out of 19 normal cases and 0/9 ITP cases did more than 10% of Mks express CD34; in contrast, 10 of 22 patients with MDS had 10% or more CD34+ Mks in their BM (Fisher's exact test: p=0.004 and 0.03, MDS versus normal controls and ITP, respectively); in three MDS cases more than 50% of Mks expressed CD34 and in a single case the vast majority of them were CD34 positive (Figure 1). The CD34+ Mks in MDS included mature forms as well as dysplastic and small Mks. Using immunostains on serial sections, we found that the CD34+ Mks also expressed FVIII-RA and CD61 (data not shown). *In vitro* studies have demonstrated that CD34 expression on Mks is limited to earlier progenitor cells and declines during maturation, while CD61 expression progressively increases;[4,5] in normal conditions, the CD34+CD61+ phenotype is found on a small subset of Mks precursors, morphologically identifiable as immature blasts.[4,6] Late Mks precursors still expressing CD34 have been recovered from peripheral blood of patients with acute myeloid leukemia after intensive chemotherapy,[7] and peripheral blood CD34+ stem cells from normal individuals contain a minor subset that expresses CD61.[8] The rare mature CD34+ Mks we found in a minority of biopsies from normal individuals might correspond to these circulating Mks precursors. In MDS the CD34 positivity on Mks correlated with the platelet count (mean platelet count of patients with <10% and ≥ 10% CD34+ Mks: 143.4±31.5 and 58.6±14.6; unpaired t test: p=0.03). This observation suggests that the CD34+ CD61+ FVIII-RA+ Mks in MDS represent neoplastic Mks showing asynchronous phenotypic differentiation and poor platelet production; the very low numbers of CD34+ Mks occurring in ITP, a condition characterized by effective thrombocytopoiesis and increased number of marrow Mks, further supports this hypothesis. The clinical significance of CD34+ Mks in MDS is unclear. Patients with a high percentage of CD34+ Mks showed a higher frequency of marrow karyotype abnormalities, which are usually associated with a more aggressive course of disease.[9] However, the number of CD34+ Mks appeared to be unrelated to the number of CD34+ small immature cells, with the different FAB subclasses and with the clinical evolution of the disease.

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Kinetics variation of CD34+ and CD34+CD90+ in subjects following different mobilizing protocols

Since CD34+/CD90+ cells could represent an index of primitive hematopoietic progenitors, we designed a study to analyze the yield of CD34+/CD90+ cells during leukapheresis courses, in response to different mobilization regimens employed in healthy donors and in patients with hematologic neoplasias for allogeneic and autologous hematopoietic stem cell transplantation, respectively.

Sir,

To evaluate the impact of $CD34^+/CD90^+$ cells on engraftment, we studied the kinetics of the $CD34^+/CD90^+$ subset in pre-leukapheresis samples, in response to different mobilizing regimens employed in healthy donors for allogeneic transplants and in patients with hematologic neoplasias, who were to be submitted to autologous transplantation.[1-5]

From January 1998 and May 1999, peripheral blood stem cells (PBSC) were collected from 21 normal individual donors and 51 consecutive patients after different mobilizing protocols. The donors' mean age was 42 years: 11 were female, 10 male, and all were treated with granulocyte colony-stimulating factor (G-CSF). The main clinical features of the 51 patients are summarized in Table 1. Evaluation of $CD34^+$ cells and $CD45^{lo+}/CD34^+/CD90^+$ cells was performed following ISHAGE recommendations.[6] Main pre-collection $CD34^+$, $CD34^+/CD90^+$ cell counts and apheresis yield of $CD34^+$ hematopoietic precursors are summarized in Table 2. Six out of 21 donors were submitted to a single harvest, while in a second group of donors two consecutive PBSC collections were carried out. In the last case, pre-apheresis $CD34^+$ and $CD34/CD90^+$ cell counts were significantly lower in the second consecutive collection (mean value $p<0.05$). As regards patients with lymphoma (NHL) or multiple myeloma (MM) mobilized with G-CSF alone, heavily pretreated or relapsed subjects had significantly lower $CD34^+$ levels and $CD34^+/CD90^+$ proportion than patients responsive to first-line chemotherapy: two harvests were performed to obtain at least $1.5\pm1\times10^6$/kg hematopoietic progenitors. Finally, high-dose-cyclophosphamide (HD-Cy) +G-CSF treated NHL and MM patients showed significant differences in $CD34^+$ and $CD34^+/CD90^+$ mobilized cells, according to total amount of the chemotherapy cycles and/or radiotherapy received and clinical status at the time of mobilizing treatment. In fact, in ten patients in

Table 1. Main clinical features, mobilizing treatments employed and overall peripheral blood stem cells harvests performed in 51 patients studied.

Disease (N. pts)	*Status (N. pts.)*	*HD-Cy* 7 g/m²* *Pts (harvests)*	*HD-Cy* 4 g/m²* *Pts (harvests)*	*G-CSF 10 µg/day* *Pts (harvests)*
MM (39)	PR (16)	16 (20)		
	Refr-Rel(20)	18 (38)		2 (4)
	CHT-RES(3)			3 (3)
NHL (12)	PR (2)		2 (2)	
	Refr-Rel(6)		2 (2)	4 (8)
	CHT-RES(4)			4 (4)

Table 2. Patients mobilized with G-CSF alone (A) or with chemotherapy plus G-CSF. (B): pre-collection and collection per leukapheresis / kg bw, $CD34^+$ and $CD34^+/CD90^+$ cells.

