Peripheral Blood Stem Cell Transplants for Multiple Myeloma: Identification of Favorable Variables for Rapid Engraftment in 225 Patients

By Guido Tricot, Sundar Jagannath, David Vesole, Jean Nelson, Sharon Tindle, Langdon Miller, Bruce Cheson, John Crowley, and Bart Barlogie

Transfusion of autologous peripheral blood stem cells (PBSCs) of good quality ensures fast hematopoietic engraftment after myeloablative therapy with a decrease in procedure-related morbidity and mortality. We have analyzed variables influencing the kinetics of engraftment, and therefore reflecting the quality of PBSC collections, in 225 patients with newly diagnosed or refractory multiple myeloma (MM) who received an autotransplant in support of high dose melphalan (200 mg/m^2); 132 of these patients also completed a second transplant. All PBSCs were collected before the first transplant after high-dose cyclophosphamide (6 g/m^2) and hematopoietic growth factors, mainly granulocyte-macrophage colony-stimulating factor. PBSCs were administered either alone (91 patients) or with bone marrow (134 patients). A highly significant correlation was observed between the number of CD34$^+$ cells per kilogram infused and prompt recovery of both granulocytes (P = .0001) and platelets (P = .0001). After correction for the proportion of patients with ≥2 × 10^6/kg CD34 PBSCs infused and with ≤12 months of prior therapy, no difference in engraftment kinetics was seen between patients receiving PBSCs only and those also receiving bone marrow. Exposure to chemotherapy, even to ≤6 months of alkylating agents, significantly delayed hematopoietic recovery posttransplantation. The threshold dose of CD34 cells necessary for prompt engraftment was ≥2.0 × 10^6/kg for patients with ≤24 months of chemotherapy before the first transplant, whereas greater than 5 × 10^6/kg CD34 cells were required to assure rapid recovery also in those with longer exposure. Such quantities, easily collected in the large majority of patients with shorter exposure (91%), were obtained in only 28% of patients with more than 24 months of prior chemotherapy. Rapid platelet recovery within a narrow range of time (before day 14) was almost invariably seen (94%) when greater than 5 × 10^6/kg CD34 cells were infused, irrespective of the duration of prior therapy, whereas the range widened progressively when less CD34 cells were infused. In the absence of CD34 measurements, fast recovery of platelets to greater than 50 × 10^9/L within 14 days after high-dose cyclophosphamide and ≤12 months of prior chemotherapy were the best predictors of early engraftment. Prudent use of stem cell-damaging agents, such as melphalan and nitrosoureas, is recommended in MM patients who might be candidates for autotransplantation. Alternatively, PBSCs should be collected early after diagnosis.

LITTLE PROGRESS has been made during the last 25 years in overall survival of patients with multiple myeloma (MM) using standard chemotherapy; no cures have been observed. Less than 10% of patients attain complete remission and only 5% live longer than 10 years.[1-4] A dose-response effect for alkylating agents has been shown in MM, first using melphalan at 100 to 140 mg/m^2 without transplant support,[5,6] and more recently with higher doses of melphalan or melphalan with total body irradiation, requiring autologous hematopoietic stem cell support.[7-13] Initially, autologous bone marrow was used. Subsequently, investigators have used peripheral blood stem cells (PBSCs) preferentially to bone marrow to achieve more rapid restoration of marrow function after myeloablative therapy. However, this advantage is attained only if the stem cells are collected after mobilization either with robust doses of cytotoxic agents, mainly cyclophosphamide,[14] or with recombinant hematopoietic growth factors.[15,16] High doses of cyclophosphamide[17] in combination with hematopoietic growth factors[18,19] result in maximal expansion of the pool of circulating hematopoietic progenitors. The low mortality rate associated with autologous transplantation in MM[7] encouraged wider application not only in refractory but also in newly diagnosed disease. In the latter group of patients, a complete remission rate of 40% to 50% has been observed and extended event-free and overall survival is likely.[11-13]

In an earlier study of previously treated MM patients who subsequently underwent autotransplantation, Jagannath et al[7] have shown that a "good" stem cell mobilization, based on the number of colony-forming units granulocyte-macrophage (CFU-GM), was associated with early platelet recovery after high-dose cyclophosphamide. Prompt posttransplant engraftment could be anticipated when prior treatment exposure had not exceeded 1 year and if granulocyte-macrophage colony-stimulating factor (GM-CSF) was used after high-dose cyclophosphamide.

We have now performed 225 PBSC autografts, either alone (91 patients) or in conjunction with autologous bone marrow transplantation (ABMT; 134 patients). Of those 225 patients, 132 received a second transplant within 12 months. We now report on the variables associated with prompt engraftment after the first and second transplant.

