

Mobilization of CD34⁺ cells in elderly patients (≥ 70 years) with multiple myeloma: influence of age, prior therapy, platelet count and mobilization regimen

CHRISTOPHER L. MORRIS, ERIC SIEGEL, BART BARLOGIE, MICHELE COTTLER-FOX, PEI LIN, ATHANASIOS FASSAS, MAURIZIO ZANGARI, ELIAS ANAISSIE AND GUIDO TRICOT *Myeloma Institute for Research and Therapy, University of Arkansas for Medical Sciences, Little Rock, AR, USA*

Received 26 June 2002; accepted for publication 14 August 2002

Summary. The mobilization of peripheral blood stem cells was studied in 984 multiple myeloma patients, including 106 patients aged ≥ 70 years. Increasing age correlated inversely with CD34⁺ yield ($P < 0.0001$), but also with ≥ 12 months of prior standard chemotherapy ($P = 0.0001$), $< 200 \times 10^9/l$ platelets ($P = 0.0006$) premobilization and mobilization with growth factors only ($P = 0.0001$). After controlling for these age covariates, multivariate analysis identified ≤ 12 months standard therapy and platelet count $\geq 200 \times 10^9/l$ premobilization as favourable variables (both $P < 0.0001$), while increasing patient age remained an unfavourable factor ($P = 0.0009$). With both favourable variables, 85% of elderly patients collected $\geq 4 \times 10^6/kg$ CD34⁺ cells in a median of one collection. The effect of age was incremental with no age threshold showing acceleration in the decline of CD34⁺ yield. Chemotherapy signifi-

cantly increased CD34⁺ yield compared with growth factors only. However, the subgroup of patients with > 12 months prior therapy and premobilization platelet count $< 200 \times 10^9/l$ mobilized as many CD34⁺ cells with granulocyte colony-stimulating factor (G-CSF) alone as with chemotherapy and haematopoietic growth factors. Increasing patient age had no effect on post-transplant neutrophil recovery, but significantly delayed platelet recovery ($\geq 50 \times 10^9/l$) if $< 2 \times 10^6/kg$ CD34⁺ cells were infused, but this effect was eliminated completely with infusion of $\geq 4 \times 10^6/kg$ CD34⁺ cells. Increasing age adversely affected CD34⁺ yield even with limited premobilization therapy, indicating that early collection is important in elderly patients.

Keywords: multiple myeloma, stem cell collection, age, platelets, standard therapy.

Treatment of multiple myeloma with high-dose therapy (HDT) has significantly improved complete remission rates, event-free and overall survival compared with standard chemotherapy (Attal *et al.*, 1996; Barlogie *et al.*, 1997). Retrospective analyses suggest that the benefit of HDT extends to older patients with myeloma (Palumbo *et al.*, 1999; Siegel *et al.*, 1999), which is an important finding considering that the median age of patients with multiple myeloma is 67 years. HDT resulted in superior disease control in a significant fraction of elderly patients with acceptable toxicity and minimal treatment-related mortality (TRM) (Palumbo *et al.*, 1999; Badros *et al.*, 2001). The reduction of TRM in patients receiving HDT for myeloma is due in large part to the rapidity of haematopoietic recovery

with peripheral blood stem cell transplants. The ability to collect large numbers of haematopoietic progenitors is a prerequisite for the timely application of tandem transplants, as practised at our Institution.

Although the ability to mobilize stem cells from healthy donors shows little deterioration with age, the influence of patient age on autologous stem cell collection is unclear and studies in patients ≥ 70 years who have received prior chemotherapy are completely lacking. Numerous studies have failed to show an independent effect of patient age on CD34⁺ mobilization. These include six studies specific to multiple myeloma (Demirer *et al.*, 1996; Goldschmidt *et al.*, 1997; Guba *et al.*, 1997; Marit *et al.*, 1998; Desikan *et al.*, 2001; Perea *et al.*, 2001) and five studies that contained over 200 patients each (Bensinger *et al.*, 1995; Guba *et al.*, 1997; Ketterer *et al.*, 1998; Pecora *et al.*, 1998; Clement *et al.*, 2000). A handful of small studies (with ≤ 70 patients/study) have shown a correlation between patient age and CD34⁺ mobilization (Bensinger *et al.*, 1994; Canales *et al.*,

Correspondence: Guido Tricot, MD, PhD, University of Arkansas for Medical Sciences, Myeloma Institute for Research and Therapy Slot 776, 4301 W. Markham Street, Little Rock, AR, 72205, USA. E-mail: TricotGuidoJ@uams.edu

2000; Corso *et al*, 2000). However, subsequent studies from these same centres, with larger numbers of patients, have failed to find any correlation between patient age and CD34⁺ cell yields (Bensinger *et al*, 1995; Demirer *et al*, 1996; Canales *et al*, 2001).

