 days (range, 10 to 100 days). Neutrophil recovery was faster than that of a historical control group treated with autologous bone marrow transplantation and filgrastim, but platelet recovery times were halved in the PBPC group. There was no secondary engraftment failure. Requirements for blood and platelet transfusions, antibiotic use, and parenteral nutrition were similar in the three patient groups. The median number of days in the hospital was 13 (range, 10 to 85) in the PBPC patients, compared with 19 (range, 14 to 51) in the historical controls. Leukapheresis yields (MNC, GM-CFC, and CD34+ cell numbers) were not useful for predicting the times to engraftment. We have shown that sufficient PBPC for transplantation can be obtained at a single leukapheresis after mobilization with routine chemotherapy and filgrastim in patients with non-Hodgkin's lymphoma, Hodgkin's disease, and acute lymphoblastic leukemia, even those heavily pretreated. Rapid and sustained hematopoietic reconstitution was seen in all patients. If confirmed with chemotherapy regimens used in other groups, this simple and safe approach makes PBPC transplantation practicable for a wider range of patients.

ALTHOUGH A MAJORITY of lymphomas and leukemias respond to combination cytotoxic chemotherapy, substantial numbers of patients still die from their disease. Increasing dose intensity has been proposed to improve survival. Myeloablative chemotherapy can be used to deliver maximal dose-intensive therapy to patients with chemosensitive lymphomas and leukemias. It is hoped that the extension of this approach to patients with poor prognostic features will improve their survival. High-dose therapy and autologous bone marrow transplantation (BMT) are inadvisable in patients with previous pelvic radiotherapy or BM contamination. The limited indications and high toxicity of this technique have restricted its adoption.

Peripheral blood (PB) is an alternative source of hematopoietic progenitors to BM for transplantation after myeloablative therapy. PB progenitor cells (PBPCs) have the advantages of availability in patients with hypocellular or tumor-involved BM and do not require a general anesthetic for their collection. Early reports suggested that the use of PB progenitors in addition to BM led to more rapid hematopoietic reconstitution after transplantation, with reduced morbidity and shorter hospital stays. Studies using PBPCs alone are now being reported. A variety of regimens has been used to mobilize hematopoietic progenitors into the circulation before leukapheresis, including different combinations of cytotoxic drugs and hematopoietic growth factors. The optimum priming regimen has not been established. To date, multiple apheresis procedures have been required to obtain sufficient progenitors for transplantation. We have previously shown that the use of granulocyte colony-stimulating factor (filgrastim) after routine outpatient chemotherapy for non-Hodgkin's lymphoma (NHL) leads to the release of large numbers of progenitors and stem cells with the capacity for long-term hematopoiesis in vitro. We established the optimum time to perform leukapheresis in this setting. We predicted that sufficient hematopoietic progenitors for transplantation could be collected at a single outpatient leukapheresis.

The definition of an adequate harvest is itself uncertain. Different investigators have suggested that total mononuclear cell (MNC) count, weight-adjusted MNC count, number of granulocyte-macrophage colony-forming cells (GM-
CFCs), or CD34+ cells will predict for hematopoietic recovery after transplantation.7,17,18

We now report the results of a phase II clinical study in patients with NHL, Hodgkin’s disease (HD), and acute lymphoblastic leukemia (ALL) eligible for high-dose chemotherapy and autologous hematopoietic cell transplantation. Routine chemotherapy and filgrastim were used for progenitor cell mobilization. One leukapheresis was performed at the time of predicted maximal release. Cells were stored in liquid nitrogen and reinfused after myeloablative chemotherapy. This simple approach has proved safe and practicable, enabling high-dose therapy to be offered to a wider range of patients.

PATIENTS AND METHODS

Patients aged between 15 and 55 years with high-grade NHL of poor prognosis, relapsed HD, or ALL were eligible for high-dose therapy and autologous hematopoietic cell rescue. Those with other uncontrolled serious medical conditions were excluded. A single leukapheresis was performed for transplantation and a reserve BM harvest or leukapheresis was performed.