MATERIALS AND METHODS

Patients. Between November 1990 and December 1993, 264 patients with an established diagnosis of MM were enrolled in autotransplant trials at our institution, using high-dose cyclophosphamide (6 g/m^2)[7] and hematopoietic growth factors for PBSC mobilization: GM-CSF at 250 μg/m^2 was started on day +1 in 215 and granulo-

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Table 1. Patient Characteristics (N = 225)

Parameter	%
>50 yr	57
Stage III at Dx	50
>1 yr from Dx	44
Resistant to prior therapy	37
B2M >2.5 mg/L	51
Newly diagnosed patients	45
PBSCs only	40

cyte-CSF (G-CSF) at 5 μg/kg in 49 patients. Before therapy, all patients signed an informed consent to participate in the study as approved by the institutional review board. Of those 264 patients, 225 had received one and 132 completed two transplants before the date of analysis. Before the first autotransplant, 126 patients had received ≤12 months, 38 between 13 and 24 months, and 61 more than 24 months of standard chemotherapy. Pertinent patient characteristics of the 225 transplant patients are summarized in Table 1. PBSC collections for both first and second transplant were performed before the first transplant through a double lumen Quinton catheter (Quinton Instruments Co, Seattle, WA). Apheresis was initiated upon recovery of leukocytes to 0.5×10^9/L and platelets to 50×10^9/L. Fenwal CS3000 (Baxter Health Care Corp, Deerfield, IL) or Cobe Spectra (Cobe Laboratories Inc, Lakewood, CO) blood cell separators were used for PBSC collections. The collections were performed daily (except on Sundays) to obtain at least 6×10^8 mononuclear cells/kg body weight to support two cycles of marrow-ablative therapy. Before July 1993, if marrow plasmacytosis was less than 30% after recovery after high-dose cyclophosphamide, autologous bone marrow was collected by multiple needle aspirations from both posterior iliac crests under general anesthesia. Every attempt was made to collect at least 3×10^8 mononuclear bone marrow cells/kg. After July 1993, bone marrow was only collected in patients with a maximum CD34 of less than 2% in their PBSC collections.

Pretransplant cytoreduction consisted of melphalan at 200 mg/m^2 administered in two equally divided doses on days −3 and −2 for the first transplant and again for the second transplant for the 92 patients in complete remission (CR) or partial remission (PR) (as defined below). For the 40 patients not attaining a PR, melphalan at 140 mg/m^2 with total body irradiation (850 to 1,125 cGy in 5 to 9 fractions over 3 days) (36 patients) or melphalan at 200 mg/m^2 with cyclophosphamide at 120 mg/m^2 (2 patients) or BEAM chemotherapy (BCNU, etoposide, cytarabine, melphalan) (2 patients) was used with the second transplant.

PBSCs were equally split between the first and second transplant and infused on day 0. If the total volume exceeded 500 mL, PBSCs were also administered on day +1. Autologous bone marrow, if infused, was administered on day 0. After transplantation, patients received hematopoietic growth factor (GM-CSF or G-CSF) at a dose of 250 μg/m^2 to start on day +1 until the absolute neutrophil count (ANC) exceeded 2×10^9/L. Only patients experiencing severe toxicity on GM-CSF either after high-dose cyclophosphamide or after transplantation received G-CSF.

Patients were nursed in a private room. Antibiotic prophylaxis included oral ciprofloxacin (500 mg twice daily) and penicillin VK (250 mg every 6 hours), whereas acyclovir (5 mg/kg every 8 hours) was administered intravenously from day +1 until the ANC was greater than 2×10^9/L.

Methods. The quantities of CD34 cells in the freshly collected and the cryopreserved apheresis product were determined. A 0.5 to 1 mL heparinized sample was washed with cold phosphate-buffered saline containing 0.5% azide and 2% fetal calf serum and stained with a directly labeled anti-CD34 antibody (HPCA-2; Becton-Dickinson, Mountain View, CA). At least 10,000 cells were then analyzed using the FACScan (Becton Dickinson). CD34$^+$ cells were defined by displaying fluorescence versus side scatter and gating on cells with low side scatter (lymphoid or lymphoblastoid). CD34 was determined on all the infused PBSC collections and at least on the first 3 days of the freshly collected apheresis products. CD34 was not measured on the bone marrows collected or infused.

The in vitro assay for CFU-GM was performed on PBSC collections at least during the first 3 days. Briefly, 10^5 light-density cells from apheresis products were cultured in a base mixture of 0.8% methylcellulose (Fisher Scientific, Pittsburgh, PA) containing Iscove's modified Dulbecco's medium, 10% heat-inactivated fetal calf serum, 1% deionized bovine serum albumin (Sigma, St Louis, MO), and 100 U of human recombinant GM-CSF (Immunex Co, Seattle, WA) in a total volume of 1 mL in 35-mm plates (Falcon, Lincoln Park, NJ). The plates were incubated in a humidified atmosphere of 5% CO_2, 10% O_2, and 88% N_2 at 37°C. Colonies were scored using an inverted microscope on day +14. Assays were performed in triplicate.

Response criteria. Improvement was defined as at least a 50% reduction in myeloma protein concentration in serum and/or a greater than 90% reduction in Bence Jones proteinuria. PR required ≥75% tumor mass reduction, including the disappearance of Bence Jones proteinuria and a marrow plasmacytosis of less than 5%. CR required the absence of monoclonal gammopathy in serum and urine on at least 2 occasions 2 months apart and a monoclonal marrow plasmacytosis on marrow aspirate and biopsy of less than 1%.

