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Clonogenic potential and phenotypic analysis of CD34+ cells mobilized by different chemotherapy regimens

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ABSTRACT

Background and Objective. **Since limited data concerning quantitative and qualitative differences of CD34+ cells collected after different mobilization schedules are available, we investigated phenotype, proliferative capacity and primitive progenitor cell content of CD34+ cells mobilized with four different regimens.**

Design and Methods. **The number, phenotype, and progenitor cell content of CD34+ cells were investigated in 46 patients mobilized with cyclophosphamide (CY) 7 g/m² plus granulocyte colony-stimulating factor (G-CSF, 5 µg/kg) (CY7+G-CSF) (n=16), CY 4 g/m² plus G-CSF (CY4+G-CSF) (n=8), IVE [ifosphamide (2.5 g/m² for 3 d), etoposide (150 mg/m² for 3 d), epirubicin (100 mg/m² on day 1)] plus G-CSF (IVE+G-CSF) (n=9), or G-CSF (10 µg/kg) alone (n=13).**

Results. **The number of CD34+ cells collected per liter of processed blood was significantly higher in the CY7+G-CSF group than in the CY4+G-CSF and G-CSF groups ($p \leq .005$), but not the IVE+G-CSF group. As compared to patients in the CY4+G-CSF group, those mobilized with CY7+G-CSF and IVE+G-CSF produced significantly lower percentages of CD34+ cells lacking CD38, CD33, CD45RA, and HLA-DR ($p \leq .016$, at least). In addition, CY4+G-CSF mobilized CD34+ cells had a significantly higher plating efficiency than the cells mobilized in other ways ($p \leq .036$). In the G-CSF group, colony-forming cells and long-term culture-initiating cells were significantly lower than in the CY groups ($p \leq .0014$ and $\leq .013$, respectively).**

Interpretation and Conclusions. **Our data demonstrate that: (i) different mobilization regimens allow the collection of CD34+ cells with distinct phenotypic and proliferative features; (ii) evaluation of the absolute number of CD34+ cells by itself is not a reliable indicator of the clonogenic content of blood mobilized with different chemotherapy regimens; (iii) because of the substantial impact that chemotherapy regimens have on the quantity and quality of collected CD34+ cells, anticancer effects and optimal blood progenitor cell yields should be evaluated for each chemotherapy schedule.**

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Randomized and non-randomized clinical trials have shown that the reinfusion of autologous blood progenitor cells (BPC), as compared to bone marrow, leads to faster hematopoietic reconstitution after high-dose chemotherapy.[1-3] Since the majority of conditioning regimens have not been proved to be myeloablative and pluripotent stem cells surviving the conditioning radio-chemotherapy may contribute to post-transplant hematopoiesis, the ability of mobilized BPC to maintain long-term engraftment has been a matter of debate.[4] Nevertheless, a significant correlation between sustained hematologic engraftment and the quantity and quality of reinfused CD34+ cells has been recently reported, indicating that collection of CD34+ cells with high viability and clonogenicity is likely to be crucial for safe engraftment.[5-8]

Number and type of pre-mobilization and pre-transplant chemotherapy schemes, pre-mobilization and pre-transplant radiotherapy, age and marrow involvement have been previously reported to affect the mobilization and reconstituting potential of autologous BPC in patients with both hematologic and non-hematologic malignancies.[6,8-11] Although a number of reports have shown that the mobilization schedule may substantially influence CD34+ cell collection,[10-13] data regarding the effect of different regimens on the qualitative and quantitative aspects of mobilized CD34+ cells are still limited.[14-16]

Hematopoietic growth factor administration following chemotherapy as well as growth factors alone are currently used to mobilize BPC. In addition to cyclophosphamide (CY) in doses ranging from 1.5 to 7 g/m²,[8,15,17] a variety of chemotherapy schemes, including myelosuppressive doses of single agents as well as polychemotherapy regimens, are increasingly used for mobilizing BPC.[5,10,18-20] Although it is accepted that mobilizing chemotherapy exerts a therapeutic effect at the time of mobilization, few data are available regarding the mobilization potential of polychemotherapy regimens as compared to the mobilization potential of schedules using high-dose

monochemotherapy or growth factors alone.