From studies of healthy elderly human subjects, it is known that steady state haematopoiesis is preserved with age, but that significant changes occur in how basal haematopoiesis is maintained (Globerson, 1999). Up to the age of 100 years, peripheral blood CD34⁺ cell numbers show only a slight decline in numbers, and the quality of these cells was indistinguishable from CD34⁺ cells obtained from younger individuals in terms of *ex-vivo* colony formation as measured by number, size and colony morphology (Bagnara *et al*, 2000). However, the profile of haematopoietic cytokines that are important in the maintenance of progenitor cells changes significantly with age (Fagiolo *et al*, 1993; Bagnara *et al*, 2000). These studies show that the number of stem cells does not decline much with increasing age, but the functional capacity of the early stem cell compartment may decrease. It is reasonable to hypothesize that as people age, chemotherapy may have differential effects on the stem cell compartment and its functional capacity.

This retrospective analysis was performed to identify the parameters predicting the ability to mobilize adequate numbers of peripheral blood stem cells in elderly patients (aged ≥ 70 years) with myeloma. These parameters were then adjusted across age groups to measure how CD34⁺ cell mobilization was affected by patient age as opposed to other risk variables already known to influence peripheral blood stem cell mobilization.

PATIENTS AND METHODS

Patients and data sources. All patients had documented symptomatic multiple myeloma and were enrolled on Institutional Review Board approved autotransplant studies after signing informed consent prior to initiation of therapy. Data on age, premobilization platelet count, duration of preceding standard therapy, mobilization regimen, CD34⁺ cell yield, percentage of bone marrow plasma cells, and serum levels of creatinine, beta-2-microglobulin (B2M), C-reactive protein (CRP), and serum albumin, were collected on all 984 patients. Several additional parameters reflecting disease burden or organ function were collected on patients aged ≥ 70 years at the time of stem cell harvest. For patients mobilized with growth factor only, the pre-mobilization platelet count was defined as the measurement closest to the start of growth factor therapy but < 30 d prior to the start of CD34⁺ cell collection. For patients receiving chemotherapy as part of the mobilization regimen, pre-mobilization platelet count was defined as the measurement closest to the d 1 of the mobilization chemotherapy but no more than 30 d prior to the start of CD34⁺ cell collection. More than 90% of values were obtained within 3 d of the start of mobilization. Additional parameters considered in patients ≥ 70 years included: haemoglobin, serum and urine monoclonal protein levels, lactate dehydrogenase (LDH),

aspartate-transaminase (AST), alanine-transaminase (ALT), alkaline phosphatase, and bilirubin. The measurements used for these parameters were obtained at the time points closest to the start of CD34⁺ cell mobilization therapy as previously defined above for pre-mobilization platelet count.

The combined group of 984 patients (878 patients < 70 and 106 patients ≥ 70 years old) comprised the overall patient denominator. Breakdown by age decades was as follows: under 30 years, six patients; 30–40 years, 68 patients; 40–50 years, 256 patients; 50–60 years, 327 patients; 60–70 years, 221 patients; 70–80 years, 102 patients; and > 80 years, four patients. For this study, the six patients in the under 30 year age group were combined with the 68 patients in the 30–40 year age group and the four patients in the > 80 year age group were combined with the 102 patients in the 70–80 year age group. Patient characteristics are shown in Table I.

Patient data on CD34⁺ cell collection was also obtained for a second group of 98 newly diagnosed patients treated on Total Therapy II. The group was selected from the entire Total Therapy II cohort entered on the study prior to August 2000, the last month for inclusion in the main group of 984 patients. The 98 patients selected included only those who had no chemotherapy or radiation therapy prior to study enrolment, to analyse the impact of age on stem cell mobilization after minimal standard therapy. All 98 patients were mobilized with CAD (cyclophosphamide, adriamycin and dexamethasone) chemotherapy (see below) and granulocyte colony stimulating factor (G-CSF, 10 $\mu\text{g}/\text{kg}/\text{d}$), after having received only one cycle each of VAD (vincristine, adriamycin and dexamethasone) and DCEP (dexamethasone, cyclophosphamide, etoposide and cisplatin) chemotherapy. CD34⁺ cell yields were expressed as the total number collected divided by the number of days of collection.

Mobilization regimens. Peripheral blood stem cells (PBSC) were collected with growth factors only in 317 patients, 299 patients received G-CSF (5–16 $\mu\text{g}/\text{kg}$ s.c.) and 18 patients received stem cell factor (SCF, 750 $\mu\text{g}/\text{m}^2/\text{d}$) plus G-CSF (10 $\mu\text{g}/\text{kg}/\text{d}$). Patients received growth factors daily with PBSC collection starting on the d 5 of administration. Chemotherapy-based regimens included high-dose cyclophosphamide (HD-CTX) 6 g/m^2 in 516 patients and was combined with granulocyte-macrophage colony stimulating factor (GM-CSF, 250 $\mu\text{g}/\text{m}^2$ s.c.) for all newly diagnosed patients ($n = 202$) as previously described (Barlogie *et al*, 1997). For previously treated patients, HD-CTX was combined with G-CSF (5–10 $\mu\text{g}/\text{kg}$ s.c., $n = 187$), G-CSF and GM-CSF (5 $\mu\text{g}/\text{kg}$ s.c. plus 250 $\mu\text{g}/\text{m}^2$ s.c. respectively, $n = 73$), or PIXY and GM-CSF (750 $\mu\text{g}/\text{m}^2$ s.c. plus 250 $\mu\text{g}/\text{m}^2$ s.c. respectively, $n = 54$). Multi-agent chemotherapy regimens were used for mobilization of 151 patients. These regimens included CAD (cyclophosphamide, adriamycin and dexamethasone: 750 mg/m^2 , 15 mg/m^2 , 40 $\text{mg}/\text{d} \times 4$ d, $n = 60$) (Barlogie, 2001) for newly diagnosed patients on Total Therapy II, while previously treated patients received either DCEP (dexamethasone, cyclophosphamide, etoposide and cisplatin: 40 mg p.o., 400 mg/m^2 , 40 mg/m^2 , 10 mg/m^2 i.v. daily $\times 4$ d,

