

# Autologous Peripheral Hematopoietic Stem Cell Transplantation Restores Hematopoietic Function Following Marrow Ablative Therapy

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From ten patients with advanced malignant disease involving the bone marrow, autologous hematopoietic stem cells were collected from the peripheral blood during eight four-hour pheresis procedures and cryopreserved. No manipulations to increase the number of stem cells circulating in the blood were used during the collections. Following marrow ablative chemotherapy or chemoradiotherapy, the autologous cells were thawed and infused intravenously (IV). WBCs reappeared in the circulation at a median of eight days (range seven to 11 days) after stem cell infusion. Two patients died early, whereas the other eight reached normal numbers of circulating granulocytes that have persisted for up to >20 months. These eight

patients became independent of RBC transfusions (hemoglobin concentration >10 g/dL) at a median of 27 days (range 11 to 58 days) after transplantation. One patient received platelet transfusions for counts <50 × 10<sup>9</sup>/L, one patient developed a clinical picture of idiopathic thrombocytopenic purpura, and six patients maintained a platelet count >20 × 10<sup>9</sup>/L at a median of 23 days (range 14 to 25 days) following stem cell infusion. This technique allows patients ineligible for autologous bone marrow transplantation due to unacceptable anesthetic risks, prior pelvic irradiation, or bone marrow metastases to receive marrow ablative therapy.

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**I**DENTIFICATION of committed hematopoietic progenitor cells in human peripheral blood suggested that pluripotent hematopoietic stem cells could be present in the circulation and generated interest in the potential of these cells to restore hematopoiesis following marrow aplasia.<sup>1</sup> Granulocyte-macrophage progenitor cells (CFU-GM) in peripheral blood were present in low concentrations, ~1% to 10% of those found in the marrow.<sup>2</sup> Methods to collect and preserve them in numbers comparable to the numbers collected in marrow used for transplantation were devised using cytopheresis and cryopreservation techniques.<sup>3</sup>

In 1979, Goldman<sup>4</sup> reported successful autologous peripheral stem cell transplantations (PSCTs) for patients with chronic granulocytic leukemia (CGL). The cells were collected and cryopreserved early in the disease course. When the CGL transformed to an acute phase, high-dose therapy was given, followed by infusion of cryopreserved autologous peripheral stem cells. Engraftment resulting in the reestablishment of the chronic phase of the disease showed that in CGL stem cells capable of restoring hematopoietic function were present in circulating human blood. Two reports of syngeneic peripheral stem cell transplants failing to engraft in 1979 and 1980,<sup>5,6</sup> however, caused concern about the ability of nonleukemic peripheral stem cells to restore marrow function. The reasons for the failures were not entirely clear, but in retrospect may have been due in part to the protracted period of stem cell infusion (1 to 2 weeks). Encouraged by observations that nonleukemic peripheral stem cells restored hematopoiesis in laboratory animals that had received marrow ablative therapy,<sup>7</sup> efforts to achieve similar results in humans continued. This report describes autologous PSCT in ten patients treated at the University of Nebraska Medical Center.

## MATERIALS AND METHODS

The patients had malignancies refractory to conventional curative therapies and were candidates for high-dose therapy with autologous bone marrow transplantation (ABMT) except that they had histopathologic evidence of metastatic disease in the marrow. Written informed consent was obtained from each patient, and the study was approved by the institutional review board. Between June 8, 1984, and October 31, 1986, ten patients received autologous PSCT; an additional patient had stem cells collected but later elected to forego

high-dose therapy for her malignancy. Six patients had advanced breast cancer, three had refractory Hodgkin's disease, and one had diffuse intermediate cell non-Hodgkin's lymphoma. The median age of the patients was 40 years (range 18 to 54 years). Two patients were males.

Autologous peripheral stem cells were collected with eight separate pheresis procedures done no more often than three times weekly. None of the phereses were specifically timed to follow chemotherapy so that cells would be collected when the peripheral blood counts were recovering from cytopenia. One patient received cytotoxic chemotherapy to prevent disease progression during a portion of the peripheral stem cell collection period and three of eight collections for that patient were timed to occur 2 weeks following the chemotherapy, after the peripheral blood count had recovered from cytopenia. No other patient received chemotherapy while peripheral stem cells were being collected. One patient had never received cytotoxic chemotherapy and, for the remaining eight patients, the median time from the last course of chemotherapy to the initial collection of peripheral stem cells was 14 weeks (range 3 to 78 weeks). Autologous marrow was harvested and cryopreserved to serve as a backup in case engraftment with peripheral stem cells did not occur or did not restore marrow function permanently (ie, was not durable).