Cytotoxic agents are known to damage both hematopoietic and mesenchymal marrow stem cell compartments,[21-23] thus suggesting that different chemotherapy agents might affect not only the quantity but also the quality of mobilized progenitors, and that careful assessment of collected BPC is required when a given chemotherapy schedule is used for mobilization purposes. The inverse correlation between incidence and plating efficiency of mobilized $CD34^+$ cells as well as the requirement of higher $CD34^+$ cell numbers for delayed engraftment in heavily pre-treated patients strongly confirm the heterogeneity of $CD34^+$ cells whose absolute number might not always reflect the quality of the graft.[24,25] Since the clonogenic potential of $CD34^+$ cells depends on the relative frequency of $CD34^+$ subsets that are not clonogenic *in vitro*, such as mature $CD34^+$ cells, lymphoid progenitors and stem cells,[26] the parallel evaluation of plating efficiency and primitive progenitor content of mobilized $CD34^+$ cells is likely to give a reliable qualitative assessment of BPC engraftment potential.

$CD34^+$ cell subsets lacking CD38, CD33, CD45RA, and HLA-DR expression are considered early hematopoietic cells capable of generating committed progenitors.[27-30] Mobilized $CD34^+CD33^-$ and $CD34^+HLA\text{-}DR^-$ subpopulations are enriched in long-term culture-initiating cells (LTC-IC),[31] that are arguably the most primitive human hematopoietic cells assessable by *in vitro* functional assays.[32] The amounts of reinfused $CD34^+CD33^-$ cells have recently gained clinical importance since they correlate with neutrophil and platelet recovery following autografting.[5] Thus, both the phenotype and the LTC-IC content of blood $CD34^+$ cells may reflect the hematopoietic stem cell content and the reconstitution ability of the grafts.

The aim of this study was, therefore, to compare the number, phenotype, committed progenitor cell content and LTC-IC content of $CD34^+$ cells mobilized by four different regimens; 7 g/m^2 CY plus granulocyte colony-stimulating factor (G-CSF) (CY7+G-CSF), 4 g/m^2 CY plus G-CSF (CY4+G-CSF), IVE (ifosfamide, etoposide, epirubicin) plus G-CSF (IVE+G-CSF) and G-CSF alone.

Design and Methods

Patients, mobilization regimens and harvesting criteria

From January 1996 to December 1997, forty-six consecutive patients (24 males, 22 females) affected by non-Hodgkin's lymphoma (n = 16), multiple myeloma (n = 20) and solid tumors (n = 10) were mobilized using the following regimens: G-CSF (5 µg/kg/day, until completion of leukaphereses) after CY 7 g/m^2 (n = 16), CY 4 g/m^2 (n = 8), or IVE (ifosfamide 2.5 g/m^2 for 3 days, etoposide 150 mg/m^2 for 3 days and epirubicin 100 mg/m^2 on day 1) (n = 9) and G-CSF alone (10 µg/kg/day, until completion of leukaphereses) (n = 13). Clinical characteristics of the patients are summarized in Table 1. Collections were started when circulating $CD34^+$ cells were $\geq 20/\mu L$ in the first three groups and at day +5 of G-CSF administration in patients treated with G-CSF alone. Leukaphereses were continued until at least a total threshold of 5×10^6 $CD34^+$ cells/Kg body weight were collected. Apheretic procedures were performed using a Cobe-Spectra continuous flow cell separator (Cobe Laboratories, Lakewood, Colorado, USA).