NHL. Previously untreated patients with histologically documented high-grade NHL (Kiell classification) with the following adverse prognostic features were entered: stage 4 disease; stage 3 with a serum lactate dehydrogenase (LDH) of ≥ 1,000 IU/L at presentation or stage 2 bulk thoracic or abdominal disease (defined as a maximum diameter greater than 10 cm and, for thoracic disease, greater than one-third the diameter at T5/6 on chest X-ray); and lymphohistioclastic involvement of any stage. All patients had to have normal renal and hepatic function unless the abnormal parameter was greater than one-third the diameter at T5/6 on chest X-ray; and lymphohistioclastic involvement. Patients with central nervous system involvement were excluded.

They were treated with 7 weeks of outpatient induction chemotherapy (VAPEC-B):4 adriamycin (35 mg/m²) and cyclophosphamide (350 mg/m²) on weeks 1 and 5; vincristine (1.4 mg/m²) and bleomycin (10 mg/m²) on weeks 2, 4, and 6; adriamycin (35 mg/m²) and etoposide (100 mg/m² orally [PO]) daily for 5 days on weeks 3 and 7; prednisolone (50 mg PO) daily for 4 weeks and then tapered off to zero. Intrathecal methotrexate (12.5 mg) was administered at weeks 1, 3, 5, and 7 in patients with lymphohistioclastic involvement and at week 1 for other histologies. Prophylactic cotrimoxazole and ketoconazole were administered throughout.

Patients with chemoresponsive disease started filgrastim at 300 µg/d subcutaneously (SC) or 5 µg/kg if body weight was greater than 75 kg, on the day after the last dose of oral etoposide (week 7). VAPEC-B and continued until leukapheresis. The dose was chosen on the basis of laboratory mobilization data obtained using filgrastim with the VAPEC-B regimen.16,20

Consolidation chemotherapy comprised three cycles of ifosfamide (3 g/m²) with mesna (3 g/m²) and cystosine arabinoside (800 mg/m²) mixed and infused through an indwelling intravenous (IV) cannula over 96 hours using an ambulatory pump. This was repeated every 21 days. Patients with lymphohistioclastic lymphoma then received methotrexate (3 g/m²) with folic acid rescue weekly for three cycles as central nervous system prophylaxis.

Myeloablative chemotherapy comprised busulphan (4 mg/kg/d PO) for 4 days as an outpatient, followed by cyclophosphamide (50 mg/kg/d) with mesna IV for 4 days. Forty-eight hours after the last dose of cyclophosphamide, the autologous PBPCs were thawed and reinfused.

HD. Consecutive patients with histologically confirmed HD who had progressed during or relapsed after an Adriamycin-containing regimen were entered. Pulmonary function and cardiac ejection fraction were measured. Cytoreductive chemotherapy for all patients was composed of 7 weeks of VAPEC-B chemotherapy with filgrastim priming before leukapheresis, as described above.

Myeloablative chemotherapy comprised cyclophosphamide (1.5 g/m²/d IV) for 4 days and then BCNU (600 mg/m² IV) (7 patients) or of cyclophosphamide (1.5 g/m²/d IV) with etoposide (200 mg/m²/d IV) for 4 days and then carmustine (BCNU; 300 mg/m² IV) (3 patients). Autologous PBPCs were reinfused 48 hours after the BCNU.

ALL. Patients of “adverse prognosis” were defined as all males aged more than 15 years; all females aged more than 20 years; females aged 15 to 20 years with high initial white blood cell count (WBC), abnormal karyotype, or noncommon ALL phenotype. Such patients in first remission or any patient in second remission were eligible if no suitable allogeneic donor was available. Induction chemotherapy comprised daunorubicin (60 mg/m² IV) for 3 days, with a fourth dose when the neutrophil count reached 1 × 10⁹/L (around day 21); vincristine (2 mg/wk IV); and prednisolone (50 mg/d PO) for 6 weeks. Consolidation chemotherapy was based on HiCCOM:11, on day 1, methotrexate (3 g/m²) with folic acid rescue; on day 8, cyclophosphamide (1 g/m² IV) and cytosine arabinoside (3 g/m² IV) every 12 hours for 8 doses; filgrastim (300 µg/d SC) started on day 21 and continued until leukapheresis. A second cycle was started on day 28 in which cytosine arabinoside was administered for only 6 doses, and completed with a third administration of high-dose methotrexate. Intrathecal methotrexate (12.5 mg) was administered on clearance of the blasts from the PB, when patients had achieved complete remission, after high-dose methotrexate, and after intensification. Patients proceeded to high-dose therapy within 2 months of completing HiCCOM.