Statistical analysis. Time to recovery of granulocytes and platelets posttransplantation was estimated from the date of transplantation until recovery using the Kaplan-Meier product limit method. Recovery times were compared between groups using log rank tests. Multivariate regression analysis was applied to determine, in a stepwise fashion, the relative rank of independently significant pretreatment variables.

RESULTS

Figure 1 shows the relationship between the number of CD34 cells infused and the time to reach an untransfused platelet count of $\geq 50 \times 10^9$/L after the first transplant. Eighty-six percent (162/188) of patients receiving $\geq 2 \times 10^6$/kg CD34 cells attained such a platelet count by day 21, whereas 62% (23/37) receiving less than 2×10^6/kg CD34 cells showed delayed (>21 days) platelet recovery. However, in 26 patients (11.6%), a slow platelet recovery was observed despite an adequate number of CD34 cells ($\geq 2 \times 10^6$/kg), whereas 14 patients (6.2%) recovered their platelets promptly with a low number of CD34 cells infused ($< 2 \times 10^6$/kg). The median times to recover platelets of 50×10^9/L after the first transplant were 12 and 44 days, respectively, for patients with ≥ and $< 2 \times 10^6$/kg CD34. Almost identical results were observed after the second transplant (Fig 2A and B). Ten of the 37 patients with less than 2×10^6/kg CD34 cells still had a platelet count less than 50×10^9/L by day +100, although only 2 patients had $\leq 25 \times 10^9$/L platelets by that time. Further analysis of patients receiving an adequate number of CD34 cells ($\geq 2 \times 10^6$/kg) showed a 93% evidence of prompt platelet recovery to $\geq 50 \times 10^9$/L by day 21 with greater than 5×10^6/kg, compared with 68.5% with 2 to 5×10^6/kg CD34 cells. As can be seen in Fig 3A, quantities of $\geq 2 \times 10^6$/kg CD34 cells were adequate for most patients with ≤24 months of cytotoxic therapy, whereas those with greater than 24 months of prior therapy

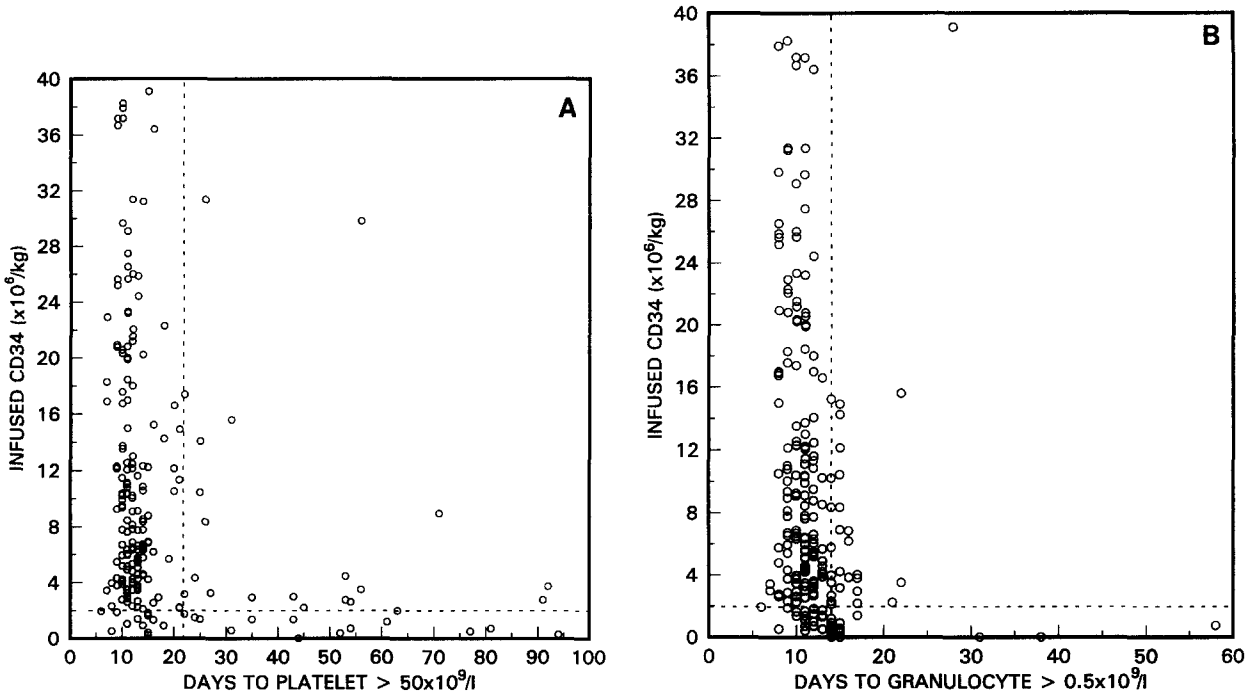

Fig 1. Correlation between the number of CD34⁺ cells per kilogram infused and the time to recover platelets to greater than 50 × 10⁹/L (A) and granulocytes to greater than 0.5 × 10⁹/L (B) after the first transplant.

required greater than 5 × 10⁶/kg CD34 cells for prompt platelet recovery (≥50 × 10⁹/L by day 21). The higher number of CD34 cells required in heavily pretreated patients was not related to the intensity of the preparative regimen, because all patients received only high-dose melphalan with their first transplant. Adequate levels of CD34 cells were obtained in 97%, 85%, and 28%, respectively, of patients with ≤12, 13 to 24, and greater than 24 months of prior therapy (Fig 3B).

