ANTICANCER RESEARCH *16:* 3089-3096 (1996)

Mobilisation of Blood Progenitor Cells with Ifosfamide and Etoposide (VP-16) in Combination with Recombinant Human G-CSF (Filgrastim) in Patients with Malignant Lymphomas or Solid Tumours

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Abstract. The mobilisation characteristics of ifosfamide and etoposide followed by Granulocyte Colony-Stimulating Factor fGCSF, filgrastim) were analysed in 17 patients with malignant lymphoma and 24 patients with solid tumours, with respect to the optimum time to harvest progenitor cells and to the yields of progenitor cells that could be achieved. In addition, we analysed patient characteristics which could influence the size of the progenitor cell harvest. Clinical parameters which were corelated with the size of the circulating progenitor cells (CPC) harvests were: the dose of G-CSF, dose of if osfamide, sex, age, diagnosis and extent of pretreatment. CPC were mobilised with 3 g/m2 (n=11) or 4 g/m2 (n=30) ifosfamide on day 1 and etoposide 100 mg/m2/day, on days 1-3 i.v., followed by daily s.c. injections withfilgrastim 5 μg/kg (n=26) or 10 μg/kg (n=15) from day 4. The maximal progenitor cell harvest was achieved on either day 12 or day 13 after the start of the ifosfamide/etoposide course. The median number of leukaphereses necessary to harvest the target quantity of 3 × 10⁶ CD34+ cells/kg body weight was 1 (range 1-9). Thirteen/41 (32%) of the patients did not achieve the target yield of 3 × 10⁶ CD34+ cells/kg. By multivariate analysis, the dose of GCSF and prior irradiation were associated with the number of progenitor cells harvested, while all other parameters, induding the dose of if osfamide and number of previous chemotherapy courses, were not. Sixteen patients received two or more mobilisation courses. Despite the fact that the same mobilisation schedule was used, the progenitor cell yields after the first mobilisation course did not predict the results after the subsequent mobilisation courses, indicating that unknown transient factors may significantly influence the CPC yield.

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Key Words: Stem cell mobilisation, ifosfamide, etoposide, G-CSF, CD34, bone marrow recovery

Circulating progenitor cell transplantations (CPCT) are increasingly used instead of bone marrow transplantations for autografting patients with malignant diseases to circumvent the myelotoxic effects of high-dose chemotherapy (1-5).

Efficient mobilisation of progenitor cells can be achieved by the use of conventially dosed chemotherapy in combination with haematopoietic growth factors such as Granulocyte Colony Stimulating Factor (G-CSF) and Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) (6-8).

The potential clinical advantages of mobilisation with chemotherapy are the reduction of tumour bulk as well as an increase in the progenitor cell content of the autograft as compared with mobilisation by haematopoietic growth factors alone (1-8). The chemotherapy regimen used for mobilisation may be one of the factors that determines the mobilisation pattern of the circulating progenitor cells (CPC) into the peripheral blood. As single agents, high-dose cyclophosphamide and etoposide have been reported to be the best mobilisers (9), although sufficient numbers of CPC could also

be harvested with other chemotherapy regimens (8,10).

Here we report our experience with a mobilisation regimen consisting of a combination of ifosfamide and etoposide followed by G-CSF in patients with recurrent or refractory germ cell cancer, Hodgkin's disease or non-Hodgkin's lymphoma (NHL). Ifosfamide and etoposide are both agents known to be effective in these patients (11-16). Ifosfamide was used instead of cyclophosphamide based on the claim that it is more effective in certain diseases, such as germ cell cancer (17), although this premise has recently been challenged (18). Ifosfamide, like cyclophosphamide, has been reported to be an effective mobilising agent (8).

We found that there was good correlation between the number of granulocyte/macrophage colony forming units (CFU-GM) and the number of CD34+ cells harvested (19,20). We therefore used the CD34+ assay as the most important parameter in this study to monitor and guide the CPC harvests, because this method is simpler than the CFU-GM determinations and the results are known within a few hours instead of two weeks (2,19).

We have analysed our experience with this mobilisation regimen to answer the following questions. First, we wished to determinate the mobilising properties of the ifosfamide/etoposide regimen used in combination with G-CSF with respect to the best time to harvest progenitor cells and the yields of CD34+ cells that could be achieved. In addition, we evaluated individual patient properties which could influence the size of the harvest of progenitor cells.