Myeloablative therapy comprised 2 days of cyclophosphamide (60 mg/kg/d IV) followed by fractionated total body irradiation (1,200 cGy in 6 fractions at 7 to 14 cGy/min by linear accelerator). The PB progenitors were infused on completion of the radiotherapy. Maintenance chemotherapy with 6-mercaptopurine was administered after transplantation.

Leukapheresis procedure. Leukapheresis was performed on the day of anticipated maximum hematopoietic progenitor release.16 This was usually 7 to 9 days after the last myelosuppressive treatment for patients primed with VAPEC-B, when the total WBC was rapidly increasing from the nadir and was between 3 and 10 × 10⁹/L.

Leukapheresis was performed with a Spectra cell separator (Cobe Laboratories, Lakewood, CO) using a continuous collection procedure until 10 to 12 L had been processed. This usually took 3 to 4 hours. An aliquot from the bag was diluted and an automated MNC count obtained. An estimate of the number of progenitors in the harvest was determined by fluorescence-activated cell separation analysis of CD34 + cells (HPCA-1; HPCA-2; Becton Dickinson, Mountain View, CA) and short-term clonogenic assay of GM-CFC.22 MNCs (1 × 10⁹/kg) were considered sufficient for transplantation if the harvest contained GM-CFC. If fewer MNCs were harvested, a second apheresis would be performed the next day. A reserve leukapheresis was performed in ALL patients after the second HiCCOM cycle and a BM harvest was performed in all other patients.

The apheresis product was centrifuged and the plasma removed to leave a cell suspension of 80 mL. This was divided equally between two freezing bags. Dimethyl sulfoxide (20% in the patient’s own plasma) was added to achieve a final concentration of 10%. These aliquots were frozen in a controlled rate freezer in the vapor phase of liquid nitrogen (Kryo 10; Planer Biomed Products Ltd, Middlesex, UK). The frozen cells were then transferred to liquid nitrogen and stored at −196°C for a period of no more than 5 months.
Postablative therapy. After myeloablative therapy, patients were nursed in a positive pressure filtered-air environment. Progenitor cells were thawed rapidly in a 37°C water bath and immediately reinfused. Filgrastim (300 µg) administered daily as a single subcutaneous injection was started 24 hours later. It was continued until the absolute neutrophil count (ANC) was less than 1 x 10^9/L on 2 successive days. A full blood count was measured daily on inpatients. Patients were transfused to maintain a hemoglobin level (Hb) ≥ 90 g/L and a platelet count ≥ 20 x 10^9/L. Other supportive care procedures were standard for the unit.

The reserve leukapheresis product or BM was kept for use in case of failure to engraft, defined as failure to achieve ANC ≥ 0.5 x 10^9/L by day 20 after PBPC reinfusion or a sustained decrease in ANC to < 0.5 x 10^9/L for at least 1 week after initial engraftment. On discharge from hospital, the full blood count was checked every 2 days until the platelet count was ≥ 20 x 10^9/L, and then weekly until the platelet count was ≥ 100 x 10^9/L. At this time radiotherapy was administered to areas of previous bulk disease in NHL and HD patients.

Analysis. In all three diagnostic groups, consecutive eligible patients were recruited. The study was approved by the local medical research ethics committee. All patients gave informed consent.

Tumor response rates were documented according to Union Internationale Contre le Cancer criteria on completion of induction therapy, before ablative therapy, and 6 weeks after transplantation. Patients were followed-up at least every 2 months for the first year, and every 3 months for the second year after transplantation.

Standard statistical methods were used. Leukapheresis yields were compared between groups using Kruscal-Wallis tests. Recovery times were compared between groups using logrank tests. Associations with recovery times were observed using Spearman's rank correlations. Results are expressed as the median (range) unless otherwise stated.

RESULTS

Patients. From November 1990 to October 1992, 54 patients were eligible. Pretreatment characteristics of the patients are shown in Table 1. The 31 NHL patients had the following adverse prognostic factors: 26 (84%) had stage IV disease, 25 (81%) had B symptoms, 29 (94%) had an increased LDH, and 21 (68%) had a Karnofsky performance score of 70 or less. Of the 12 patients with relapsed HD, 8 (67%) had received two or more previous chemotherapy regimens and 7 of these (10 in total) had received radiotherapy to the mediastinum, at least. The remaining 4 patients had relapsed within 6 months of their initial chemotherapy and radiotherapy. Five of the 11 adults with ALL were in second remission with no available allogeneic donor.

