

TREATMENT OF NORMAL DONORS WITH rhG-CSF 16 $\mu\text{g}/\text{kg}$ FOR MOBILIZATION OF PERIPHERAL BLOOD STEM CELLS AND THEIR APHERETIC COLLECTION FOR ALLOGENEIC TRANSPLANTATION

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ABSTRACT

Background. Utilization of peripheral blood stem cells (PBSC) in allogeneic transplantation requires a method for their mobilization and collection that is not inconvenient for the donor.

Methods. We administered rhG-CSF (filgrastim) 16 $\mu\text{g}/\text{kg}$ subcutaneously for 4 days in five normal subjects (age 18-31, M=3, F=2), previously selected as HLA-identical donors of siblings with leukemia. All the donors gave written informed consent. On days 4 and 5 (in one donor on day 6 too), 10:l leukapheresis collection was performed with a CS-3000 (Baxter) or an AS-104 (Fresenius) cell separator through the antecubital vein.

Results. The WBC count reached a median peak of $57.0 \times 10^9/\text{L}$ on day 5. The peripheral blood CFU-GM peaked to a median level of 8908/mL on day 5 with a median increase over baseline values of 39.1 times. The $\text{CD}34^+$ cells peaked to (median) $147.0 \times 10^6/\text{L}$ on day 4 with a median increase of 65.3 times. A lesser enrichment was recorded for BFU-E (median increase 12.7 times) and CFU-GEMM (median increase 15.2 times). Even $\text{CD}3^+$ and $\text{CD}56^+\text{CD}3^-$ cells increased (median 1.7 and 1.5 times, respectively). A median of 771×10^8 MNC (range 672-1378), 116.4×10^6 CFU-GM (range 47.7-145.1) and 754×10^6 $\text{CD}34^+$ cells (range 477-2599) were apheretically collected. Concerning side effects, mild to moderate back pain and general minor discomfort were reported by all donors. The platelet level regularly but transiently decreased after completion of the apheretic procedures with a median nadir of $69 \times 10^9/\text{L}$ (range 43-126) on (median) day 7, but in no case did thrombocytopenia cause bleeding. The thrombocytopenia was more pronounced with the CS-3000 than the AS-104 apparatus.

Conclusions. rhG-CSF 16 $\mu\text{g}/\text{kg} \times 4$ days is an efficient schedule for PBSC mobilization in healthy donors, but lower doses and even a single apheresis procedure might prove similarly adequate.

Key words: PBSC, rhG-CSF, transplantation, apheresis, donor

Hemopoietic progenitors circulate at low concentration,^{1,2} but their number can be dramatically increased by chemotherapy and/or hematopoietic growth factors.³⁻⁷ These primed peripheral blood stem cells (PBSC) can rapidly repopulate the marrow after high-dose therapy, increasing the speed of neutrophil and platelet recovery.⁸⁻¹⁰ In recent years the PBSC technique has become popular for autologous transplants,^{11,12} but it has been appli-

ed only occasionally in the allogeneic setting,¹³⁻¹⁵ due in part to fear of increasing GVHD and in part to the lack of direct evidence for the presence of uncommitted, self-repopulating stem cells in the peripheral blood. However, peripheral blood contains early progenitors¹⁶ and PBSC can permanently engraft marrow-ablated animals, establishing full donor chimeras.¹⁷⁻¹⁹

A strong argument in favor of PBSC in the allogeneic setting may be the high number of

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progenitors. In a recent study,²⁰ patients receiving suboptimal ($< 2.4 \times 10^4/\text{kg}$) numbers of CFU-GM showed significantly lower platelet counts on day +80, +100 and +150, more CMV infections and a significantly greater transplant related mortality. Therefore, increasing the number of allogeneic hemopoietic progenitors would not only accelerate hemopoietic recovery and shorten hospital stay, but it may also lead to reduced transplant complications. Since a high number of hemopoietic progenitors can only be obtained from the peripheral blood, the modality of PBSC mobilization and collection in healthy donors represents a key issue.²¹