The rate of haematological reconstitution following the high-dose chemotherapy in relation to graft size and subsets of CD34+ cells have been reported elsewhere (19,20) and will not be repeated in this article.

Patients and Methods

Patients and schedule. A total of 41 patients were included in the study. Eleven patients received 3 g/m^2 ifosfamide i.v. on day 1, etoposide 100 mg/m^2 i.v. on days 1, 2, 3 followed by G-CSF (filgrastim, Neupogen^R, Amgen-Roche, Breda, The Netherlands) 5 μg/kg/day by s.c. injection from day 4 till the day of the last leukapheresis. Thirty patients received 4 g/m^2 ifosfamide on day 1 i.v., etoposide 100 mg/m^2 i.v. on day 1, 2 and 3, followed by either 5 μg/kg (15 patients) or 10 μg/kg (15 patients) G-CSF s.c. from day 4 till the day of the last leukapheresis. The ifosfamide was given in combination with mesna.

All patients scheduled to undergo high-dose chemotherapy with stem cell support, were required to have adequate renal function (creatinine clearance > 60 ml/minute), adequate hepatic function (bilirubin < 25 μmol/l, ASAT < 60 U/I) and adequate bone marrow function (white blood cell count > 3.5 × 10^9/l, platelets > 100 × 10^9/l).

All patients had WHO performance status 0 or 1. The patients' characteristics are shown in Table I.

The study was approved by the medical ethical and scientific review committees of the Netherlands Cancer Institute. Informed consent was obtained from all patients.

CPC harvest and cryopreservation procedure. From the 8th day of G-CSF administration, the percentage of CD34+ cells was determined daily. As soon as the white blood cell count (WBC) exceeded 3 × 10^9/l and a consistent rise in the percentage of CD34+ cells was observed, the leukaphereses were started and continued for 1-5 consecutive days depending on the number of CD34+ cells harvested and on the continued presence of CD34+ cells in the peripheral blood. The target yield for the harvest was a minimum of 3 × 10^6 CD34+ cells/kg for each transplantation procedure, since it is known that with this quantity rapid and sustained bone marrow recovery can be achieved (19,20,21). A second mobilisation procedure was performed and/or bone marrow was harvested if this target yield was not obtained. Prior to the mobilisation procedure, a double lumen Hickman catheter (13.5 French) was inserted in the subclavian or femoral vein. The leukaphereses were performed on an outpatient setting with a continuous flow blood cell seperator (Fenwall CS3000, Baxter, Utrecht, the Netherlands). The total blood volume processed in each session was 101 at a flow rate of 50-70 ml/minute.

The cell yield, containing more than 95% mononuclear cells (MNC) in most patients, counted and the CD34+ cell percentage was determined by flow cytometry. The number of CFU-GM was measured only in cases of a poor mobilisation result or in patients for whom

Table I. *Patients' characteristics.*

Characteristic	G-CSF 5 μg/kg I* : 3 g/m^2	5 μg/kg I : 4 g/m^2	10 μg/kg I : 4 g/m^2
No of patients	11	15	15
Male : female	8 : 3	7 : 8	13 : 2
Diagnosis			
Germ cell tumour	6	4	11
Hodgkin's	3	2	3
NHL, high grade	1	5	0
NHL, low grade	0	3	0
Other solid tumours	1	1	1
Median age in years	36	44	30
Range	20 - 45	24 - 57	17 - 41
Dose G - CSF/body weight in μg/kg			
Median	4.29	4.35	10
Range	3.61 - 6.38	3.66 - 5.26	8.16 - 11.29
Previous chemotherapy			
No, cycles, median	6	8	7
Range	3 - 17	4 - 14	4 - 13
No regimens,median	2	2	2
Range	1 - 3	1 - 4	1 - 3
Radiotherapy			
< 20% bone marrow	1	3	3
>20% bone marrow	1	4	1
Bone marrow involvement (all NHL)			
at any time 1	3	0	
at time of harvest	0	2	0

*I=Ifosfamide

Table II. *CPC harvests after the mobilisation scheme with ifosfamide, etoposide and G-GSF.*