Figure 4 addresses the range of days to platelet recovery to ≥50 × 10⁹/L with the median as well as 25th and 75th percentiles. Those receiving greater than 5 × 10⁶/kg CD34

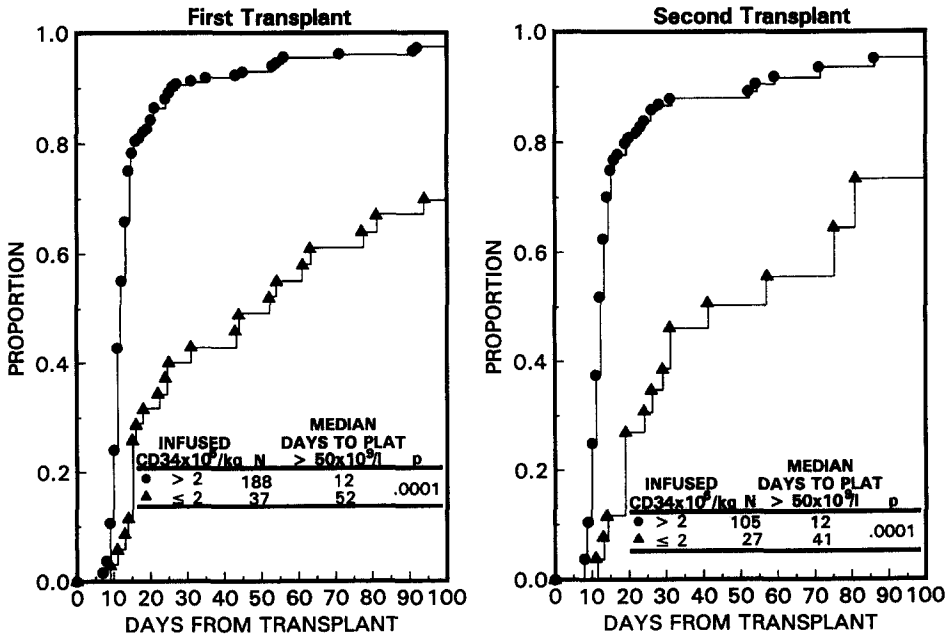

Fig 2. Proportion of patients who recovered platelets to greater than 50 × 10⁹/L with time after the first (A) and second (B) autologous transplant, according to the number of CD34 cells per kilogram infused.

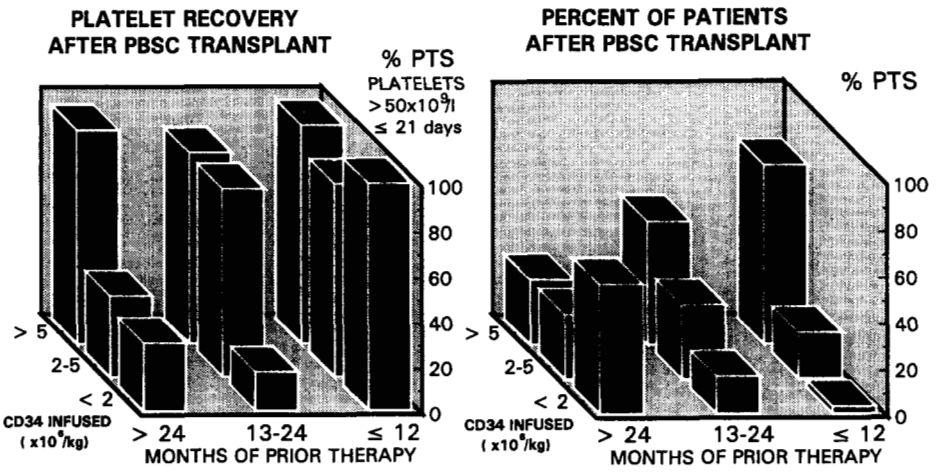

Fig 3. (A) Proportion of patients recovering platelets to greater than 50 × 10⁹/L within 21 days after the first transplant according to the number of infused CD34 cells per kilogram and the duration of prior therapy. The number of patients in each subgroup is indicated. (B) Distribution of patients (%) according to quantities of infused CD34 per kilogram and duration of prior therapy. The percentage of patients in each subgroup is provided. *, Total population in subgroups; **, actual percentages in subgroups.

cells engrafted within a narrow window (75% of patients recovered within 14 days), irrespective of the duration of prior chemotherapy, whereas this window widened progressively with less CD34 cells infused. This inverse relationship between CD34 quantity and recovery time was much less prominent for granulocytes.