Eight patients were withdrawn before leukapheresis (Table 2). One patient was excluded because of hepatitis B antigen positivity; 1 HD patient who had received full mantle radiotherapy with a mediastinal boost and two previous courses of chemotherapy was excluded because of inadequate pulmonary reserve; 1 NHL patient refused high-dose treatment on religious grounds; and 3 patients had progressive disease. Two patients with ALL were withdrawn because of treatment toxicity. A further 5 NHL patients, 4 with lymphoblastic histology, were withdrawn from the program after leukapheresis but before high-dose therapy because of disease progression. One ALL patient was withdrawn before high-dose therapy because of reactivation of hepatitis B.

Leukapheresis. After the last myelosuppressive dose of induction chemotherapy, 27 patients with high-grade NHL, 10 with HD, and 9 with ALL underwent leukapheresis. Filgrastim priming was administered for a median of 7 days (range, 5 to 11) in the NHL group, 8 days (range, 7 to 9) in the HD group, and 6 days (range, 5 to 7) in the ALL group before leukapheresis. It was well tolerated, with mild bone pain as the only side effect.

All patients had a single leukapheresis at the anticipated time of peak progenitor release. The WBCs on the day of apheresis were 7.4 x 10^9/L (range, 3.5 to 55 x 10^9/L) for the NHL group, 4.9 x 10^9/L (range, 3 to 30.4 x 10^9/L) for the HD group, and 6 x 10^9/L (range, 2.7 to 30 x 10^9/L) for the ALL group. Platelet counts were 177 x 10^9/L (range, 11 to 382 x 10^9/L) for NHL, 132 x 10^9/L (range, 29 to 370 x 10^9/L) for HD, and 36 x 10^9/L (range, 14 to 50 x 10^9/L) for ALL. The blood volumes apheressed were 14.4 L (range, 9.4 to 20.6 L) for the NHL group, 14.2 L (range, 5.6 to 23 L) for the HD group, and 13.3 L (range, 10 to 15 L) for the ALL group. No adverse events were related to the leukapheresis. Symptomless decreases in platelets were observed in all patients, but no bruising or bleeding occurred.

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<thead>
<tr>
<th>Table 1. Patient Characteristics</th>
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<td>Sex (M/F)</td>
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<td>Histology</td>
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<tr>
<td>Performance KP</td>
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<td>LDH (IU/L)</td>
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Abbreviations: KP, Karnofsky performance; LDH, lactate dehydrogenase.
The number of MNCs, CD34+ cells, and GM-CFCs were measured in the PB before harvest and in the apheresis product (Table 3). Sufficient hematopoietic progenitors for reconstruction were obtained in all individuals from a single leukapheresis. There was substantial variation in yields among patients, but these were comparable in the three groups. In patients with relatively low progenitor yields, these were attributable to poor venous access or apheresis technique rather than to low levels of progenitors in the PB, as judged by GM-CFC ratios.

The number of MNCs per kilogram, CD34+ cells per kilogram, and GM-CFCs per kilogram in the leukapheresis product were lower in the heavily pretreated HD patients than in the NHL or ALL patients, but only the numbers of MNCs per kilogram reached statistical significance ($P = .01$).

The median times to achieve neutrophil and platelet engraftment were similar in the three groups (Fig 1 and Table 4). There was a small but significant ($P = .02$) delay in achieving a neutrophil count of $\geq 0.5 \times 10^9$/L in the HD patients (median, 11 days) compared with the NHL and ALL groups (median, 9 days).

There was a weak correlation between the number of MNCs per kilogram harvested and the time taken to achieve a neutrophil count of $\geq 0.5 \times 10^9$/L ($R = -.46$, $P = .01$; Fig 2), but the number of MNCs harvested was not significantly correlated with time to platelet recovery. Similarly, there was a weak correlation between the number of CD34+ cells per kilogram harvested and the time taken to achieve a neutrophil count of $\geq 0.5 \times 10^9$/L ($R = -.41$, $P = .01$; Fig 2), but the number of CD34+ cells per kilogram was not significantly correlated with time to platelet recovery. Consistent with this, the numbers of MNCs per kilogram and CD34+ cells per kilogram were correlated ($R = .65$, $P = .001$). No significant correlations were seen between granulocyte-macrophage progenitors harvested and time to neutrophil or platelet recovery. Approximately 6 weeks after initial engraftment, a secondary trivial decrease in circulating platelet numbers $\geq 40 \times 10^9$/L was observed in 2 NHL and 2 ALL patients. This was not predicted by the number of MNCs, CD34+ cells, or GM-CFCs infused. No patient required transfusion support or hospitalization as a result. No patient had secondary graft failure defined as ANC greater than $0.5 \times 10^9$/L or platelets greater than $20 \times 10^9$/L.