Characteristic	G-CSF 5 µg/kg I* : 3 g/m²	5 µg/kg I : 4 g/m²	10 µg/kg I : 4 g/m²
No. of patients	11	15	15
No. of LF@ necessary to obtain 3 × 10⁶ CD34+ cells/kg			
Median	2	2	1
Range	1 - 5	0 - 9	1 - 9
No. of mobilisation courses necessary to obtain 3 × 10⁶ CD34+ cells/kg			
Median	1	1	1
Range	1 - 3	1 - 3	1 - 3
No. of patients who harvested < 3 × 10⁶ CD34+ cells/kg	1	9	3
Complete failure to mobilise CD34+ cells	0	4	1
Bone marrow harvest required	1	7	2
Start LF@ after course			
Median day after course	12	12	11
Range	9 - 12	10 - 13	11 - 13
Maximal yield CD34+ cells/kg			
Median day after course	13	12	12
Range	11 - 15	12 - 14	11 - 14
Max yield CD34+ cells/kg/LF@			
Median	2.2	3.32	6.5
Range	0.8 - 7.5	0 - 16	0.1 - 13.2

*I = Ifosfamide @LF = Leukapheresis

Table III. *Association between CD34+ cells/kg harvested and clinical parameters.*

	p value
Dose of G-CSF#	0.0172
Ifosfamide dose#	0.6804
Diagnosis	
Germ cell tumour#	0.0008
Hodgkin's disease#	0.2113
Non-Hodgkin lymphomal#	0.0077
Sex#	0 0049
Prior radiotherapy#	0.0009
Prior radiotherapy > 20% bone marrow#	0.0022
Number of prior chemotherapy courses#	0.0088
Age (years)*	0.0018

#Spearman correlation test
*Mann/Whitney/Kruskal Wallis test

Table IV. *The influence of the dose of G-CSF used and irradiation on the CPC harvests.*

G-CSF	RT	No of patients	CD34+ cells × 10⁶/kg harvested	
			Mean ± SD	Range
5 µg/kg	+	9	2.56 ± 4.76	0 - 14.7
5 µg/kg	-	17	8.37 ± 5.12	0.46 - 18.6
10 µg/kg	+	4	5.8 ± 4.86	1.4 - 12.1
10 µg/kg	-	11	14.08 ± 6.32	0.1 - 21.2

RT=radiotherapy
SD=standard deviation

multiple transplantation procedures were planned. The cells were cryopreserved in physiologic saline solution, enriched with 0.1% glucose, 0.38% trisodium citrate, 10% human serum albumin and 10% dimethylsulfoxide (DMSO) at a cell concentration of approximately 50-100 × 10⁶ MNC/ml. For cryopreservation, the cell suspensions were frozen at a controlled rate using a Kryo10 (Cryotech, Schagen, the Netherlands). The frozen cells were stored in the vapor phase of liquid nitrogen until reinfusion.

CD34+ cell counts. Ten ml of EDTA blood was centrifuged to remove the platelet rich plasma, followed by lysis of the pellet in 25 ml of isotonic NH_4Cl for 10 minutes at 0°C. For the leukapheresis material, 1-2 ml NH_4Cl was added to 300 µg leukapheresis cell suspension containing 4-8 × 10⁶ cells. Phosphate buffered saline (PBS) containing bovine serum albumin (BSA) 0.2% (w/v) was added to a final volume of 50 ml, followed by centrifugation and resuspension of tbe pellet in PBS/BSA 0.2% to a cell concentration of 20 × 10⁶/ml. 0.5-1 × 10⁶ cells were incubated for 30 minutes at 4°C with tbe fluorescein isothiocynate (FITC) conjugated monoclonal antibody 8 G 12 (CD34, kindly donated by Dr. P.M. Lansdorp, Terry Fox Laboratory, Vancouver, Canada). When peripheral blood was analysed, incubation with biotinylated CD66 antibody was performed as well to exclude CD66+ granulocytes, thus enhancing the sensitivity of the assay. The cells were incubated with streptavidine-phycoerythrin (streptavidine-PE) for 20 minutes at 4°C. Flow cytometry was performed using a FACScan (Becton and Dickinson, San Jose, USA). For the determination of the percentage CD34+ cells in the leukocyte fraction, at least 20.000 cells were acquired in the list mode; in the mononuclear cell fraction, the percentage of CD34+ cells was determined setting a live gate using a single histogram on PE-negative cells and storing the data of at least 10.000 CD66 negative cells.