Univariate analysis identified the following factors favorable for early granulocyte and platelet recovery: infusion of $\geq 2.0 \times 10^6$/kg CD34 cells, collection of $\geq 4.0 \times 10^6$/kg CD34 cells during the first 3 days of apheresis, maximal CD34 ≥2% in any of the apheresis products, recovery of platelet counts to greater than 50×10^9/L within 14 days after high-dose cyclophosphamide, ≤12 months of prior cytotoxic therapy, no exposure to alkylating agents, collection of $\geq 4 \times 10^4$ CFU-GM/kg during the first 3 days of apheresis, and the infusion of PBSCs only (Table 2). The proportion of patients with early platelet recovery after their first transplant progressively decreased with more extensive exposure to alkylating agents (Fig 5). Platelet recovery to greater than 50×10^9/L after transplantation in the 29 patients with only 1 month of alkylating therapy before PBSC collection was comparable to that of the 107 patients with no prior exposure to alkylating agents ($P = .9$). However, the 34 patients with 2 to 6 months (median, 4 months) of prior alkylating therapy already showed a significant delay in platelet recovery when compared with those never exposed to alkylators ($P = .04$), and the difference in platelet recovery for the 29 patients with 4 to 8 months (median, 6 months) was highly significant ($P = .004$; Table 3). Age ≤ 50 years was only significant for platelet recovery ($P = .02$). Although any of the 3 measurements of CD34 was significantly correlated with recovery of platelets and granulocytes, they were not completely overlapping: 68% of patients with less than 2×10^6/kg CD34 cells infused had a maximal CD34 of ≥2%, although only 2.5% of the patients with $\geq 2 \times 10^6$/kg CD34 cells infused had a maximal CD34 less than 2%. The finding that infusion of PBSCs only (without bone marrow) was associated with a more rapid recovery posttransplantation was surprising. Further analysis showed significantly higher proportions of patients with $\geq 2 \times 10^6$/kg CD34 cells infused (98% *v* 74%; $P = .0001$) and with ≤12 months of prior therapy (66% *v* 49%; $P = .01$) among patients receiving PBSCs only, versus those in whom bone marrow was added. Once these two variables were corrected for, the two groups had comparable engraftment kinetics (Fig 6). Because of the few patients in the PBSCs-only group (2%), the benefit of infusing bone marrow in addition to PBSCs could not be assessed in patients receiving a low number of CD34 cells per kilogram. Parameters of tumor load at diagnosis, such as staging according to Durie-Salmon and B-2-microglobulin (B2M), had no impact on hematopoietic recovery. Although not significant, a trend towards a slower platelet recovery was seen after the second transplant when compared with the first ($P = .3$). This finding was mainly due to slower platelet recovery in patients who received total body irradiation or cyclophosphamide in addition to melphalan as conditioning for their second transplant ($P = .2$).

In a multivariate analysis (Table 4), the two most significant favorable variables for prompt platelet recovery after the first and second transplant were identical and included infusion of ≥2.0/kg CD34 cells as well as rapid platelet recovery after high-dose cyclophosphamide. In addition, up to 12 months of prior cytotoxic therapy was also important for platelet recovery after the first transplant. The most significant favorable variable for granulocyte recovery after the first and second transplant was the procurement or infusion of an adequate number of CD34 cells; again ≤12 months of prior cytotoxic therapy was only significant after the first transplant.

When CD34 measurements are not available, fast recovery (≤14 days) of platelets to greater than 50×10^9/L after high-dose cyclophosphamide ($P = .0001$) and duration of prior chemotherapy ($P = .0001$) are the best predictors of early recovery of platelets after autotransplantation.

DISCUSSION

More than 750 autotransplants have been reported in MM worldwide, initially with bone marrow support and later with PBSCs with or without bone marrow.[20] PBSC transplants may result in faster engraftment[16,18,21] and allow autologous transplantation to be performed in patients with inadequate bone marrow cellularity due to local radiotherapy or with