Supportive care. Requirements for platelet transfusions showed wide variation between patients but were comparable in the three patient groups (Table 5). There were no significant differences in days of filgrastim administration, parenteral feeding, or intravenous antifungal or antibiotic use.

Toxicity. Hepatic veno-occlusive disease developed in 2 NHL patients, defined as the occurrence of two of the following features within 30 days of transplantation: jaundice, hepatomegaly with right hypochondriacal pain, and ascites. One patient developed hemorrhagic cystitis. One other patient developed immune thrombocytopenia that resolved on steroids. In these patients, examination of bone marrow trephines confirmed trilineage engraftment, although PB counts recovered slowly.

Four deaths (10%) occurred within 100 days of high-dose therapy. One NHL patient died with respiratory complications after liver transplantation for hepatic veno-occlusive disease. One HD patient died 6 weeks after transplantation. Pulmonary consolidation and diffuse petechial hemorrhages were seen at postmortem. He had previously received four regimens of chemotherapy for HD and extensive radiotherapy. A bone marrow trephine taken on day 20 and at postmortem showed trilineage engraftment but reduced megakaryocyte numbers. Two HD patients died at 9 and 13 weeks, respectively, of pulmonary fibrosis.

Disease response. Forty-one percent (9 of 22) of the NHL patients achieved complete response; 23% (5 of 22) achieved complete response (uncertain), denoting complete resolution of all disease but residual radiologic abnormalities of uncertain significance; and 36% (8 of 22) achieved partial response. Seven of the 8 patients who achieved a partial response had radiotherapy to the residual abnormalities. The median follow-up is 224 days for NHL and 107 days for ALL.

Of the 10 HD patients, 1 died during transplantation, 4 achieved partial response, 4 achieved complete response (uncertain), and 1 achieved complete response after high-

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Table 2. Reasons Precluding High-Dose Therapy and Autologous Transplantation

<table>
<thead>
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<th>HL (31 patients)</th>
<th>HD (12 patients)</th>
<th>ALL (11 patients)</th>
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<tbody>
<tr>
<td>Disease progression</td>
<td>7 (6 lymphoblastic)</td>
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<td>0</td>
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<tr>
<td>Treatment toxicity</td>
<td>0</td>
<td>0</td>
<td>2</td>
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<tr>
<td>Organ dysfunction</td>
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<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Other</td>
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<tr>
<td>Total excluded</td>
<td>9</td>
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Table 3. Leukapheresis Yields

<table>
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<tr>
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<th>HL (27 patients)</th>
<th>HD (10 patients)</th>
<th>ALL (8 patients)</th>
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<tr>
<td>Volume apheresed (L)</td>
<td>14.4 (9.4-20.6)</td>
<td>14.2 (5.6-23.2)</td>
<td>13.3 (10-14.9)</td>
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<tr>
<td>Total MNCs ($\times 10^6$)</td>
<td>167.5 (63.4-476)</td>
<td>119 (60.5-235)</td>
<td>338 (112-760)</td>
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<tr>
<td>MNCs ($\times 10^9$/kg)</td>
<td>3.0 (1.0-9.7)</td>
<td>1.78 (1.3-2.8)</td>
<td>4.9 (1.8-12.1)</td>
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<tr>
<td>GM-CFC cells ($\times 10^4$/kg)</td>
<td>1.1 (0.02-5.2)</td>
<td>0.26 (0.13-1.84)</td>
<td>1.42 (0.026-4.8)</td>
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<tr>
<td>CD34 cells ($\times 10^9$/kg)</td>
<td>11.6 (4.3-368)</td>
<td>4.65 (1.9-28)</td>
<td>25, 120</td>
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Fig 1. Recovery of neutrophil and platelet counts after high-dose therapy and reinfusion of autologous hematopoietic cells. Cumulative percentage of patients attaining neutrophils $\geq 0.5 \times 10^9/L$ (-), platelets $\geq 20 \times 10^9/L$ (---), and platelets $\geq 50 \times 10^9/L$ (-----). Top, NHL (22 patients); middle, HD (10 patients); bottom, ALL (8 patients).

dose therapy. The median follow-up is 234 days from transplantation. All of the 8 ALL patients were in remission at the time of transplantation. The median follow-up is 166 days from transplantation.