(A)

Disease (Pts)	*G-CSF 300 µg twice a day x 5-7 days*	*Harvests*	*$CD34^+$ % Mean±SD*	*$CD34^+$ µL x µL Mean±SD*	*$CD34/CD90^+$ % Mean±SD*	*$CD34/CD90^+$ µL Mean±SD*	*$CD34^+$ collected $x10^6$/kg BW Mean±SD*	*$CD34/CD90^+$ $x10^6$/kg BW Mean±SD*
MM (3)	G-CSF	single	0.1±0.06	82±48	20.1±11	18±11	8.1±4	1.3±1.1
MM (2)*	G-CSF	1st of 2	0.07±0.05	25±17	27±16	10±8	1.5±1.2	0.4±0.1
MM (2)	G-CSF	2nd of 2	0.04±0.02	11±10	24±14	6±3	0.7±0.5	0.1±0.1
NHL (4)	G-CSF	single	0.1±0.01	99±57	24±10	22±10	9±2	2±1.3
NHL (4)*	G-CSF	1st of 2	0.05±0.02	29±19	25.5±9	12.5±10.4	1.1±1	0.3±0.1
NHL (4)	G-CSF	2nd of 2	0.03±0.01	10±4	24±5	4±1	0.7±0.7	0.1±0.09
DON (6)	G-CSF	single	0.2±0.07	121.6±41	30.6±14	40.6±23	8.2±2.9	1.3±0.7
DON (15)	G-CSF	1st of 2	0.2±0.04	109±91	36±15.5	42±29	8.5±3.2	1.8±1.8
DON (15)	G-CSF	2nd of 2	0.19±0.09	73.5±26	31.6±18	27±19	4.3±1.3	1±1

(B)

Disease (Pts)	*Mobilizing regimlen*	*Harvests*	*$CD34^+$ % Mean±SD*	*$CD34^+$ µL x µL Mean±SD*	*$CD34/CD90^+$ % Mean±SD*	*$CD34/CD90^+$ µL Mean±SD*	*$CD34^+$ collected $x10^6$/kg BW Mean±SD*	*$CD34/CD90^+$ $x10^6$/kg BW Mean±SD*
MM (16)	HD-7 Cy+G	single	2.1±1	511±136	37.1±12.6	155±117	31.3±20	7±5
MM (18)	HD-7 Cy+G	1st of 2	0.7±0.5	155±117	37±16	62±48	3.5±2-2	4±6
MM (18)	HD-7 Cy+G	2nd of 2	0.6±0.5	144±104	34±14	56±43	3.7±1.5	0.1±0.1
NHL (4)	HD-4 Cy+G	single	1±0.1	249±57	44±10	112±20	10±2	7±3

MM= multiple myeloma; NHL= non-Hodgkin's lymphoma; DON= healthy donors; §only one harvest performed; @first of two harvests performed; #second of two harvests performed; BW=body weight.

advanced-relapsed status of disease or refractory to first-line treatment, mobilizing rate and magnitude were significantly impaired in comparison to patients responsive to induction therapy and two PBSC collections were needed to reach the hematopoietic progenitor threshold dose of 2.0×10^6/kg. Linear regression analysis, performed on 10^5 PBSC collections, demonstrated a strong correlation between absolute $CD34^+$ and/or $CD34^+/CD90^+$ detected in pre-harvest samples and the yield of hematopoietic progenitors in the apheresis products, since the correlation coefficients (CC) calculated in patients submitted to a standard 10 L processed collection were 0.88 and 0.70, respectively.

Pre-collection $CD34^+$ absolute counts correlated closely to both $CD34^+/CD90^+$ pre-collection values and hematopoietic progenitors yielded in apheresis products.

In MM and NHL patients, HD-Cy + G-CSF was demonstrated to be very efficient in promoting marrow release of both $CD34^+$ and $CD34^+/CD90^+$ subsets. However, the rate and the magnitude of response to HD-Cy + G-CSF were confirmed as strictly dependent on disease status and overall dose of chemo-radiotherapy previously received by the patients. In G-CSF treated subjects, significantly lower absolute $CD34^+$ counts were detected, and, in particular the proportion of $CD34^+/CD90^+$ cells was significantly lower than that recorded in HD-Cy treated patients.

Concerning the impact of the $CD34^+CD90^+$ subset on hematopoietic recovery time, our data seem to show that recovery time of >500 polymorphs/μL and >50,000 platelets/μL are independent of $CD34^+$ and $CD34^+/CD90^+$ cells infused per kg of patient body weight, while a slight positive correlation was found between the CFU-GM dose infused and short-term hematopoietic reconstitution (p=0.002). In conclusion, the $CD34^+/CD90^+$ subset might be a sensitive index of potential residual hematopoiesis in heavily treated patients and a sign of the magnitude of response to mobilizing treatment in healthy donors, but its impact on hematopoietic recovery was not demonstrated to be of crucial importance in our series of allotransplanted patients. We believe that the co-expression of other antigens, such as AC133 or CD26 on the $CD34^+/CD90^+$ subset could lead to identification of a more staminal subsets.[7]

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CD34+ cells, CD34+/CD90+ cells, G-CSF, leukapheresis, PBSC.

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