A variety of blood cell separators, including a CS-3000 continuous flow device (Fenwal, Deerfield, IL) and Models 30, 30+, 30S, and V-50 intermittent flow devices (Haemonetics, Braintree, MA) were used. The endpoint of collection was either 10,000 mL whole blood processed (CS-3000) or four hours (Haemonetics devices). Sedimenting agents were not used. Owing to the large extracorporeal volume required for collections with the Haemonetics devices, three patients with low body weights and low hematocrits had procedures performed with 125-mL disposable bowls rather than the 225-mL adult bowls. For the Haemonetics Model 30 devices, the bowl was

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filled with peripheral blood at a rate of 40 to 80 mL/min. After the plasma was discharged and the buffy coat reached the shoulder of the bowl, the flow was decreased to 40 mL/min until the platelet band began to discharge. The flow rate was then reduced again to 20 mL/min. When half of the platelet band was discharged, cell collection began and continued until two minutes after the RBCs began to discharge. The discharged plasma and platelets were returned intravenously (IV) to the patient, and the procedure was repeated until four hours had passed. The product was allowed to settle between passes, and a portion of the RBCs was returned to the patient. Immediately following the four-hour collection, a cell count and differential count were done on an aliquot of the total product to determine the number of mononuclear cells collected.

Next, the product was centrifuged at 400 g for ten minutes in the processing laboratory. Enough plasma was removed to give a cell concentration of  $1 \times 10^8$  cells/mL, and the product was cooled to 4 °C. A precooled cryoprotectant mixture containing 40% dimethylsulfoxide (DMSO) and 6 µg/mL DNA'se in Medium 199 (GIBCO, Grand Island, NY) was added at a ratio of one part cryoprotectant to three parts concentrated leukapheresis product, and the cells were frozen in a controlled rate liquid nitrogen freezer (Cryo-Med, Mt Clemons, MI). The average total volume of cryopreserved cells for these patients was 1,278 mL.

Aliquots of all eight collections from each patient were thawed and pooled. CFU-GM assays were performed on the pooled specimens using an agar culture system<sup>8</sup> with normal peripheral blood mononuclear cells ( $1 \times 10^6$  cells/mL) in an underlayer as a source of colony-stimulating factors (CSFs). One-milliliter aliquots of feeder cell suspension in 0.5% agar medium were incubated in 35-mm Petri dishes at 37 °C in 7% CO<sub>2</sub> humidified air for at least 24 hours. One-milliliter aliquots of target cells ( $1 \times 10^5$  cells/mL) in 0.3% agar medium were then pipetted onto the semisolid feeder layers. The cultures were incubated at 37 °C in 7% CO<sub>2</sub> humidified air for 14 days, stained with Wright's stain, and counted (>50 cells = one colony). In our laboratory, the normal number of pooled peripheral blood CFU-GM obtained by leukapheresis prior to cryopreservation is  $26 \pm 5.6$  colonies/ $1 \times 10^5$  cells plated.

The high-dose therapy administered varied according to the type of neoplastic disease and prior treatment received. All anticancer drugs were given IV. Four patients (patients 1, 2, 5, and 8 in Table 1) with breast cancer received cis-platinum, 125 mg/M<sup>2</sup> in a 24-hour infusion followed by two consecutive daily doses of cyclophosphamide, 60 mg/kg. On the following day, total body irradiation (TBI) was begun for five daily fractions of 2.2 to 2.5 Gy. Patients 3 and 4 received six doses of etoposide 150 mg/M<sup>2</sup> every 12 hours followed by cis-platinum and cyclophosphamide as given above. All three patients with Hodgkin's disease received cyclophosphamide 6 g/M<sup>2</sup> in four divided doses over 4 days, six doses of etoposide, 150 mg/M<sup>2</sup> at 12-hour intervals over 3 days, and carmustine 300 mg/M<sup>2</sup> given on the day of the first doses of cyclophosphamide and etoposide. The patient with non-Hodgkin's lymphoma received six doses of cytarabine 3 g/M<sup>2</sup> every 12 hours, followed 2 days later by cyclophosphamide 90 mg/kg. Forty-eight hours after the last dose of cyclophosphamide, 9 Gy of TBI fractionated into five doses was given over 2½ days. Following these various therapies, the patients' peripheral stem cells were rapidly thawed in a 40 °C water bath at the bedside and immediately infused IV in a one- to four-hour period. A bone marrow biopsy was done for patient 1 on day +7 (ie, seven days following stem cell infusion) to document marrow aplasia. Because this biopsy showed marrow recovery, subsequent biopsies to demonstrate aplasia were performed on day +4.