Immunophenotype analysis

Cells in EDTA anticoagulated blood and leukapheresis samples were counted, their concentration adjusted to 0.5×10^6/mL by dilution with Dulbecco's phosphate buffered saline without Ca^{++} or Mg^{++} (PBS) and their phenotype determined by immunofluorescence analysis. Cells were incubated for 30 min on ice

Table 1. Clinical characteristics of the patients at mobilization.

Characteristics	*CY7+ G-CSF*	*CY4+ G-CSF*	*IVE+ G-CSF*	*G-CSF*	*p*
No. of subjects *	16	8	9	13	
Age (years)					
median	49	53	57	46	.03
range	30-67	19-62	41-67	17-62	
Males:Females	10:6	4:4	3:6	7:6	
Diagnosis					
non-Hodgkin's lymphoma	8	4	0	4	
multiple myeloma	3	2	9	6	
solid tumor	5	2	0	3	
Bone marrow involvement					
yes	4	2	8	6	
no	6	6	1	5	
ND	4	0	0	2	
Previous CT cycles					
median number	5	3	3	2	.18
range	0-18	0-12	2-4	0-16	
Disease status					
complete remission	0	4	1	4	
partial remission	8	3	7	2	
active disease	6	0	1	6	
stable disease	2	1	0	1	
Days from diagnosis					
median	180	615	398	239	.6
range	45-3,390	120-1,530	70-770	15-1,020	
Days from last CT	66	60	55	65	.8
median	15-960	30-450	30-90	45-210	
range					
Total processed blood (Lt)					
median	13	17	16	15	.33
range	6-28	9.6-20	9.8-26	5.3-26	

*Abbreviations: ND, not determined; CT, chemotherapy; *some patients in the CY7+G-CSF (n = 2), CY4+G-CSF (n = 6), and G-CSF (n = 5) groups have been previously reported (ref. #15) .*

with 10 mL fluorescein isothiocyanate (FITC)- and/or phycoerythrin (PE)-conjugated monoclonal antibodies (MoAb). The following antibody combinations were used: CD4-FITC/CD8-PE; CD45-FITC/CD14-PE; CD38-FITC/CD34-PE; CD33-FITC/CD34-PE; CD45 RA-FITC/CD34-PE; HLA-DR-FITC/CD34-PE. Each fluorescence analysis included a double negative isotype control (IgG1-FITC/IgG1-PE). Directly conjugated MoAb and isotype controls were obtained from Becton Dickinson Immunocytometry Systems (San Jose, CA, USA), except for CD38-FITC and CD33-FITC (Caltag Laboratories, San Francisco, CA, USA). Red blood cells were lysed with FACS lysing solution (Becton Dickinson), washed in PBS, and then acquired in listmode. The threshold was set on the forward scatter (FSC) to exclude cellular debris. A minimum of 40,000 events and a minimum of 100 $CD34^+$ events were analyzed. Analysis was performed by setting two gates: the first gate was set on a plot of CD45 vs CD14 and drawn to include the lymphocyte and monocyte cells. The second gate was set in the plot of FSC vs side scatter (SSC) to include the lymphocyte and monocyte cells. This gate was activated and used to generate a plot of SSC vs anti-CD34 fluorescence intensity. The percentage of positive cells was determined by subtracting the percentage of fluorescent cells in the control from the percentage of cells positively stained with the MoAb. Phenotypic analysis was performed with a FACSort flow cytometer (Becton Dickinson). Data were processed with an Apple Macintosh Quadra 650 computer (Cupertino, CA, USA) using CELLQuest Software.

