PMCA-immunoreactive band of about 140 kDa was detected in platelet lysates; however, the amount of PMCA detected in platelets from NIDDM patients was found to be 26.7±7.9% lower than that in platelets from controls (Figure 2A; $p<0.05$; n = 5).

We have previously shown that activation of platelets causes rapid tyrosine phosphorylation of PMCA.[7] Consistent with this, treatment with TG + ionomycin increased the phosphotyrosine content of PMCA by 418.2±35.2% of basal (Figure 2B). Here we show for the first time that PMCA tyrosine phosphorylation was significantly higher in platelets from NIDDM patients than in platelets from controls both in resting conditions (185.6±16.8% of platelets from healthy donors) and after stimulation with TG + ionomycin (721.8± 45.9% of resting control platelets; Figure 2B, $p<0.05$; n=3). These findings suggest that platelet PMCA activity in NIDDM patients might be reduced by tyrosine phosphorylation.

We conclude that the lower expression and increased phosphotyrosine content of platelet PMCA in NIDDM subjects result in decreased Ca^{2+} extrusion, which might explain the altered ionic homeostasis described in insulin-resistant conditions.[10]

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Stem Cell Transplantation

Granulocyte-colony stimulating factor after autologous CD34+ immunoselected peripheral blood stem cell transplantation

Granulocyte colony-stimulating factor (G-CSF) can be administered after a peripheral blood stem cell transplantation with the aim of accelerating neutrophil recovery. In a randomized, single-blind study we studied a new administration schedule of G-CSF in this context.

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High-dose chemotherapy with autologous peripheral blood stem cell transplantation has been used for the treatment of several malignancies because of the more rapid hematopoietic recovery than after autologous bone marrow transplantation (ABMT), and also because it requires less supportive care and a shorter period of hospitalization.[1] CD34+ cell selection has been developed in order to reduce contaminating neoplastic cells in the graft; on the other hand this procedure eliminates hematopoietic precursors from leukapheresis products, consequently causing a slower hematopoietic recovery than that following unfractionated PBSCT. Since the incidence of infection is proportional to the duration of neutropenia,[2] several measures directed at shortening the duration of neutropenia have been evaluated. Myeloid growth factors (G-CSF and GM-CSF) were introduced into clinical practice to accelerate neutrophil recovery after PBSCT although there is no consensus about their indications and schedules of administration.[3] In order to reduce costs and drug exposure, several researchers[4-6] have evaluated different schedules of G-CSF administration after unmanipulated PBSCT; they compared delayed (day +3 to day +7) to early (day +1) administration; these studies reported contradictory results for the end-points considered, such as time to neutrophil recovery, antibiotic therapy, hospital stay, infections and G-CSF use.

In our experience, G-CSF, administered from day +1 after CD34+ PBSC infusion, significantly improved granulocyte recovery, approaching the results observed after unmanipulated PBSCT, while its late administration (day +7) induced a significant delay in reticulocyte recovery, a decrease of the high fluorescent reticulocytes (HFR)% peak, a delay in platelet and hemoglobinn recovery, and an increase of requirements of packed red blood cell units (pRBCu) and single donor platelet units (SDu).[7]

On the other hand, early daily G-CSF administration resulted in striking and, probably excessive G-CSF increase in serum levels (7-12 fold greater than those in untreated patients in the same setting).[8] Therefore we designed a clinical trial in order to verify the likelihood of a safe reduction of G-CSF combined with the advantages of early (day +1) administration. The trial design was a randomized single blind study evaluating early daily G-CSF administration vs every other day G-CSF, extending the interval between the doses to 48 hours. From April 1999 to September 2002 we enrolled 33 consecutive patients submitted to immunoselected CD34+ PBSCT. The patients were allocated randomly into two groups in a 1:1 ratio. Group A patients were assigned to receive G-CSF (lenograstim, rHuG-CSF, Chugai-Rhone-Poulenc Rorer) 263 μg/day standard dose from day +1; group B received G-CSF 263 μg/day standard dose from day +1 on alternate days. Given the low number of patients submitted to CD34+ immunoselected PBSCT, patients were not stratified (Table

Table 2. Results.