Patients received irradiated RBC transfusions when their hemoglobin concentrations were <10 g/dL and irradiated platelet transfusions when their platelet counts were <20 × 10<sup>9</sup>/L except for patient 10, whose platelet count was maintained above 50 × 10<sup>9</sup>/L because of pulmonary alveolar hemorrhage. All patients were nursed

with a dedicated nursing staff in a special care unit in single rooms supplied with high-efficiency particulate-filtered (sterile) air at a rate of exchange that provided continuous positive pressure to the corridor. Appropriate antibiotics were administered for identified infections and for unexplained fevers >38.4 °C. All patients received parenteral hyperalimentation from the time high-dose therapy was instituted until granulocyte recovery (>0.5 × 10<sup>9</sup>/L granulocytes) occurred.

A complete response was defined as disappearance of all detectable malignant disease. Partial responses were defined as a decrease in tumor volume of ≥50%. Patients with bone metastases had a partial response if all measurable disease decreased in volume by 50% and persistent or improved bone abnormalities were present on radiographs.

## RESULTS

A median of  $8.4 \times 10^8$  mononuclear cells/kg patient weight was collected from 11 patients that contained a median of  $8 \times 10^4$ /kg CFU-GM after thawing. All patients who had a bone marrow biopsy done on day +4 had histopathologic evidence of marrow aplasia, and all ten patients developed WBC counts < 0.1 × 10<sup>9</sup>/L and became RBC and platelet transfusion dependent.

Following peripheral stem cell infusion, all patients demonstrated engraftment. Patients 1 and 8 died on day +12; bone marrow examination at autopsy showed that both patients had all hematopoietic cell lines. Circulating WBCs reappeared at a median time of day +8 (range day +7 to +11). Eight patients reached normal numbers of circulating granulocytes (ie,  $2 \times 10^9$ /L) that persisted for up to 20+ months. The median time to reach  $0.5 \times 10^9$ /L granulocytes was day +22 (range day +11 to +58). The patients maintained a hemoglobin concentration of 10 g/dL without transfusions at a median time of day +27 (range day +11 to +58). One patient (patient 2) developed a clinical picture of idiopathic thrombocytopenic purpura with adequate numbers of megakaryocytes in the bone marrow biopsy, one patient was transfused to maintain a platelet count of  $50 \times 10^9$ /L (patient 10), two patients had an early death, and six patients maintained a platelet count of >20 × 10<sup>9</sup>/L at a median time of day +23 (range day +14 to +36). WBC counts and platelet counts not supported by transfusions are shown for each patient in Fig 1.

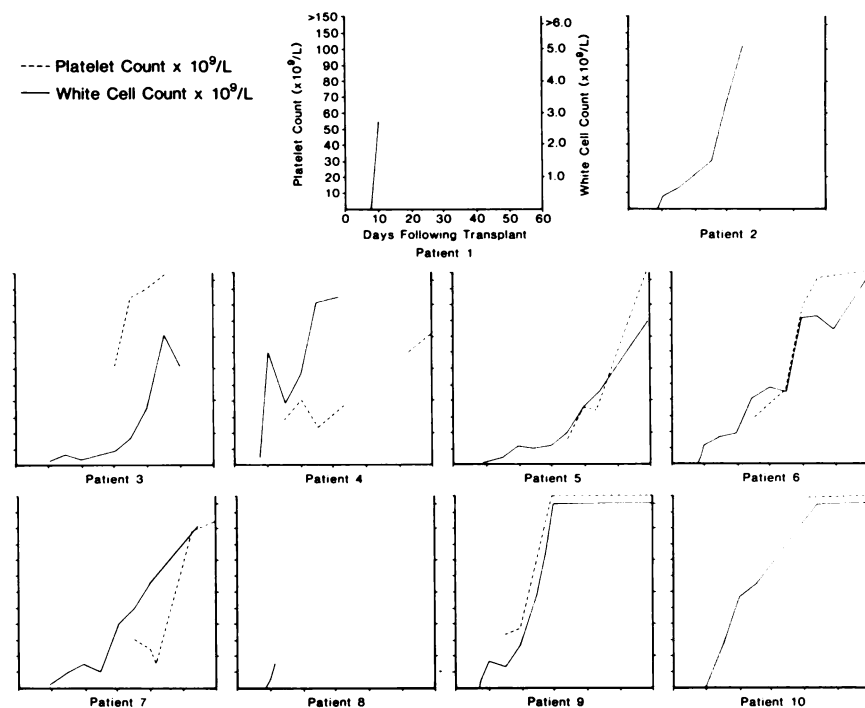
When normal peripheral counts were reached, they persisted until an event to explain cytopenia other than failure of the graft to endure occurred; the backup marrow was never used. One patient developed thrombocytopenia ( $28 \times 10^9$ /L) and anemia (hemoglobin concentration 8.1 g/dL) on day +409 (patient 4). A bone marrow biopsy revealed extensive replacement with malignant cells, and she died of progressive disease 16 days later. Patients who received systemic therapy for relapsed disease also had the expected depression of circulating WBC and platelet numbers. Patient 7 developed thrombocytopenia with a viral illness. A bone marrow biopsy revealed normal numbers of megakaryocytes, and the platelet numbers rose spontaneously as he recovered from his illness.