CFU-MIX, BFU-E, and CFU-GM assay

The assay for CFU-Mix, BFU-E, and CFU-GM was carried out as described elsewhere.[33] Briefly, 1 to 5×10^4 nucleated cells were plated in 35 mm Petri dishes in 1 mL aliquots of IMDM containing: 30% FBS (Stem Cell Technologies); 10^{-4} M 2-mercaptoethanol (Gibco); and 1.1% (w/v) methylcellulose. Cultures were stimulated with interleukin-3 (10 ng/mL, Sandoz), granulocyte colony-stimulating factor (10 ng/mL, Amgen Inc.), granulocyte-macrophage colony-stimulating factor (10 ng/mL, Sandoz) and erythropoietin (3 U/mL, Amgen Inc.). Progenitor cell growth was evaluated after 14-18 days of incubation (37°C, 5% CO_2) in a humidified atmosphere. Four dishes were set up for each individual data point per experiment. CFU-Mix defined as containing at least erythroid and granulocytic cells, BFU-E with 500 cells and CFU-GM with 40 cells were scored from the same dish.

LTC-IC assay

The long-term culture-initiating cell (LTC-IC) assay was performed according to a procedure used by Sutherland *et al*.[29] Briefly, a test cell (5×10^6 nucleated cells) suspension was seeded into cultures containing a feeder layer of irradiated (8,000 cGy) murine M2-10B4 cells ($3\times10^4/cm^2$, kindly provided by Dr. C. Eaves) engineered by retroviral gene transfer to produce human IL-3 and human G-CSF.[34] Test cells were resuspended in complete medium consisting of α-medium (Gibco) supplemented with fetal bovine serum (12.5%), horse serum (12.5%), L-glutamine (2 mM), 2-mercaptoethanol (10^{-4} M), inositol (0.2 mM), folic acid (20 µM) and freshly dissolved hydrocortisone (10^{-6} M). Cultures were fed weekly by replacement of half of the growth medium containing half of the non-adherent cells with fresh complete medium. After 5 weeks in culture, nonadherent cells and adherent cells harvested by trypsinization were pooled, washed, and assayed together for clonogenic cells in standard methylcellulose cultures at an appropriate concentration. The total number of clonogenic cells (i.e., CFU-Mix plus BFU-E plus CFU-GM) present in 5-week old LTC provides a relative measure of the number of LTC-IC originally present in the test suspension.[35] Absolute LTC-IC values were calculated by dividing the total number of clonogenic cells by 4, which is the average output of clonogenic cells per LTC-IC, according to limiting dilution analysis studies reported by others.[36]

Statistical analysis

Four plates were scored for each data point per experiment and the results were expressed as the mean ± SEM. Statistical analysis was performed with the statistical package StatView (BrainPower Inc., Calabasas, CA, USA) running on a Macintosh Performa 6300 personal computer (Apple Computer Inc., Cupertino, CA, USA). Multiple group comparisons were made using the Kruskal-Wallis one way analysis of variance (ANOVA) calculated with the SPSS software (SPSS Inc., Chicago, IL, USA). If a significant difference was established, a Wilcoxon's Rank Sum test was applied (a p value ≤ 0.05 was considered statistically significant).

Results

Forty-six patients receiving either CY7+G-CSF (n = 16), CY4+G-CSF (n = 8), IVE+G-CSF (n = 9) or G-CSF (n = 13) and undergoing a total number of 82 leukapheretic procedures were studied. As shown in Table 1, the four treatment groups were not significantly different in terms of time from diagnosis, time from last chemotherapy, number of chemotherapy cycles before mobilization, and liters of processed blood. Although no major difference was evident among the CY7+G-CSF, CY4+G-CSF, and G-CSF groups in terms of age, diagnosis and percentage of patients with marrow involvement, the former two groups included 50% and <10% patients with active or stable disease, respectively. The IVE+G-CSF group included only patients with multiple myeloma and a median age of 57 years.

$CD34^+$ cells

The incidences of $CD34^+$ cells in peripheral blood and leukapheresis products are summarized in Table 2. Although the absolute number of $CD34^+$ cells per

Table 2. CD34+ cells in peripheral blood and leukapheresis products.