**DISCUSSION**

The role of high-dose therapy with autologous hematopoietic rescue for lymphoma and leukemia is controversial. A controlled trial of high-dose versus conventional-dose chemotherapy in relapsed and resistant HD was stopped early when a significant difference in relapse-free survival was observed in patients receiving autologous bone marrow transplantation and because subsequent patients refused randomization. No overall survival difference has yet been seen in this study. The use of PBPCs in transplantation promises to improve the risk/benefit ratio of this procedure. To make PBPC transplantation cost effective, it is necessary to optimize the conditions for priming, collection, storage, and engraftment of the leukapheresis product.

Cytotoxic chemotherapy with or without hematopoietic growth factors has been used to mobilize hematopoietic progenitors into the PB. Seriously myelosuppressive chemotherapy has been used to mobilize PBPCs. We have shown that chemotherapy and filgrastim act synergistically to increase the number of hematopoietic progenitors in the PB. Using only moderately myelosuppressive chemotherapy, we obtained at least 2.5 times the number of PBPCs with filgrastim that could be obtained with chemotherapy alone. This enabled PBPCs to be collected during scheduled treatment from patients in whom delays in chemotherapy would be unacceptable.

If apheresis can be timed to coincide with the time of maximal progenitor release, the number of leukaphereses can be minimized. We have used a rapidly increasing WBC after the chemotherapy-induced nadir to guide the timing of leukapheresis. This has proved to be a robust indicator in

| Table 4. Time to Hematologic Recovery After Transplantation |
|---------------------------------|-----------------|-----------------|
|                                 | HL (22 patients) | HD (10 patients) | ALL (8 patients) |
| Neutrophils $\geq 0.5 \times 10^9/L$ | 9 (8-11)         | 11 (9-16)        | 9 (8-13)        |
| Neutrophils $\geq 1 \times 10^9/L$ | 9 (8-11)         | 11.5 (10-17)     | 10 (8-13)       |
| Platelets $\geq 20 \times 10^9/L$ | 9 (6-88)         | 11 (6-68)        | 13 (8-30)       |
| Platelets $\geq 50 \times 10^9/L$ | 15.5 (10-100)    | 16.5 (11-80)     | 16 (12-40)      |

Values are the median (range) number of days to reach the indicated level.
different groups of patients. The day of maximal PBPC release is remarkably consistent after the same priming regimen, occurring at day 8 (range, day 7 to 9) after VAPEC-B with filgrastim in HD, day 7 (range, day 6 to 8) after VAPEC-B with filgrastim in NHL, and day 16 (range, day 15 to 17) after cyclophosphamide and cytosine arabinoside in HicCOM with filgrastim in ALL.

In all previous published series of PBPC transplantation, multiple aphereses have been required, regardless of the priming regimen used. In addition to the time involved for patients and staff, cryopreservation facilities may limit the number of patients that can be treated in this way. The reinfusion of large volumes of dimethyl sulfoxide and free hemoglobin from multiple aphereses also pose potential problems in transplantation.

The yield of GM-CFCs varied substantially between individuals but did not correlate with engraftment after PBPC reinfusion. Quantitative assessment of the leukapheresis product is difficult. GM-CFC and CD34+ cell assays are highly variable between laboratories. Some investigators have argued strongly that the number of CD34+ cells is the best predictor of engraftment capacity, but our results and those of others do not support this. We found that the number of CD34+ cells in the leukapheresis product correlated with the number of MNCs. Although there was a weak correlation between these parameters and time to neutrophil engraftment, neither was useful to predict the time to hematopoietic recovery after transplantation. Other investigators have suggested that the minimum CD34+ cell dose necessary for prompt engraftment is between 2.5 and 5 x 10^6 cells/kg. Only 2 of the 34 leukapheresis harvests in this series in which CD34+ cells were estimated contained less than 2.5 x 10^6 cells/kg, and 8 contained less than 5 x 10^6 cells/kg. The poor correlation found between numbers of CD34+ cells infused and time to hematopoietic recovery may be explained by the high number of our patients whose leukapheresis yields exceeded the supposed threshold dose.