The hemopoietic growth factor rhG-CSF has an excellent mobilizing effect^{7,9,22,23} and a safe clinical profile.²⁴⁻²⁶ In normal donors, doses of 2 to 16 $\mu\text{g}/\text{kg}/\text{day}$ of rhG-CSF have produced good PBSC yields with little or no side effects.^{25,27-31} We tested the feasibility of a scheme for PBSC mobilization and collection in normal donors that consisted of administering rhG-CSF 16 $\mu\text{g}/\text{kg}/\text{day} \times 4$ days, followed by 2 apheretic harvests, and we report here the results in 5 healthy subjects. We also studied the variations in peripheral blood cell populations during and after rhG-CSF treatment in these subjects by means of *in vitro* colony assays and flow cytometry analysis.

Materials and Methods

Normal donors. Five healthy adult donors entered the protocol (Table 1). They were HLA-identical, MLC non-reactive sibling donors either of patients with advanced (beyond 1st

remission, 2 patients) or high-risk (Ph^+ ALL, 1 patient) acute leukemia or of patients who had experienced a relapse after allogeneic bone marrow transplantation (CML, 1 patient; AML, 1 patient). For these last patients the donors were the same ones who had previously donated marrow to them, and the mobilization procedure was undertaken with the intent of infusing the hosts with donor leukocytes enriched in PBSC without myeloablation. All the donors were thoroughly informed and gave written consent.

rhG-CSF administration. rhG-CSF (*recombinant human granulocyte colony-stimulating factor*, filgrastim, Amgen) was administered subcutaneously to donors at a dosage of 16 $\mu\text{g}/\text{kg}/\text{day}$ in two divided daily injections for 4 subsequent days. The first day of rhG-CSF administration was conventionally labelled as day 1. This treatment was always performed on an outpatient basis.

PBSC apheretic collection. The CS-3000 *plus* (Baxter) or the AS-104 (Fresenius) was employed. The CS-3000 was equipped with a *large* collection chamber. Aphereses were run through the antecubital veins. Ten litres of blood were processed at a time. The aphereses were started on day 4 of rhG-CSF administration, soon after the morning dose. The evening of the same day the donor received his last dose of rhG-CSF. The second apheresis was regularly run on day 5, approximately 12 hours after the last rhG-CSF dose. A third apheresis on day 6 was run only in one case (donor #2). The PBSC bags were heparinized (calcium heparin 5,000 units) immediately after the apheretic run. The

Table 1. Donor characteristics, reported complaints, number of apheresis procedures, and lowest platelet counts during PBSC mobilization and collection. All the donors received rhG-CSF 16 $\mu\text{g}/\text{kg}$ for 4 consecutive days, and none interrupted the program. Note that mild to moderate thrombocytopenia developed in 4, with bleeding complications in none.

Donor #	Age/sex	Symptoms	Cell separator	No. aphereses	Platelet nadir $\times 10^9/\text{L}$ and (day)
1	28/M	fatigue, sweating	CS 3000 large ch	2	69 (6)
2	26/M	fatigue, fever, back pain, chest pain	AS 104	3	87 (9)
3	31/F	back pain	AS 104	2	126 (9)
4	22/M	fatigue, fever, headache	CS 3000 large ch	2	54 (7)
5	18/F	fatigue, dizziness, back pain	CS 3000 large ch	2	43 (7)

Large ch = large chamber.

day 4 apheresis was kept at 4°C until the following day, when it was infused in the recipient together with the day 5 apheresis. In one case, a third collection (day 6) was cryopreserved in liquid nitrogen for future use by means of a computerized freezer (Planer R203) at the rate of -1°C/min to -40°C in Gambro DF-700 bags.

Cell characterization. Peripheral blood (day 0, 1, 2, 3, 4, 5, 7, 9 and 11) and the leukapheresis product samples were immunofluorescence labeled using standard protocols.