	CY 7+G-CSF (n = 26)	CY 4+G-CSF (n = 18)	IVE+G-CSF (n = 14)	G-CSF (n = 24)
Circulating CD34+ cells per µL				
Mean±SEM	193±32	143±37	140±29	122±17
Range	32-714	11-487	26-336	36-333
CD34+ cells (x10⁷) collected per liter processed blood				
Mean±SEM	9.9±1.9	5.5±1.4*	7.2±1.6	3.5±0.7*°
Range	0.3-39	0.3-21	1.3-17	0.5-14

**Significantly lower as compared to CY7+G-CSF; °significantly lower as compared to IVE+G-CSF. For p values, refer to the text.*

Table 3. Phenotype of CD34+ cells mobilized by different regimens.

	CY 7+G-CSF (n = 26)	CY 4+G-CSF (n = 18)	IVE+G-CSF (n = 14)	G-CSF (n = 24)
Percentage of CD34+ cells lacking				
CD38				
Mean±SEM	4±1.6*	21±7	3.3±0.9*°	14±3
Range	0-22	0-70	0-9.4	0-64
CD33				
Mean±SEM	22±6*	59±10	48±9.6*	37±6
Range	0-106	0-100	2.7-110	0-100
CD45RA				
Mean±SEM	17±8*	63±10	5.2±1.5*°	21±5*
Range	0-83	9-100	0-21	0-94
HLA-DR				
Mean±SEM	1.3±0.5*°	9±2.5	3.2±1*°	18±3
Range	0-6.1	0-29	0-8.9	0-58
Yield per liter processed blood				
CD34+CD38- (×10⁶)				
Mean±SEM	5.6±2.4	24±11	2±0.4	3.4±1
Range	0-33	0-133	0-4.4	0-19
CD34+CD33- (×10⁶)				
Mean±SEM	32±9.5	50±18	38±10.8	10±2
Range	0-170	0-197	1-112	0-33
CD34+CD45RA- (×10⁶)				
Mean±SEM	41±24	49±17	4.1±1.5*	8±2
Range	0-316	0-181	0-17.1	0-300
CD34+HLA-DR- (×10⁶)				
Mean±SEM	2.3±1.4	4.2±1	2.2±0.5	8±2.6
Range	0-21	0-13	0-5.8	0-386

**Significantly lower as compared to CY4+G-CSF; °significantly lower as compared to G-CSF. For p values, refer to the text.*

µL was higher in the CY7+G-CSF group this difference did not reach the level of statistical significance ($p \leq .26$). The mean number of CD34+ cells collected per liter of processed blood from the CY7+G-CSF mobilization group was significantly higher than that from either the CY4+G-CSF or G-CSF group ($p \leq .005$), but not the IVE+G-CSF group.

Phenotypic analysis of CD34+ cells

The percentages and yields of CD34+ cell subsets per liter of processed blood in the differently treated groups are shown in Table 3. As compared to the CY4+G-CSF group, patients mobilized with CY7+G-CSF and IVE+G-CSF produced a significantly lower mean percentage of CD34+ cells lacking CD38 ($p \leq .02$), CD33 ($p \leq .016$), CD45RA ($p \leq .0001$), and HLA-DR ($p \leq .0002$). In addition, the percentages of IVE+G-CSF-mobilized CD34+ cells lacking CD38, CD45RA and HLA-DR were significantly lower than those of G-CSF-mobilized CD34+ cells. G-CSF mobilized CD34+ cells had significantly higher HLA-DR expression than those mobilized by CY7+G-CSF.

Mean absolute numbers of CD34+CD38- and CD34+CD33- cells collected per liter of processed blood were higher in the CY4+G-CSF group, but differences were not statistically significant ($p \leq .86$ and $\leq .1$, respectively). Mean absolute number of CD34+CD45RA- cells collected per liter of processed blood was significantly higher in the CY4+G-CSF group than in the IVE+G-CSF group ($p \leq .03$). No significant difference was found in the number of CD34+HLA-DR- cells collected per liter of processed blood mobilized by the different regimens ($p \leq .1$).