Previous investigators have considered more MNCs and GM-CFCs to be necessary for PBPC transplantation than for bone marrow transplantation. A minimum of 6 x 10^8 MNCs/kg have been recommended when using nonmobilized cells and 4 x 10^8 MNCs/kg when using mobilized cells. Fifteen- to 50-fold more GM-CFCs have been recommended when using chemotherapy-mobilized PBPCs compared with bone marrow. We have already shown that PBPCs mobilized with chemotherapy and filgrastim have an in vitro engraftment potential equivalent to that of bone marrow. We therefore used the same criterion to define an adequate harvest as is used for bone marrow, ie, 1 x 10^8 MNCs/kg. We now show that sufficient cells for hematopoietic reconstitution were obtained at a single apheresis in all patients studied, even in heavily pretreated HD and ALL patients. No further manipulation of the apheresis product was necessary and no reinfusion toxicity was seen. Rapid and sustained engraftment was seen in all patient groups, including those receiving total body irradiation. The single apheresis procedure has made PBPC transplantation...
tion simpler than bone marrow harvesting in our institution. Other patient groups, such as myeloma, may behave differently.

Benefits of PBPC transplantation were also seen after high-dose treatment. The time taken to attain neutrophil and platelet engraftment was shorter than a historical control group of 7 high-grade NHL patients treated with VA-PBC-B and receiving transplants in first remission who were also treated with the busulphan and cyclophosphamide conditioning regimen but received autologous bone marrow followed by filgrastim post-transplant. Their median time to reach a neutrophil count of ≥0.5 × 10^9/L was 13 days (range, 11 to 23 × 10^9/L); platelets ≥ 20 × 10^9/L were reached in 20 days (range, 12 to ≥282 × 10^9/L); and platelets ≥ 50 × 10^9/L were reached in 41 days (range, 20 to ≥282 × 10^9/L). Neutrophil recovery was faster in the NHL patients treated by PBPC transplantation (Table 4), but, most strikingly, platelet recovery times were halved.

No secondary graft failures were seen. There were no late decreases in circulating neutrophils, but 4 patients experienced trivial and brief reductions in platelet numbers at 6 to 10 weeks posttransplant. These remain unexplained, but were not associated with low leukapheresis yields, as postulated by To et al. This supports the contention that sufficient progenitors to sustain long-term hematopoiesis in vivo were obtained at a single apheresis.

Supportive care requirements were reduced as a result of rapid hematologic engraftment. The duration of hospital stay was markedly shorter in all three patient groups studied than in the historical controls, with a median of 13 days (range, 10 to 55 days) compared with 19 days (range, 14 to 51 days). A reduction in parenteral antibiotic requirements from 12 days (range, 0 to 38 days) to 8.5 days (range, 0 to 22 days) was also observed. Red blood cell transfusions, days of parenteral antifungal therapy, and parenteral nutrition requirements were low but similar to our previous experience with autologous bone marrow transplantation.

PBPC mobilization was achieved in this study using routine chemotherapy with filgrastim. Mild bone pain was the only adverse effect attributable to the priming regimen, leukapheresis, or PBPC reinfusion. There were no unexpected toxicities from the induction and consolidation chemotherapy in any group. Patients who have previously received radiotherapy in addition to chemotherapy require careful cardiopulmonary evaluation before high-dose therapy. Of the three high-dose therapy regimens here, that using BCNU and cyclophosphamide in the HD patients was associated with excessive pulmonary toxicity and has been discontinued.

The median follow-up in this study is only 213 days, so it is still too early to comment on survival. However, if better prognosis patients are to be treated, we must reduce transplant morbidity to a minimum. We have shown that primed PBPCs can improve the safety of myeloablative therapy. It remains to be investigated whether high-dose chemotherapy in multiple cycles could substitute for or improve on the results of a single cycle of an ablative regimen. This study has shown that PBPC harvesting after priming with routine chemotherapy plus filgrastim is a simple and effective procedure both for the patient and the clinician. Adequate numbers of PBPCs for hematopoietic rescue after myeloablative therapy were harvested at a single leukapheresis. This was possible even in a heavily pretreated group of HD patients. Rapid and complete engraftment was seen in all patients. In addition PBPCs with filgrastim support post-transplant is effective in reducing the morbidity associated with ablative therapies.

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