In brief, cells ($0.5-1 \times 10^6/100 \mu\text{L}$ per tube) were incubated for 30 minutes at 4°C with monoclonal antibodies conjugated to phycoerythrin, fluorescein or peridinin chlorophyll protein, then hemolyzed with ammonium chloride hemolytic buffer and washed twice with PBS. The monoclonal antibodies used were: CD3 (Leu-4, PerCP), CD4 (Leu-3a, FITC), CD8 (Leu-2a, Pe), CD19 (Leu-12, PE), CD20 (Leu-16, FITC), CD56 (Leu-19, PE), CD34 (HPCA-2, FITC), CD45/CD14 (LeucoGate) and Ig isotype controls (Becton Dickinson). The samples were acquired on a FACSCAN and analyzed using Lysis II software (Becton-Dickinson). Analysis of CD34⁺ cells was performed using large contiguous gates, as previously described.³² Lymphocyte subset analysis was evaluated using LeucoGATE. A gate was set around the lymphocyte population expressing high levels of CD45 but low levels of CD14. A light-scatter gate was then set to include $\geq 98\%$ of the lymphocytes in the fluorescence gate. The lymphocyte subsets were calculated on this latter gate.

In vitro colony assay. CFU-GM, BFU-E and CFU-GEMM were grown by plating in 35 mm Petri plastic culture dishes (Falcon) $0.5-1 \times 10^5$ MNC in 1 mL of Iscove's methylcellulose (Terry Fox Laboratories, Vancouver, Canada) containing 0.9% methylcellulose, 30% fetal calf serum, 1% bovine serum albumin, 150 U/mL erythropoietin, 10% agar leukocyte conditioned medium, 10^{-2}M 2-mercaptoethanol, 200 mM L-glutamine and 8% Iscove's medium. All cultures were carried out in duplicate. After 14 days of incubation in a humidified atmosphere of 5% CO₂ at 37°C, colonies (aggregates of ≥ 40 cells) were counted using an inverted microscope.

Results

Procedure-related toxicity. All the donors completed the program of rhG-CSF treatment and PBSC apheretic collection. However, mild to moderate disturbances were reported by all and these included fatigue, low-grade fever, sweating, back pain, headache, dizziness and chest pain (Table 1). Complaints began on day 2 or 3 of rhG-CSF treatment and subsided one-two days after stopping the treatment. The two subjects who had previously donated their marrow under general anesthesia tolerated the PBSC mobilization/collection procedure better than the marrow harvest.

Cell mobilization. The levels of WBC, platelets, CFU-GM, BFU-E, CFU-GEMM and CD34⁺ cells in the peripheral blood before and during mobilization and collection are reported in Figure 1 and Table 2. The WBC count sharply increased during rhG-CSF administration in all the donors, reaching a peak of 34.0 to 102.0 (median $57.07 \times 10^9/\text{L}$ on day 4 to 5 (median 5)). Peripheral blood CFU-GM peaked at a level of 2686 to 23,067/mL (median 8908/mL) on day 4 to 5 (median 5), with a median increase of 39.1 times baseline values. A substantial though less abundant enrichment was also recorded for circulating BFU-E (median 12.7 times) and CFU-GEMM (median 15.2 times). CD34⁺ cells were present at low levels (0.9 to $9.2 \times 10^6/\text{L}$, median 3.7) in base samples, but peaked at 68.2 to $353.0 \times 10^6/\text{L}$ (median 147.0) on day 4 to 5 (median 4). These cells were enriched by 18.4 to 118.7 times (median 65.3) after rhG-CSF. The variations in lymphocyte subsets are reported in Table 2. T cells were expressed as total CD3⁺, B cells as total CD19⁺ and NK cells as total CD56⁺ CD3⁻. Though the elevation of lymphoid cells was not comparable to that of total WBC, a definite increase was recorded in all donors, with CD3⁺ values increasing 1.5 to 2.9 times (median 1.7), CD19⁺ 1.3 to 4.5 times (median 1.6) and CD56⁺ 1.0 to 3.0 times (median 1.5) baseline values.