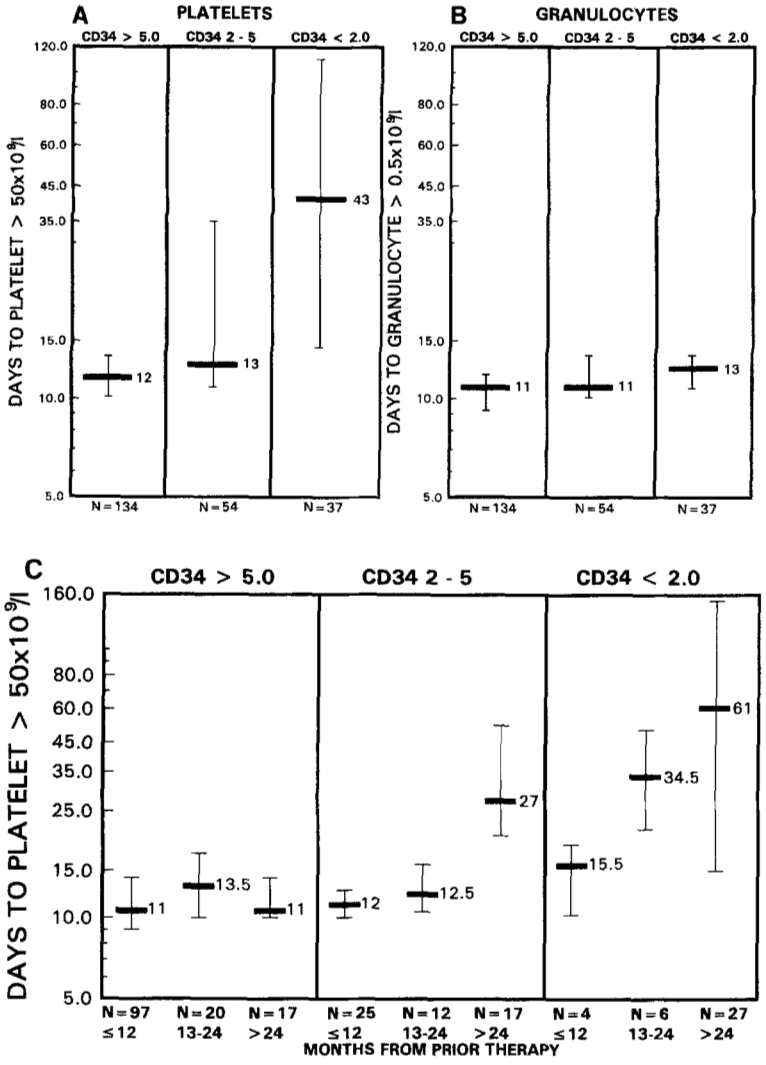

Fig 4. Recovery of platelets to greater than 50 × 10^9/L (A) and of granulocytes to greater than 0.5 × 10^9/L (B) after first autotransplant. Depicted are the median as well as the 25th and 75th percentiles. (C) Platelet recovery in relationship to CD34 cells per kilogram and, in addition, according to the duration of prior therapy (n = 225).

extensive marrow disease.[22] Optimal mobilization of peripheral blood progenitor cells is attained with a combination of high-dose cytotoxic therapy followed by administration of hematopoietic growth factors.[18,19,23]

This is the first detailed report on a large number of patients with a single hematologic malignancy that examines potentially important factors associated with hematopoietic recovery after transplantation with PBSCs mobilized with high-dose cyclophosphamide and hematopoietic growth factors. The strongest predictor of rapidity of granulocyte and platelet recovery was the number of CD34 cells infused. The closer association between the quantity of CD34 cells and platelet, rather than granulocyte recovery, as well as the link between platelet recovery after high-dose cyclophosphamide and transplantation, does support the notion that megakaryopoiesis is a better indicator for the quality of stem cell function than granulopoiesis. In the presence of growth factors that stimulate granulocytes, prompt granulocyte recovery (≤21 days) occurs in almost all patients (97%) irrespective of the dose of CD34 cells infused (Fig 1B). In patients with ≤24 months of prior cytotoxic therapy, a threshold quantity of 2.0 × 10^6/kg $CD34^+$ cells was required for prompt platelet recovery after myeloablative therapy. However, with more extensive prior therapy, greater than 5 × 10^6/kg CD34 cells were necessary to ensure rapid recovery. These threshold numbers, although easily collected in the large majority (94%) of patients with ≤24 months of prior therapy, were obtained in only 28% of patients with more extensive prior treatment.

Prior cytotoxic chemotherapy adversely affected the yield of CD34 cells. Haas et al[24] calculated an average decrease of 0.2 × 10^6/kg CD34 cells per leukapheresis with each cycle of chemotherapy in malignant lymphoma patients. The most severe effects on the hematopoietic system are seen with exposure to BCNU or busulfan, intermediate effects with cyclophosphamide and cisplatin, and the least effect with cytosine arabinoside, hydroxyurea, and 5-fluorouracil.[25] Exposure to cytotoxic agents affects self-renewal capacity of stem cells more than their ability to differentiate. Therefore, clonogenic assays such as CFU-GM and CFU-granulocyte, erythroid, monocyte, megakaryocyte measuring the quantity of more differentiated hematopoietic progenitors have only

Table 2. Univariate Analysis of Variables Potentially Associated With Granulocyte and Platelet Recovery After the First Transplant