Colony-forming cells

Committed progenitors in leukapheresis products are summarized in Table 4. The incidence of CFU-Mix per 5×10^4 nucleated cells was significantly higher in the CY4+G-CSF group than in the G-CSF group ($p \leq .004$). The incidences of BFU-E, CFU-GM and CFC were significantly lower in the G-CSF group than in the other groups ($p \leq .0002$, $\leq .0001$ and $\leq .0001$, respectively). Mean absolute numbers of BFU-E, CFU-GM and CFC collected per liter of processed blood were significantly lower in the G-CSF group than in the other groups ($p \leq .04$, $\leq .0009$ and $\leq .0014$, respectively). To calculate the plating efficiency of the CD34+ cells, CFC concentration was divided by the number of plated CD34+ cells. As shown in Table 5, CY4+G-CSF mobilized CD34+ cells had a significantly higher plating efficiency than cells mobilized by the other regimens ($p \leq .036$).

Long-term culture-initiating cells

The growth of LTC-IC was investigated in leukapheresis products obtained after mobilization with CY7+G-CSF (n = 18), CY4+G-CSF (n = 16), IVE+G-CSF (n = 8) and G-CSF (n = 18) (Table 6). In the G-CSF group, LTC-IC incidence per 2×10^6 nucleated cells (38±17) was significantly lower than in other groups ($p \leq .0001$), and the absolute number of LTC-IC collected per liter of processed blood was significantly lower than the numbers collected from the CY7+G-CSF and CY4+G-CSF groups ($p \leq .013$). The number of LTC-IC collected in the IVE+G-CSF group was also low, although not statistically different from the yield in the CY groups, probably because of the limited number of samples which were analyzed.

Table 4. Clonogenic cells collected after different mobilization regimens.

	CY 7+G-CSF (n = 26)	*CY 4+G-CSF (n = 18)*	*IVE+G-CSF (n = 14)*	*G-CSF (n = 24)*
Concentration per 50,000 nucleated cells				
CFU-Mix				
Mean±SEM	7±2	8±2 *	4±1	2±0
Range	0-26	1-24	0-8	0-8
BFU-E				
Mean±SEM	85±14	62±12	67±20	17±4°
Range	0-201	5-164	2-305	0-60
CFU-GM				
Mean±SEM	212±30	162±21	145±42	28±5°
Range	0-551	41-294	12-692	2-80
CFC				
Mean±SEM	305±41	231±29	216±62	47±9°
Range	0-649	50-451	14-942	3-133
Yield per liter processed blood				
CFU-Mix ($\times 10^4$)				
Mean±SEM	24±5	40±14	13±3	14±5
Range	0-94	1-194	0-28	0-91
BFU-E ($\times 10^5$)				
Mean±SEM	36±8	31±8	22±6	13±3°
Range	0-145	1-124	1-100	0-62
CFU-GM ($\times 10^6$)				
Mean±SEM	11±3	8±2	5±1	2±0.6°
Range	0.001-57	0.6-29	3-21	0.6-15
CFC ($\times 10^6$)				
Mean±SEM	15±3	12±3	8±2	4±1°
Range	0.001-67	0.7-36	0.3-31	0.3-22

**Significantly lower as compared to CY4+G-CSF; °significantly lower as compared to G-CSF. For p values, refer to the text.*

Table 5. Plating efficiency of CD34+ cells collected after different mobilization regimens.

	CY 7+G-CSF (n = 26)	*CY 4+G-CSF (n = 18)*	*IVE+G-CSF (n = 14)*	*G-CSF (n = 24)*
Mean±SEM	15±2	26±4 *	13±2	15±3
Range	1.5-39	3.3-74	0.4-27	1-48

**Significantly higher as compared to CD34+ cells mobilized by other regimens. For p values, refer to the text.*

Table 6. LTC-IC collected after different mobilization regimens.