Cell harvest. Table 3 shows the number of cells harvested with the apheretic procedures. A median of 771×10^8 MNC (range 672-1378), 116.4×10^6 CFU-GM (range 47.7-145.1) and 754×10^6 CD34⁺ cells (range 477-2599) were

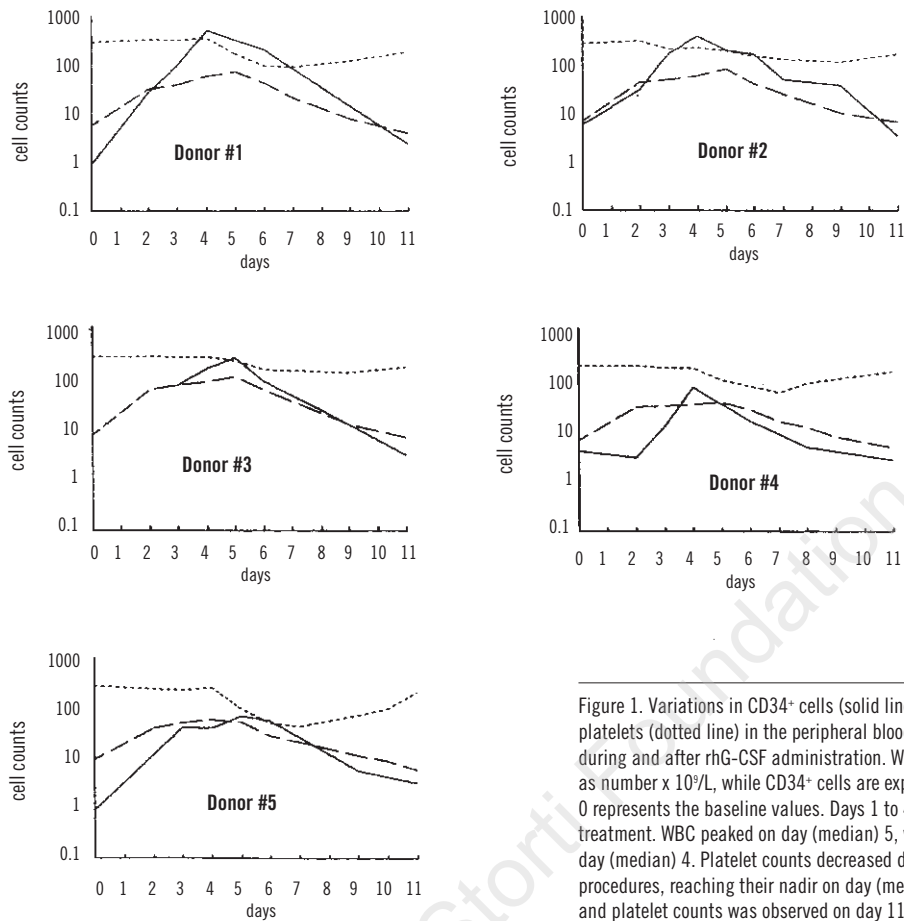


Figure 1. Variations in CD34⁺ cells (solid line), WBC (dashed line) and platelets (dotted line) in the peripheral blood of the five healthy donors during and after rhG-CSF administration. WBC and platelets are expressed as number $\times 10^9/L$, while CD34⁺ cells are expressed as number $\times 10^6/L$. Day 0 represents the baseline values. Days 1 to 4 were the days of rhG-CSF treatment. WBC peaked on day (median) 5, while CD34⁺ cells peaked on day (median) 4. Platelet counts decreased during and after the apheresis procedures, reaching their nadir on day (median) 7. Normalization of WBC and platelet counts was observed on day 11. See text for further details.

collected. The number of progenitors collected, as judged by the CD34 antigen, varied from donor to donor by a factor of 5, with a median of 754×10^6 (range 477 to 2599), while the number of CD3⁺ showed only modest donor-to-donor variation (median 270×10^8 , range 191 to 372). The same was true for CD19⁺ cells (median 60.2×10^8 , range 31.0-124.7) and CD56⁺CD3⁻ cells (median 20.5×10^8 , range 20-32).