Complete responses occurred in the patient with non-Hodgkin's lymphoma and in a patient with Hodgkin's disease. Four breast cancer patients and two Hodgkin's disease patients had partial responses. Two patients were not evalua-

**Table 1. Characteristics of Ten Patients With Malignancies Treated With Marrow Ablative Therapy, Their Peripheral Stem Cell Transplantations, and Their Courses**

Patient No.	Sex	Age (yr)	Malignancy	Days to First WBC	Most Recent GRN Count		Most Recent HG Value		Days to >20 × 10 <sup>9</sup> /L PLTS	Days to >150 × 10 <sup>9</sup> /L PLTS	Most Recent PLT Count × 10 <sup>9</sup> /L and Time Determined	Tumor Response	Response (days)	Survival (days)	Status
					Days to >2 × 10 <sup>9</sup> /L GRN	Time Determined	Days to 10 g/dL HG	Time Determined							
1	F	41	Breast cancer	8	NE	0.05	NE	NE	NE	NE	NE	NE	NE	12	Died of hepatic failure
2	F	54	Breast cancer	8	33	19.0	10.3	34	NA	Never	20	Partial response	104	104	Died of pulmonary fibrosis, developed idiopathic purpura following transplant
3	F	35	Breast cancer	11	45	0.4	9.3	47	25	40	23	Partial response	78	156	Died of progressive breast cancer
4	F	43	Breast cancer	7	59	3.6	14.5	23	15	59	168	Partial response	276	425	Died of progressive breast cancer
5	F	36	Breast cancer	9	89	3.0	9.4	58	36	58	198	Partial response	272	709+	Alive with progressive breast cancer, taking cytotoxic chemotherapy
6	F	18	Hodgkin's disease	8	52	10.8	11.1	26	25	59	"Adequate"	Complete response	170	509+	Progressive disease, short survival expected
7	M	53	Non-Hodgkin's lymphoma	9	56	11.6	12.6	27	21	347	day 478	Complete response	391+	391+	Alive with no evidence of malignancy
8	F	47	Breast cancer	9	NE	0.06	NE	NE	NE	NE	NE	NE	0	12	Died of pulmonary hemorrhage
9	M	40	Hodgkin's disease	7	29	9.6	8.5	19	14	29	258	Partial response	95	216	Died of progressive disease
10	F	26	Hodgkin's disease	8	26	10.8	13.2	11	NE	43	87	Partial response	165	272	Died of progressive disease

Days, days following transplantation; NE, not evaluable; NA, not available; PLTS, platelets; GRN, granulocytes; HG, hemoglobin.



**Fig 1. WBC and platelet recovery unsupported by transfusion following autologous peripheral stem cell infusion for 10 patients.**

ble for response due to early death. Details of each patient's course are given in Table 1.

#### DISCUSSION

The number of mononuclear cells and CFU-GM have been used as indicators,<sup>9-13</sup> but specific cell numbers that accurately reflect the number of pluripotent stem cells in a peripheral blood collection may or may not exist. For our ten patients, no statistically significant correlation between the number of mononuclear cells or the number of CFU-GM administered and the length of time to hematopoietic recovery of any cell line was found (Spearman rank correlation coefficient), but the sample size was not large enough to detect correlations of  $\leq 0.6$ . Recently, autologous PSCT in a small number of patients with diseases other than CGL has been reported.<sup>9-15</sup> Most of these transplants<sup>11-15</sup> were done with cells collected when peripheral blood counts were beginning to recover following nonmarrow lethal chemotherapy; the number of CFU-GM circulating in the peripheral blood is increased at that time when compared with the normal population.<sup>16</sup> Five of those PSCT were successful,<sup>11-14</sup> and two of the five were done with a number of mononuclear cells equivalent to the number our patients received.<sup>11,13</sup> The number of cells infused for two transplants were not specified,<sup>14</sup> and the fifth successful PSCT was done with a relatively small number of mononuclear cells ( $1.9 \times 10^8/\text{kg}$ ).<sup>12</sup> However, two PSCTs using cells collected at the time of marrow recovery resulted in incomplete hematopoietic reconstitution<sup>15</sup>; for these two transplants, the numbers of CFU-GM collected (some may have been lost in the freezing-thawing process) were similar to the numbers given to our patients, but the numbers of mononuclear cells infused ( $1.3$  and  $3.0 \times 10^8/\text{kg}$ ) were considerably less than our patients received. This information suggests that the number