	CY 7+G-CSF (n = 18)	*CY 4+G-CSF (n = 16)*	*IVE+G-CSF (n = 8)*	*G-CSF (n = 18)*
Concentration per 2x10⁶ nucleated cells				
Mean±SEM	352±90*	471±193*	94±23*	38±17
Range	10-1,402	2-2,608	3-182	1-312
Yield per liter processed blood				
Mean±SEM	53±14*	72±39*	9±3	10±4
Range	0.2-173	0.1-63	0.2-20	0.2-62

**Significantly higher than in G-CSF group. For p values, refer to the text.*

Discussion

The expanding use of autologous BPC for hematopoietic rescue after high-dose chemotherapy has increased the interest in factors influencing the quality of collection and the mechanisms of progenitor cell mobilization in order to allow the optimization of disease-specific mobilization strategies.[37] Although several lines of evidence suggest that chemotherapy and growth factors might influence mobilized progenitor cells at the functional level, limited data concerning the quantitative and qualitative differences of CD34+ cells collected by using different mobilization regimens are available.[10,12,13-15,17,19,38,39]

In this study, we compared phenotype, proliferative capacity and primitive progenitor cell content of circulating CD34+ cells obtained by using four different mobilization regimens. We demonstrate that CY4+G-CSF mobilizes CD34+ cells with a primitive phenotype which parallels their high plating efficiency and LTC-IC content. In contrast, CD34+ cells mobilized by IVE+G-CSF reveal a more mature phenotype and a low LTC-IC content.

As previously reported, when G-CSF is administered after cytotoxic chemotherapy, the yields of blood-derived CD34+ cells and primitive and committed hematopoietic progenitors are higher than after G-CSF administration as a single mobilizing agent,[10,13,15,40,41] probably because of the mobilization of mature or lymphoid progenitors. Variable numbers of primitive and committed progenitors collected by different chemotherapy regimens strongly suggest that distinct regimens mobilize different amounts of lineage marker negative CD34+ cells that are functionally heterogeneous and variably enriched in LTC-IC.

According to previous reports,[17,41] administration of 7 g/m^2 CY followed by G-CSF resulted in the collection of higher numbers of CD34+ cells and CFU-GM as compared to 4 g/m^2 CY followed by G-CSF. As reported herein, the latter schedule mobilized CD34+ cells with higher plating efficiency and a greater proportion of both primitive CD34+ cell subsets and LTC-IC. CY has been demonstrated to damage the stem cell compartment in a dose-dependent manner in mice.[22] It is likely that a higher toxic effect of 7 g/m^2 CY on primitive cells results in a shift from early to more committed progenitors within the CD34+ cell fraction, leading to the collection of relatively more myeloid progenitors than stem cells, as assayable by the LTC-IC culture system. The CY7+G-CSF regimen seems to be recommendable in patients with large tumor bulk and/or refractory multiple myeloma.[17] Indeed, hospital admission rates of 100%

and 44% were reported in patients mobilized with CY at doses of 7 g/m^2 and 4 g/m^2, respectively, with a single death in each group and with 20% of patients in each group requiring platelet transfusions.[42] Addition of growth factors to a 7 g/m^2 CY mobilization regimen reduced toxicity, but 13% of patients still required platelet transfusions.[43] Although the results of mobilization with CY 1.5 g/m^2 plus G-CSF have been reported to be satisfactory, adequate mobilization fails to be achieved in a consistent percentage of pretreated patients,[8] and higher doses of CY are known to give rise to higher progenitor cell yields.[44] However, increasing the dose of CY is associated with significant morbidity and occasional mortality.[42,45-47] Assuming that CY4+G-CSF improves the quality of mobilized blood progenitor cells, such a schedule should be preferred to enhance release of primitive progenitors in heavily pre-treated patients with reduced bone marrow reserve.