Statistical analysis. The Spearman correlation test and the Mann Whitney/Kruskal-Wallis test were used to evaluate the association between clinical parameters and the yield of the progenitor cell harvests, as determined by the number of CD34+ cells/kg body weight harvested. Clinical parameters which were investigated in relation to the results of the progenitor cell harvest were the dose of G-CSF, the dose of

ifosfamide, sex, age, diagnosis and the extent of pretreatment. These parameters were also studied in a multivariate stepwise analysis.

The Spearman correlation test was used to study the relationship between the number of WBC, MNC and the number of CD34+ cells in the peripheral blood and the results of the progenitor cell harvests.

T-tests and the chi^2 test (Cochran Mantel Haenszel) were used to test if there was a difference in pretreatment factors (number of courses of chemotherapy, the extent of radiotherapy).

Results

Ifosfamide/etoposide regimen. The regimen was well tolerated. All patients received ondansetron (8 mg) or granisetron (3 mg) before ifosfamide, and none reported significant nausea or vomiting. Thirty-one patients already had alopecia WHO grade III due to their prior chemotherapy. The remaining 10 patients had hair loss grade II-III due to this regimen. Apart from this, nonhaematological toxicities exceeding WHO grade II were not observed. None of the patients required hospitalisation because of neutropenic fever and none of them progressed, except a single patient with a germ cell tumour. He developed symptomatic brain metastases shortly after the mobilisation procedure.

G-CSF (filgrastim). The G-CSF injections were well-tolerated. Mild bone pain and/or myalgia were the only complaints, which could easily be alleviated with paracetamol.

CPC collection. The maximal yield of CD34+ cells/kg body weight was achieved on either day 12 or day 13 after the start of the ifosfamide/etoposide course (after 8 resp 9 full days of G-CSF administration). The median number of leukapheresis necessary to harvest the target quantity of 3×10^6 CD34+ cells/kg body weight was 1 (range 1-9). The results of the mobilisation are summarised in Table II. The dose of G-CSF, sex, diagnosis, age, pretreatment, were associated with the number of CD34+ cells harvested, when analysed with the Spearman correlation test and Mann Whitney/Kruskal-Wallis test Table III. For this analysis, we used the number of CD34+ cells/kg harvested on day 12 or 13 after the start of the mobilisation course, because at that time the maximal yields were achieved in all patients.

A higher dose of G-CSF was associated with a larger harvest, males had a higher harvest than females, patients with germ cell tumours or NHL had a lower harvest than the other patients, irradiation and number of courses had a negative influence on the progenitor cell harvests. Older patients had lower harvests than younger ones.

The factors in Table III were also studied in a multivariate stepwise analysis. The dose of G-CSF used and prior irradiation were associated with the number of CD34+ cells harvested. All other factors did not improve the model in a significant way (Table IV).

Twenty-one of forty-one patients harvested $\geq 3 \times 10^6$ CD34+ cells/kg in a single leukapheresis. Thirteen of forty-one (32%) patients did not achieve the target yield of 3×10^6 CD34+ cells/kg despite several leukaphereses and mobilisation courses. All these patients were heavily pretreated. Thirteen of the 19 patients (70%) who had received more than 7 chemotherapy cycles and/or had been irradiated mobilised poorly (chi^2 test, $p<0.001$). Patients who mobilised well ($\geq 3 \times 10^6$ CD34+ cells/kg) had received an average of 6.7 chemotherapy cycles prior to the stem cell harvest, the patients who mobilised poorly ($< 3 \times 10^6$ CD34+ cells/kg) had had an average of 9 chemotherapy cycles (t-test, $p=0.0147$). Prior irradiation influenced the CPC harvests in a negative way ($p=0.001$; >20%, as calculated according to the method described in ref.22, of the bone marrow irradiated (6 out of the 13 patients who had been irradiated): $p=0.004$).

Five out of the 13 patients who mobilised poorly were not transplanted: 1 patient because of marker normalisation

Table V. *Characteristics of 14 patients who received 2 subsequent similar mobilisation courses and the results of the harvests.*