Discussion

In recent years an increasing number of patients have been autografted with peripheral blood as the sole source of stem cells.¹² This ever-growing experience has also encouraged the use of PBSC in the allogeneic setting, focusing the attention of investigators on the method of PBSC mobilization and collection in healthy

donors, as well as to the possible advantages over bone marrow harvesting.

Marrow harvesting is not completely devoid of complications, side effects or patient discomfort. In a report on 1,270 harvest procedures in Seattle,³³ six donors suffered life-threatening complications and ten showed significant operative site morbidity. As many as ten percent of donations were associated with fever, and increasing donor age was significantly linked to poor cell harvest. In a different survey, 10 percent of donors recovered completely from bone marrow donation only after more than 30 days from the procedure.³⁴ The use of PBSC, which eliminates the need for general anesthesia, is expected to reduce or abolish hospitalization and operative morbidity. On the other hand, efficient PBSC collection requires treatment with a progenitor cell mobilizing agent.

Table 2. Baseline, peak value and fold increase of peripheral blood cells during rhG-CSF mobilization. Day cell count peaked is in parentheses.

Donor	Value	WBC x 10 ⁹ /L	CFU-GM mL	BFU-E mL	CFU-GEMM mL	CD34+ x10 ⁶ /L	CD3+ x10 ⁹ /L	CD19+ x10 ⁹ /L	CD56+CD3- x 10 ⁹ /L
1	Base	4.6	589	380	209	5.4	1.0	0.2	0.1
	Peak	54.0 (5)	23067 (5) *	3495 (5)*	6990 (5)*	353.0 (4)	2.9 (4)	0.9 (4)	0.3 (4)
	Fold increase	11.7	39.1	9.1	33.4	65.3	2.9	4.5	3.0
2	Base	6	330	440	330	2.4	1.7	0.3	0.2
	Peak	64.0 (5)	10620 (5)	5610 (4)	4780 (4)	285.0 (4)	2.9 (4)	0.5 (4)	0.3 (4)
	Fold increase	10.6	32.1	12.7	14.4	118.7	1.7	1.6	1.5
3	Base	7.5	180	420	120	9.2	1.5	0.2	0.3
	Peak	102.0 (5)	8908 (4)	7540 (4)	1830 (5)	244.8 (5)	3 (4)	0.6 (4)	0.6 (4)
	Fold increase	13.6	49.4	17.9	15.2	26.6	2.0	3.0	2.0
4	Base	6.2	81	216	0	3.7	1.7	0.3	0.1
	Peak	34.0 (5)	2686 (4)	957 (3)	864 (5)	68.2 (4)	2.7 (4)	0.4 (5)	0.1 (5)
	Fold increase	5.4	33.1	4.4	—	18.4	1.5	1.3	1.0
5	Base	9.3	39	156	0	0.9	1.9	0.3	0.4
	Peak	57.0 (4)	3420 (6)	2508 (6)	1216 (6)	69.1 (5)	3.2 (4)	0.4 (3)	0.5 (3)
	Fold increase	6.1	87.6	16.0	—	76.7	1.6	1.3	1.2
Median	Base	6.2	180	380	120	3.7	1.7	0.3	0.2
	Peak	57.0 (5)	8908 (5)	3495 (4)	1830 (5)	147.0 (4)	2.9 (4)	0.5 (4)	0.3 (4)
	Fold increase	10.6	39.1	12.7	15.2	65.3	1.7	1.6	1.5

* indicates that day 4 colony-forming cell assays are missing.