Recently the IVE regimen has been demonstrated to be more effective than intermediate doses of CY for BPC mobilization in malignant lymphomas.[20] In our study, similar numbers of CD34$^+$ cells and CFC were collected in the IVE and CY groups, with the LTC-IC content being lower in the IVE-mobilized than in the CY-mobilized patients. The apparent discrepancy between our data and those of others may be the consequence of either differences in patient populations or drug dosages employed in the IVE schedules. As compared to McQuacker *et al.*,[20] we used lower ifosfamide and etoposide doses causing a mild myelosuppression and, in turn, a less potent mobilizing effect.[17,41,44] Moreover, we used a higher dose of epirubicin than that previously employed by others.[20] Since anthracyclin-induced myelosuppression has recently been suggested to cause selective damage of marrow stromal/stem cell compartment[21,48] and repeated cycles of anthracycline-containing regimens result in progressive, far lower collections of LTC-IC than of committed progenitors,[18] the poorer mobilization potential displayed by our IVE regimen might be explained by a higher anthracycline-related toxicity on the bone marrow.

A variety of mechanisms are likely to be involved in the transient release of progenitors into peripheral blood. Growth factors modulate cell adhesion molecule expression, extracellular matrix metabolism and ligand-receptor systems supporting progenitor cell adhesion to stroma.[49] Chemotherapy is known to induce the disruption of the marrow endothelial cell barrier.[50,51] Different mobilization patterns displayed by chemotherapy-based regimens with different cytoreducing potentials suggest agent- and dose-dependent mobilization effects via the degree of induced myelosuppression. The higher mobilization of LTC-IC and clonogenic progenitors by CY-based regimens suggests that marrow aplasia might enhance BPC mobilization by affecting cell cycle induction and rate of primitive cells. In addition, possible toxic effects of mobilizing agents or doses on the stromal/stem cell compartment might be relevant in determining the mobilization pattern of primitive progenitors.

In our study age, sex, diagnosis and disease status were heterogeneously distributed among the analyzed mobilization groups. In particular, < 10% of CY4+G-CSF-mobilized patients had active or stable disease vs 50% of those mobilized with CY7+G-CSF, and time from diagnosis to mobilization was longer (although not significantly) in the CY4+G-CSF group as compared to the CY7+G-CSF group. Although only previous chemotherapy and schedule have been clearly demonstrated to affect the release of primitive progenitors into the peripheral blood,[15,18] the detected superiority of the CY4+G-CSF regimen to mobilize CD34$^+$ cells with immature phenotype and LTC-IC could in part depend on the better clinical status of patients at the time of mobilization. The possible influence of pre-mobilization characteristics on both the phenotype and functional properties of mobilized CD34$^+$ cells as well as the small number of analyzed patients make any firm conclusion difficult and further evidence is needed to confirm our findings.

Although based on a retrospective analysis of a limited number of patients, our data suggest that mobilization efficiency of the examined regimens varies widely. Priming with CY plus G-CSF gives better results than priming with other regimens, especially administration of G-CSF alone. In addition, our data strongly support the relevance of functional analysis of CD34$^+$ cells when a new mobilizing regimen is introduced into clinical practice. While further knowledge of the mobilization mechanisms will help to improve the number and composition of harvested CD34$^+$ cells as well as the dosages and administration modalities of mobilizing agents, an ideal priming regimen balancing anticancer effect and optimization of BPC yield is likely to differ between various groups of patients.

Contributions and Acknowledgments

CCe: conception and design of the study, statistical analysis and interpretation of data, patients' assessment, writing of the manuscript. ER: clonogenic assays, LTC-IC assay, data collection. DG: cytofluorimetric analysis, data collection. CCa and LM: patients' assessment, critical revision of the manuscript. VR: critical revision and final approval of the version. The order of the authors reflects their contribution. The authors wish to thank Dr. Carmelo Carlo-Stella for important conceptual and laboratory contributions.

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Disclosures

Conflict of interest: none.

Redundant publications: yes, <50%: we have previously published (Bone Marrow Transplant 1998; 21:561-8) a similar analysis focusing the effects of cyclophosphamide + G-CSF alone on mobilized CD34+ cells.

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