	Lower CPC yields after		
	First course	Second course	No CPC harvested
Number of patients	5	4	5
Diagnosis			
Germ cell tumour	3	2	2
Malignant lymphoma	0	1	3
Other	2	1	0
Median age in years	23	37	40
Range	17 - 33	35 - 48	31 - 46
Prior treatment			
Radiotherapy	0	0	3
Number of chemotherapy courses			
Median	8	6	10
Range	3 - 10	5 - 9	6 - 14
Mobilisation schedule			
I* 3 g/m^2; G - CSF 5 μg/kg	2	2	0
I 4 g/m^2; G - CSF 5 μg/kg	0	2	3
I 4 g/m^2; G - CSF 10 μg/kg	3	0	2
Median number and range of CPC harvested in 10^6/kg			
After the first course	1.2	5.6	0
	0.3 - 3.68	1.6 - 6.41	0 - 0.5
After the second course	6.2	0.84	0.4
	2.35 - 12.6	0 - 2.2	0 - 0.99

*I = Ifosfamide

(germ cell tumour), 1 patient with low grade NHL with indolent disease, 3 patients because of disease resistant to subsequent standard-dosed chemotherapy (one patient with germ cell tumour, 2 patients with NHL).

Fourteen patients received a second mobilisation course with ifosfamide and etoposide, in an effort to achieve their target yields of CD34+ cells. Their characteristics are shown in Table V. Surprisingly, 5 patients mobilised considerably better during the second course than during their first, despite the fact that the same regimen and G-CSF dose were used, while four patients mobilised considerably worse (Figure 1).

Five patients mobilised none or only very few progenitor cells: their harvests were less than 1×10^6 CD34+ cells/kg. These patients tended to be older and had received more pretreatment than the other groups (Table V). One female patient with a low grade NHL achieved better CPC harvests with cyclophosphamide and 10 μg/kg G-CSF after a 5-months therapy-free interval than after two mobilisation courses with ifosfamide, etoposide and 5 μg/kg G-CSF (2.8×10^6 CD34+ cells/kg versus no CD34+ cells in circulation).

Two patients (Hodgkin's disease resp. NHL, age 42 resp. 24 years), who were heavily pretreated, received three mobilisation courses. Their harvests contained: 2, 1.7 and 0.2 $\times 10^6$ CD34+ cells/kg; 1.1, 0 and 0.3×10^6 CD34+ cells/kg.

For practical reasons we were interested in peripheral blood counts at the time of the harvest and their predictive value in respect with the number of haematopoietic progenitor cells in the leukapheresis product. As might be expected, the absolute number and percentage of CD34+ leukocytes present in the peripheral blood is the best parameter to monitor and guide the progenitor cell collection (r=0.95 resp.r=0.93, p=0.0001).

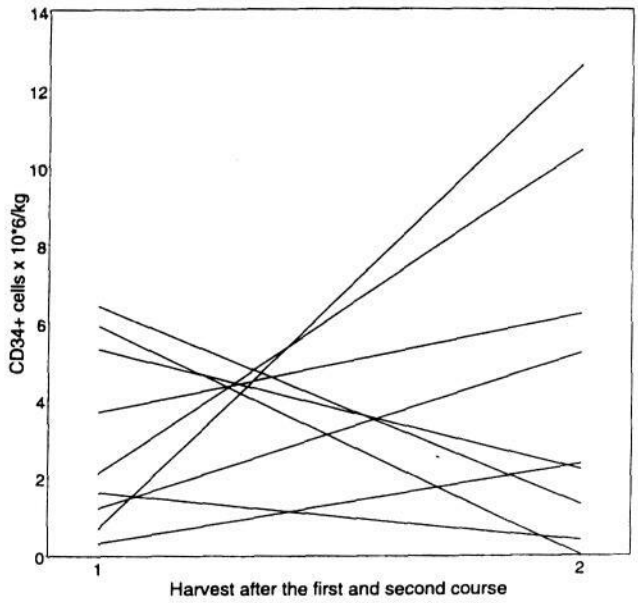

Figure 1. *The stem cell yields of 5 patients who mobilised better and 4 patients who mobilised worse after the second mobilisation course with ifosfamide and etoposide.*

Discussion

In this report we show that in 68% of the patients a sufficient progenitor cell harvest can be obtained for a single or for multiple transplantation procedure with a mobilisation regimen consisting of ifosfamide and etoposide followed by G-CSF. The maximal progenitor cell harvest was achieved on either day 12 or day 13 after the start of the ifosfamide/etoposide course. The number of CD34+ cells harvested correlated with the number of WBC, MNC and CD34+ cells present in the peripheral blood.