The peripheral blood cell dose necessary for stable engraftment has not been clearly determined. In the autologous setting a dose of $> 2 \times 10^6$ /kg CD34⁺ cells is able to produce a stable and rapid hematopoietic reconstitution.³⁵ The threshold dose might be higher in allogeneic transplantation as a consequence of the immunological mechanisms involved. For this reason, it would be unwise to collect PBSC in the steady state (i.e. with no mobilization treatment), since it would result in a high number of apheretic procedures with a low total progenitor cell count.¹³

Table 3. Total number of cells collected in the donors. Two apheretic procedures were performed in all donors but one (#2), who underwent 3 apheretic procedures over 3 days.

Donor	cells collected							
	MNC x10 ⁸	CFU-GM x10 ⁶	BFU-E x10 ⁵	CFU-GEMM x10 ⁵	CD34+ x10 ⁶	CD3+ x10 ⁹	CD19+ x10 ⁹	CD56+CD3- 3-XX x10 ⁶
1	1378	93.3	26.8	54.1	2599	372	124.7	32
2	771	47.7	16.5	20.9	754	258	70.5	20
3	672	145.1	72.6	15.4	507	191	31.0	20
4	767	nd	nd	nd	477	270	nd	nd
5	826	142.4	66	33	890	336	49.9	21
Median	771	116.4	46.4	26.9	754	270	60.2	20.5

In the present paper we show the results of PBSC mobilization with rhG-CSF in a small cohort of normal individuals previously selected as HLA-identical donors for their siblings with leukemia. The schedule of rhG-CSF administration was 16 µg/kg/day for 4 consecutive days, similar to that employed in a previous study of five syngeneic donors²⁹ reporting a harvest of 4.6 to 211.9 × 10⁴/kg CFU-GM and 1.6 to 12.6 × 10⁶/kg CD34⁺ cells. In that study, donor cells were transplanted to recipients after different preparative regimens and led to rapid hematologic engraftment: $> 0.5 \times 10^9$ /L granulocytes on day 13 and $> 20 \times 10^9$ /L platelets on day 10.

Lower daily doses have been adopted in other centers for PBSC mobilization in normal individuals. Matsunaga *et al.*²⁸ treated three healthy volunteers with rhG-CSF 2.5 µg/kg/day on days 1-6 followed by 5.0 µg/kg/day on days 7-10. These subjects obtained a CFU-GM peak on day 6, but continuing rhG-CSF administration at 5.0 µg/kg/day did not reinforce the level of circulating CFU-GM. A single dose of rhG-CSF of 15 µg/kg was given to 10 normal donors by Schwinger *et al.*,²⁷ who on day 5 obtained significant rises in CD34⁺ cells, CFU-GM and BFU-E to counts of 250/mL, 3.2×10^3 /mL and 1.75×10^3 /mL, respectively. Sato *et al.*,³⁰ in a time

course study on the optimal method for harvesting PBSC from normal donors, gave their volunteers a dose of 2 µg/kg/day rhG-CSF for 5 days and obtained a median increase of CFU-GM to more than 2,000/mL blood on day 6, representing a 30-fold maximum increase over baseline values. A lesser, but still significant increase was recorded for BFU-E and CFU-Mix. The same authors also showed that a maximum increase in progenitors takes place 6 hours after the last rhG-CSF dose and lasts up to 30 hours. Fritsch *et al.*³⁶ reported the use of 5 µg/kg of either rhG-CSF or of GM-CSF in 17 healthy volunteers for a period of 5 days, resulting in a significant elevation of CD34⁺ cells as well as clonogenic cells on day 5. They concluded that sufficient PBSC could be collected with a single apheresis. In contrast, Kessinger *et al.*¹³ collected sufficient amounts of allogeneic PBSC without cytokine amplification, but they ran 10 aphereses over 30 days.