		Days to ANC >0.5 × 10^9/L				Days to Platelets >50 × 10^9/L			
	N	25%	50%	75%	P	25%	50%	75%	P
CD34 infused									
≥2 × 10^6/kg	188	10	11	12	.0001	10	12	14	.0001
<2 × 10^6/kg	37	11	13	15		15	52	157	
Maximal CD34 in collection									
≥2%	146	9	11	12	.0001	10	12	14	.0001
<2%	79	11	13	14		13	21	54	
CD34 collected first 3 d									
≥4 × 10^6/kg	135	9	11	12	.0001	10	12	14	.0001
<4 × 10^6/kg	90	11	13	15		12	14	54	
Duration of prior therapy									
≤12 mo	126	9	11	12	.0001	10	12	14	.0001
>12 mo	99	11	12	14		11	15	52	
Prior alkylating therapy									
0 mo	107	9	11	12	.0001	10	12	14	.0001
≥1 mo	118	10	12	14		11	14	43	
Platelets >50 post-CTX									
≤14 d	142	10	11	12	.05	10	12	14	.0001
>14 d	83	10	11	14		12	18	54	
Source of transplant									
PBSCs only	91	10	11	12	.07	11	11	14	.0003
PBSCs + BM	134	10	11	14		11	13	26	
CFU-GM first 3 d									
≥4 × 10^4/kg	129	10	11	12	.03	11	12	15	.001
<4 × 10^4/kg	96	10	12	14		11	14	31	
Order of transplant									
First	225	10	11	13	.08	11	13	20	.2
Second	132	9	11	13		11	13	26	
Age (yr)									
≤50	103	10	11	13	.8	10	12	15	.02
>50	122	10	11	13		11	13	22	
Stage at diagnosis									
I + II	113	10	11	13	.6	10	13	21	.5
III	112	10	11	13		11	13	18	
B2M at diagnosis									
≤2.5 mg/L	110	10	11	13	.1	10	12	17	.04
>2.5 mg/L	115	9	11	13		11	14	21	

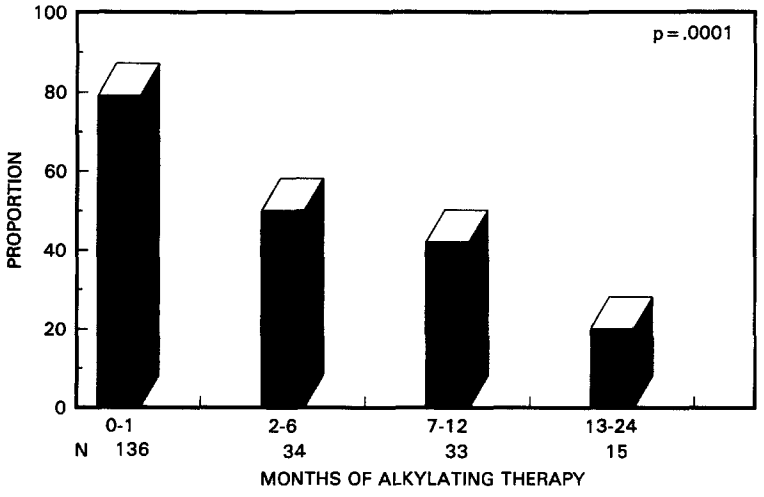

Fig 5. Proportion of patients recovering platelets to greater than 50 × 10^9/L by day 14 after transplantation according to the months of alkylating therapy before the collection of PBSCs. The number of patients in each group is provided.

Table 3. Peripheral Blood Stem Cell Mobilization

Months of Prior Alkylating Therapy	Median	N	Days to Platelets >50 × 10^9/L			P*
			25%	50%	75%	
0	0	107	10	12	14	
1	1	29	10	12	14	.9
2-6	4	34	11	14	17	.04
4-8	6	29	11	15	25	.004
9-12	11	22	11	25	52	.002
13-24	21	15	15	24	81	.0003
25-44	34	7	14	67	94	.01

*P values are for the groups indicated when compared with the 107 patients with no prior alkylating therapy.

limited value in the estimation of the hematopoietic stem cell deficit after exposure to cytotoxic agents.

In this study, 72% of patients with greater than 24 months of prior therapy failed to mobilize enough CD34 cells to ensure rapid platelet recovery to greater than 50 × 10^9/L. We observed a highly significant decrease (P = .0001) in the proportion of patients with early platelet recovery after more extensive exposure to alkylating agents (Fig 5). It has been noticed that, in heavily pretreated patients, the use of high-dose cytotoxic therapy and GM-CSF has high patient-to-patient variability with a fraction of those having little or no increase in CFU-GM or $CD34^+$ cells in the blood.[21] Such patients may have better mobilization with high doses of G-CSF than with cyclophosphamide and GM-CSF, as recently suggested by Bensinger et al.[21]

The fact that higher numbers of CD34 cells per kilogram are required for rapid platelet recovery in heavily pretreated patients shows that CD34 cells are heterogenous and represent only a surrogate marker of hematopoietic stem cell function. In the recovery phase after high-dose cyclophosphamide, a several hundred-fold increase in peripheral blood burst-forming units-megakaryocyte (BFU-Meg) and CFU-Meg has been observed, explaining the rapid platelet recovery after PBSCs.[26] The BFU-Meg is $CD34^+DR^-$, whereas the differentiated CFU-Meg is $CD34^+DR^+$.[27] It is likely that, with more extensive prior chemotherapy, a shift occurs from early to more committed progenitor cells within the CD34 fraction and that relatively more myeloid progenitors ($CD34^+$, $CD33^+$) are mobilized than megakaryocytic precursors.