In the multivariate analysis, the dose of G-CSF and prior irradiation were significantly predictive of the number of progenitor cells harvested (Table IV), while the dose of ifosfamide, age and the number of chemotherapy courses were not. Nevertheless, the extent of pretreatment with chemotherapy and age were factors that influenced the progenitor cell harvest in a negative way, as shown by the univariate analysis (Table III). Apart from the impact of the factors mentioned, the wide range in the number of CD34+ cells harvested Table IV indicates that there was a considerable inter-individual variation in the ability to mobilise progenitor cells into the peripheral blood.

Thirteen of the forty-one patients did not achieve the target quantity of 3×10^6 CD34+ cells/kg. All had received more than 7 cycles of chemotherapy and/or irradiation. This finding is consistent with the results of other authors (21,23). Damage to progenitor cells due to prior treatment was probably the main cause of the smaller yields. Bone marrow cells of mice, treated with repeated cycles of high-dose cyclophosphamide folloed by other G-CSF or GM-CS caused the loss of their ability to repopulate recipient lymphoid organs, and resulted in a dramatic loss of haematopoietic progenitors and a reduction in marrow repopulating ability (24).

Irradiation of part of the bone marrow might cause more damage to progenitor cells and their micro-environment than chemotherapy, since the multivariate analysis showed that irradiation is the most important factor influencing progenitor cell yields in a negative way.

The fact that 5/14 patients who required 2 mobilisation courses to achieve the target quantity of CD34+ cells/kg (Table V, Figure 1), mobilised better during the second, rather than during the first course without a change in regimen or G-CSF dose, supports the hypothesis that haematopoietic reserve and the mobilisation schedule are not the only factors determining the progenitor cell mobilisation into the peripheral blood. Transient changes in bone marrow function induced by the treatment given prior to the mobilisation regimen or intercurrent diseases like infections might also influence the results of the progenitor cell harvests. Possible changes may include an altered stromal

function, change in expression of adhesion molecules, change in production of and/or an altered reaction to haematopoietic growth factors.

Increasing the dose of G-CSF (filgrastim) from 5 μg/kg to 10 μg/kg by daily subcutaneous injections improved the progenitor cell yields in our study. Since our study was not randomised and the groups of patients receiving either 10 μg or 5 μg/kg filgrastim are not entirely comparable, we cannot draw a definite conclusion from this finding. Nevertheless, this dose response relationship has also been shown for lenograstim in healthy donors (25). A plateau effect at a dose of 10 μg/kg filgrastim by subcutaneous injection for 5 days was noted by Dührsen *et al* (6). At higher doses no further augmentation of CPC was observed. Thus, a daily subcutaneously administered dose of 10 μg/kg filgrastim seems to be the optimal amount for the mobilisation of progenitor cells into the peripheral blood.

In two heavily pretreated patients who failed to mobilise sufficient progenitor cells, we attempted to obtain better stem cell harvests using GM-CSF and Interleukin-3 according to the schedule described in reference 8 instead of G-CSF after the chemotherapeutic regimen. No mobilisation was achieved. These preliminary findings suggest that efforts to improve the progenitor cell harvests by employing other growth factors are probably futile in this patient population and that the total stem cell pool present in the bone marrow is more important for the outcome of the progenitor cell harvests than an altered response to a particular growth factor.

Thirteen patients did not achieve the target quantity of 3×10^6 CD34+ cells/kg, but this was not a reason to refrain from the transplantation procedure. A complete haematopoietic recovery could be achieved with lower CD34+ counts in 3 patients, and 5 other patients were transplanted with a combination of autologous bone marrow and CPC. Five of the 13 patients who mobilised poorly were not transplanted because of progressive disease, marker normalisation or disease stabilisation.

In summary, ifosfamide and etoposide in combination with G-CSF can be used to harvest progenitor cells in patients with germ cell tumours and malignant lymphoma. The regimen is well tolerated and gives a predictable peak of circulating CD34+ cells 12 and 13 days after the start of the treatment. Provided that the patients are not heavily pretreated, the progenitor cell harvests are sufficient, and enough CD34+ cells can be obtained in 1 or 2 leukapherese, for even 2 or 3 transplantation procedures. A poor harvest after the first mobilisation course does not preclude a sufficient harvest after a consecutive course, even with the same mobilisation regimen.

Acknowledgement

We are indebted to the nursing staff of the fourth floor for their excellent care for the patients and the leukapheresis assistants for their dedication and enthusiasm.

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Received March 12, 1996
Accepted May 2, 1996