of mononuclear cells given may be either a more sensitive predictor of engraftment or a more accurate reflection of the number of pluripotent stem cells present than the number of CFU-GM infused.

The time following PSCT until reappearance of circulating WBCs in these ten patients (median eight days) compares favorably with the time until reappearance of circulating WBCs for 83 patients (median 11 days) who have had ABMT at our institution ( $P = < .00001$  by the two-sided Mann-Whitney U test). This shorter period of aplasia may be important since infections during aplasia are common.<sup>17</sup> Although granulocytes reappeared earlier in these patients, comparisons of the time to reach  $0.5 \times 10^9/\text{L}$  granulocytes following PSCT (median 22 days) and ABMT (median 23 days) ( $P = .44$ ), the time to maintain 10 g/dL hemoglobin after PSCT (median 27 days) and ABMT (median 24 days) ( $P = .21$ ), and the time to recover  $20 \times 10^9/\text{L}$  platelets following PSCT (median 23 days) and ABMT (median 24 days) ( $P = .44$ ) at our institution failed to suggest an additional advantage for PSCT.

Because each patient received autologous cells and no marker to discriminate between autologous peripheral stem cell and autologous marrow stem cell progeny existed, recovery of marrow function rather than engraftment by peripheral stem cells could not be disproved. However, no explanation seems likely to account for peripheral evidence of hematopoiesis eight days following nine or more Gy of TBI (patients 1, 2, 5, 7, and 8) other than peripheral stem cell engraftment. Probably no current high-dose therapy is consistently permanently marrow ablative. Following high-dose therapy and allogeneic marrow transplantation, one patient began to produce autologous cells at 4 months and the proportion of donor cells continued to decrease through subsequent months from 90% to 50%, whereas another patient continued to produce cells exclusively of donor origin

>1 year later.<sup>18</sup> Autologous marrow transplantation is used to accelerate marrow recovery after high-dose therapy, thereby making such therapy feasible as originally described by Appelbaum et al.<sup>19</sup> Peripheral stem cell transplantation resulted in more rapid return of circulating cells than we have seen with ABMT; early hematopoiesis may have resulted from engraftment of committed rather than pluripotent stem cells and later hematopoiesis from autologous marrow recovery.

The peripheral cells were not examined for tumor cell contamination. Circulating tumor cells may have been collected, cryopreserved, and reinfused. However, no patient had an initial tumor relapse in a site different from known areas of metastases at the time high-dose therapy was given, suggesting that residual disease rather than reinfused tumor stem cells was responsible for the regrowth of malignancy. One could speculate that PSCT offers less chance of returning tumor stem cells capable of reestablishing malignancy to the patient than does ABMT. Recently, tumor stem cells were found in long-term cultures of histologically normal bone marrow collected from four of nine patients with breast

cancer.<sup>20</sup> The reported high plating efficiency of these tumor stem cells suggests that they could be especially virulent. Malignant cells in human circulation, however, are not necessarily capable of establishing metastatic disease.<sup>21</sup> Animal studies have demonstrated that most tumor cells entering the circulation are immediately trapped by the lungs, and only a small fraction of them continue in the circulation.<sup>22</sup> If the small number of tumor cells remaining in the circulation are there because of an inability to extravasate, they could not serve as metastatic progenitors.

PSCT permits patients with unacceptable anesthetic risks, prior pelvic irradiation, or metastatic disease in the bone marrow to receive marrow ablative therapy. PSCT might prove better than ABMT for all patients receiving marrow ablative therapy since the trauma associated with harvesting marrow is eliminated, the period of aplasia following transplantation is shortened, and the risk of reestablishing malignancy may be lessened; therefore, a randomized prospective study comparing PSCT to ABMT for patients with similar malignancies receiving identical high-dose therapy is underway.

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