End points	*Group A (quartile values)*	*Group B (quartile values)*	*p*
N. of CD34+ cells $\times 10^6$/kg infused	3.94 (2.6-8.3)	5.1 (3.1-11.5)	0.78
Weight of patients	63 (54.5-76)	70 (60-83)	0.29
Days to PMN >0.5×10^9/L	10.5 (9-11.2)	11 (9-11)	0.59
Days to PMN >1×10^9/L	11 (10.7-12.2)	11 (10-12)	0.81
Days of PMN <0.1×10^9/L	4 (4-5)	4 (4-6)	0.63
Days to Ret count>1%	13 (11.2-14.7)	14 (14-14)	0.15
Days to Hb >10 g/dL	60 (30-70)	31 (24-60)	0.76
Days to Plts >50×10^9/L	16 (13.7-18.2)	16 (14.7-23.2)	0.9
Days to Plts >100×10^9/L	27 (19-65)	24.5 (18.2-91.2)	0.55
Days of fever >38°C	3 (1.7-5)	3 (0-4)	0.63
Days of NPA	8 (5.75-10)	10 (7-12)	0.14
Days of hospitalization	22 (18.7-24.2)	20.5 (19.7-22)	0.71
N. of pRBCu	0 (0-1)	0 (0-2)	0.37
N. of single donor platelet unit	1 (0.87-1)	1 (0-2)	0.59
N. of G-CSF vials administered	11 (10-13)	6 (6-6)	<0.0001
Median cost of G-CSF treatment	572 (520-676)	312 (312-312)	<0.0001

All results are expressed as a median value.

1). We evaluated the following clinical outcomes: time to neutrophil recovery (0.5×10^9/L and 1.0×10^9/L), number of days with granulocytes <0.1×10^9/L, time to platelet recovery > 50×10^9/L and >100×10^9/L, time to lymphocyte recovery (0.5×10^9/L), duration of hospitalization, duration of non-prophylactic antibiotic (NPA use), number of days with fever > 38°C, incidence of sepsis, number of G-CSF vials administered, reticulocyte recovery (>1%), time to unsupported hemoglobin Hb >10 g/dL, number of pRBCu and SDu infused. The number of patients allocated allowed our study to have 60% power to detect a 1 day difference in neutrophil engraftment. Statistical analyses were performed using *GraphPad Prism* software (GraphPad Software Inc., San Diego, CA, USA). The Mann-Whitney U test was used to analyze continuous factors and the χ^2 test was chosen for the analysis of categorical factors. Statistical significance was defined as p <0.05. The results of hematopoietic recovery were calculated according to the Kaplan and Meier method.

Table 1. Characteristics of patients.

Characteristics	*Group A*	*Group B*
Number of patients	16	17
Sex		
Male	10	11
Female	6	6
Median age	48 (range 17-61)	44 (range 18-62)
Disease		
Hodgkin's disease	1	3
Non Hodgkin's lymphoma	9	8
Multiple myeloma	6	6
Conditioning regimen		
BEAM	1	3
BuMel	6	7
HDMel	6	6
TTMel	–	1
Bucy2	2	–
BuTTMel	1	–

According to the limited power of our study, hematopoietic recovery and other clinical outcomes analyzed were absolutely superimposable between the two adopted G-CSF administration schedules (Table 2). Obviously, every other day administration considerably reduced the number of G-CSF vials administered (11 vs 6 vials), thus decreasing both the procedure's cost and drug exposure. Given that every other day administration produced equivalent clinical results, this schedule is a better use of resources.