rhG-CSF has a dose dependent effect, and 10 µg/kg proved superior to 5 µg/kg in mobilizing hemopoietic progenitors in normal subjects.³⁷ In our donors who received 16 µg/kg WBC, colony forming cells and CD34⁺ cells rose sharply and rather synchronously, reaching a peak between days 4 and 5. The peak of CD34⁺ cells, however, anticipated that of WBC and CFU-GM by one day in three donors. The level of progenitors declined slowly after reaching a peak, a finding that might be exploited when there is a need for a back-up PBSC harvest. All donors responded well to the mobilization protocol, though a certain donor-to-donor variation was observed. Not only peripheral blood CFU-GM increased during rhG-CSF treatment, but BFU-E and CFU-GEMM also rose in a similar way. We regularly obtained excellent yields of progenitors with 2 aphereses. In fact, the minimum number of CD34⁺ cells collected was 477×10^6 , corresponding to 6.8×10^6 /kg transplantable CD34⁺ cells for an ideal 70 kg recipient. This implies that, with our mobilization protocol, even a single apheresis would probably be sufficient to ensure rapid engraftment in an adult recipient. With the cells collected from donors #3, 4 and 5 we have already transplanted their HLA-identical siblings recipients. Detailed results are not

reported here, but hematologic engraftment was rapid in all patients both for the granulocyte and platelet series.

We also confirm the capacity of rhG-CSF to increase the number of circulating lymphocytes. In our study the peripheral blood CD3⁺ population increased by a factor of 1.7 during rhG-CSF administration. This increment was much less pronounced than for total WBC counts, circulating colony-forming cells or CD34⁺ cells, and showed less donor-to-donor variation. Why rhG-CSF elevates the lymphocyte count is not understood, since lymphocytes are not known to express receptors for rhG-CSF. This effect has already been reported in primates³⁸ and humans,^{28,29,39} and may be due to the release of other cytokines induced by rhG-CSF.

As consequence of the combined effect of lymphocyte count increase and the large volume of blood processed, the apheretic samples from our donors contained a much higher number of T-lymphocytes than would be found in a conventional marrow harvest. This may be relevant in view of the transplant procedure, since GVHD is sustained mainly by donor T-lymphocytes. However, the specific role of the number of T-lymphocytes is debated; it has been correlated with the incidence of GVHD by some investigators,^{41,42} but not by others.^{43,44}

Interestingly, the PBSC allogeneic transplants reported so far,¹³⁻¹⁵ (Dipersio unpublished, Buckner unpublished) were not complicated by an increased incidence or severity of GVHD.

Our donors tolerated the rhG-CSF treatment and the apheretic procedures well. Fatigue and back pain were the most frequent complaints (4 and 3 donors, respectively). Apheretic procedures were always run on an outpatient basis and none of the donors interrupted the program. However, mild to moderate thrombocytopenia developed in all of them following the apheretic collections. Platelet counts reached their nadir on days 6 to 9 (median 7) following the start of the mobilization/collection procedure, but in no case did thrombocytopenia cause even mild bleeding. In this case thrombocytopenia seems to be the result of the combined action of rhG-CSF administration and

platelet removal by the cell separator. A dose-dependent depression of platelet counts has been described after several days of treatment with rhG-CSF,³⁹ and a comparison between rhG-CSF-treated and non-treated granulocyte donors suggests a role for rhG-CSF in the development of thrombocytopenia.²⁵ In our study, when the collections were run with the CS-3000 cell separator, we observed a more pronounced thrombocytopenia than with the AS-104 apparatus. One way to reduce platelet withdrawal might be to limit apheresis collections to a single procedure³⁶ or to use lower daily doses of rhG-CSF.³⁹

In conclusion, the schedule of rhG-CSF 16 µg/kg for 4 days produces a sharp increase in PB WBC and progenitors, which can easily be collected by apheresis on days 4 and 5 and employed for allogeneic transplantation. Thrombocytopenia in the donor may be relevant, but changing the rhG-CSF schedule and dosage, reducing the number of apheresis procedures and reinfusing autologous platelets into the donor are expected to minimize this problem.

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