As can be seen in Table 2, the number of infused CD34 cells per kilogram can be replaced by any of the other CD34 measurements during the collection of PBSCs. If CD34 measurements are not available, the rapidity of platelet recovery postcyclophosphamide is a reliable indicator of the speed of hematologic recovery posttransplantation, equivalent to the much more laborious in vitro CFU-GM cultures.

Rapid platelet recovery within a narrow range was almost invariably seen (97% of patients) when greater than 5 × 10^6/kg CD34 cells were infused, irrespective of the duration of prior therapy. Because it is crucial to collect an adequate number of CD34 cells for early hematopoietic recovery, we recommend that hematopoietic stem cell-damaging agents, especially melphalan, nitrosoureas, and local radiotherapy, be used prudently. There is also a need for additional growth factors acting on earlier hematopoietic progenitor cells, such as PIXY321, interleukin-3, stem cell factor, and interleukin-6 for patients with more extensive prior chemotherapy.

Finally, this study has not addressed the important question of tumor contamination in PBSCs. Using polymerase chain reaction (PCR)-based techniques, we[28] and others[29] have found sizable quantities of myeloma cells (up to >1%) in almost all PBSC collections. It is yet unknown whether more efficient mobilization of peripheral stem cell progenitors will also result in the appearance of more immature myeloma cells in the peripheral blood and whether both the hematopoietic progenitor cells and the myeloma cells are mobilized at the same time or sequentially. It will be necessary to provide virtually tumor-free grafts to achieve sus-

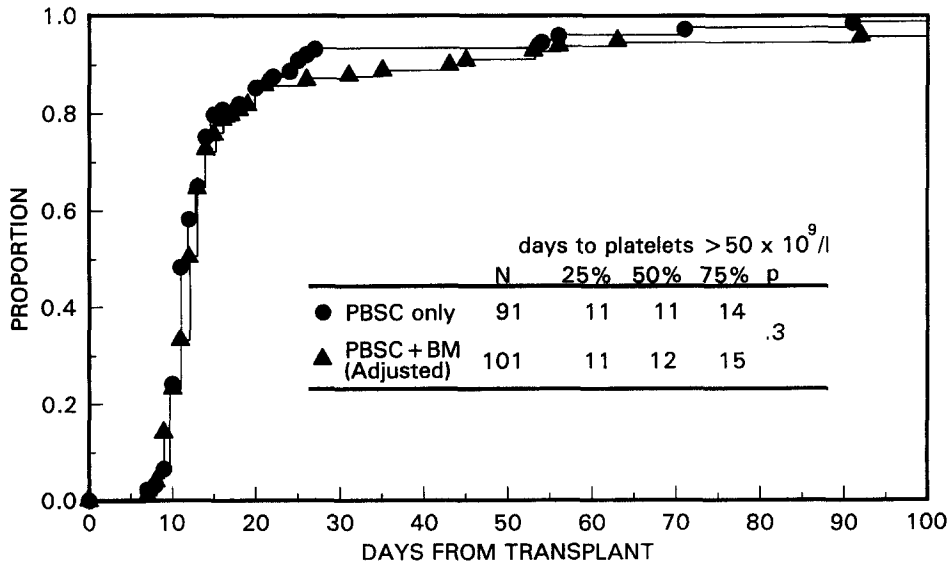

Fig 6. Recovery of platelets to greater than 50 × 10^9/L after first autotransplants in patients receiving PBSCs only versus those who received PBSCs and bone marrow. The group of patients receiving PBSCs and bone marrow was adjusted for the percentage of patients receiving ≥2 × 10^6/kg CD34 cells and the percentage of patients with ≤12 months of prior therapy; 33 patients in the PBSCs and bone marrow group were excluded to match the PBSCs only group.

Table 4. Multivariate Analysis of Potentially Important Prognostic Variables for Platelet Recovery After Transplant

Favorable Variables	
First transplant	
Infused CD34 ≥2.0	0.0001
Fast platelet recovery post-CTX	0.0001
≤12 mo of prior therapy	0.007
Age ≤50 yr	0.08
Maximal CD34 ≥2.0%	0.2
CD34 ≥4.0 during first 3 d	0.3
CFU-GM ≥4 during first 3 d	0.6
No prior alkylating agents	0.9
Second transplant	
Infused CD34 ≥2.0	0.0001
Fast platelet recovery post-CTX	0.03
CD34 ≥4.0 during first 3 d	0.08
≤12 mo of prior therapy	0.1
Age ≤50 yr	0.2
Maximal CD34 ≥2.0%	0.3
No prior alkylating agents	0.4
CFU-GM ≥4 during first 3 d	0.4

Abbreviation: CTX, cyclophosphamide.

tained complete remissions once adequate tumor cytoreduction has been accomplished with myeloablative therapy. Preclinical studies with flow-sorted $CD34^+Lin\text{-}Thy1^+$ cells have shown that such a product contains less than 1 tumor cell per 10^5 purified cells (limit of detection), based on PCR for CDRIII.[28] Because all selection processes are accompanied by a substantial loss of target cells, optimizing mobilization regimens and minimizing prior chemotherapy will be important to make such procedures clinically feasible.

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