Concerning drug exposure, clinical studies have demonstrated the potential role of G-CSF in promoting the growth of leukemia cells;[9] there is a theoretical possibility that G-CSF might induce or stimulate an abnormal or malignant clone, eventually leading to overt leukemia.[10]

Further trials are required to confirm the optimal dose and schedule of G-CSF administration after CD34$^+$PBSCT. In the meantime, pegylated G-CSF (pegfilgrastim), in which the pegylation increases the half-life of the protein, is currently being tested at our Institution after autologous peripheral blood stem cell transplant.

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Key words: CD34$^+$PBSCT, G-CSF administration schedule, cost evaluation.

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Stem cell transplantation

CD34+ cell dose predicts costs after autologous peripheral blood stem cell transplantation for breast cancer

We assessed the effect of CD34+ cell dose on costs in breast cancer patients undergoing autologous peripheral blood stem cell (PBSC) transplantation. Mean hospitalization costs were 26,992.9±9582.9 for patients receiving a CD34+ cell dose <5×10⁶ cells/kg versus 22,339.4± 5471.1 for those receiving >5×10⁶ CD34+ cells/kg (p=0.0065).

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Several studies have evaluated the use of high-dose chemotherapy followed by autologous hematopoietic cell transplantation (HCT) in primary high-risk or metastatic breast cancer.[1-3] Similarly, the association between CD34+ cell dose and hematopoietic recovery has been previously examined.[4] However, although the impact of CD34+ cell dose on costs has been previously assessed in other malignancies,[5,6] such a study has never been published in breast cancer patients. To assess this question, 55 women with high-risk primary or metastatic breast cancer transplanted with autologous PBSC after a standard Stamp V regimen were included. The protocol was approved by the Ethics Committee at the University of Liège. Patients receiving a CD34+ cell dose of less than 5×10⁶ cells/kg were included in group 1 (n=13) and those receiving a CD34+ cell dose 5×10⁶ cells/kg in group 2 (n=42). Progenitor cells were mobilized with an intensified

Table 1. Patients' characteristics and clinical parameters.

	Group 1	*Group 2*	*p value*
Age (years)	47±7	45±8	NS
Weight (kg)	66±10	67±13	NS
Body surface area (m^2)	1.7±0.1	1.7±0.1	NS
ECOG performance status : N(%)			NS
0	5 (38)	17 (40)	
1	8 (62)	25 (60)	
Disease: N (%)			NS
Adjuvant	7 (54)	23 (55)	
Metastatic	6 (46)	19 (45)	
Prior radiation therapy : N (%)			NS
Yes	4 (31)	13 (31)	
No	9 (69)	29 (69)	
Number of previous lines of chemotherapy	1.5±0.5	1.4±0.7	NS
Graft composition			
NC (×10⁸/kg)	3.9±2.1	9.2±31.5	NS
CD34+ cells ×10⁶/kg)	2.6±1.1	12.6±9.5	<0.0001
CFU-GM (×10⁴/kg)	42.9±28.8	159.4±112.3	<0.0001
BFU-E (×10⁴/kg)	62.7±41.5	295.7±245.2	<0.0001
CFU-Mix (×10⁴/kg)	4.6±4.1	26.7±19.1	<0.0001
Median time (days) to achieve :			
Neutrophil count > 0.5×10⁹/L	10	9	<0.001
Neutrophil count > 1.0×10⁹/L	10	9	<0.001
Platelet count > 20×10⁹/L	12	9	<0.001
Platelet count > 100×10⁹/L	49	14	<0.001
Reticulocytes > 1%	13	11	<0.001
Median time to last RBC transfusion	10	7	0.003
Median time to last platelet transfusion	9	8	0.029
Median time to hospital discharge	14	12	0.049
Days of G-CSF administration	14±3	10±1	<0.001
Number of platelets transfusions	5±6	3±4	0.0516 (NS)
Number of RBC transfusions	6±6	2±2	0.0027
Number of days of hospitalization	17±8	13±5	0.0098

Mean±standard deviation unless otherwise specified.