Table I. Patient demographics.

Parameters	Per cent of patients				
	< 40 years (n = 74)	40–49 (n = 256)	50–59 (n = 327)	60–69 (n = 221)	≥ 70 years (n = 106)
Male	54	56	67	67	71
Prior therapy > 12 months	10.8	21.5	27.5	34	44
Platelet count ≥ 200 × 10 ⁹ /l*	74.3	70.3	62.4	61.5	54.7
Chemotherapy mobilization	85	76	70	60	56
Creatinine ≥ 176.8 μmol/l*	8.2	8.4	8.9	11.3	12.5
B2M > 2.5 mg/l*	33.3	33.4	36.8	46.6	59.8
CRP > 4 mg/l*	37.5	51.9	52.2	40	55.9
Albumin ≤ 35 g/l*	21.6	13.7	23.5	37	40.8
Percentage of bone marrow plasma cells, median*	30	20	20	20	15
Immunoglobulin subclass					
IgG	50.7	50.6	56.6	60.9	57.0
IgA	15.9	17.2	21.0	24.9	27.0
Light chain	26.9	22.7	16.8	10.6	11.0
Non-secretory	6.0	6.9	4.9	3.3	5.0
Other IgM, IgD, IgE	< 1	2.6	< 1	< 1	0

*Premobilization.

$n = 51$) or DTPACE (dexamethasone, thalidomide, cisplatin, adriamycin cyclophosphamide, etoposide: 40 mg p.o., 400 mg p.o., 10 mg/m², 10 mg/m², 400 mg/m² and 40 mg/m² i.v. daily × 4 d, $n = 33$) (Tricot, 2001). Seven patients received cyclophosphamide 4 g/m² and etoposide 1.8 g/m² by i.v. continuous infusion over 48 h. G-CSF (10 μg/kg/d) was given to all patients receiving multi-agent chemotherapy, starting 24 h after completion of chemotherapy. Leukapheresis was initiated when the leucocyte count exceeded 1×10^9 /l. Collections were processed on Cobe Spectra (Cobe BCT, Lakewood, CO, USA) after April 1999 and on a CS3000 (Baxter Healthcare Corporation, Pinellas Park, FL, USA) prior to April 1999. Total volumes processed were between 15 and 20 l per collection. The target for CD34⁺ cell mobilization was 10×10^6 /kg for previously treated patients and 20×10^6 /kg for newly diagnosed patients. A lower target was used for previously treated patients, because of the more extensive damage to their stem cell compartment caused by prolonged chemotherapy. Peripheral blood stem cells were collected irrespective of the percentage of myeloma cells in the bone marrow. The decision to mobilize with growth factors only versus chemotherapy was mainly based on the duration of prior therapy on the one hand, with more heavily pretreated patients more likely to be mobilized with growth factors, and the tumour load on the other hand, with patients having a higher tumour load preferentially mobilized with chemotherapy and growth factors.

Toxicity. Toxicity was scored using the National Cancer Institute (NCI) common toxicity criteria version 2.0 (CANCER THERAPY EVALUATION PROGRAM, Department of Health and Human Services, April 1999. <http://ctep.cancer.gov/reporting/ctc.html>).

Statistics. All statistical analysis was performed with version 8 of the SAS software package. Logistic and standard regression was employed to perform univariate analysis of the effect of the factors listed below on stem cell collection. The response variables for logistic regression were dichotomized average CD34⁺ cell yield, ≥ 2 and $< 1 \times 10^6$ /kg/d, calculated from the total CD34⁺ cells/kg collected for the entire mobilization divided by the number of days to collect. For standard regression, the response variable was the common logarithm of the average CD34⁺ yield, as the log-transformed variable had better variance properties than the untransformed variable. Factors assessed in univariate analysis for their prognostic significance were: use of chemotherapy in the mobilization regimen, age, length of prior standard chemotherapy, the premobilization laboratory parameters (B2M, creatinine, CRP and percentage of plasma cells in the bone marrow). On multivariate analysis, all prognostic factors that were univariately significant at $P \leq 0.1$ were entered into multivariate regression models with variable selection by backward elimination. Factors retained by the variable selection procedure at $P \leq 0.05$ were considered multivariately significant and used as a guide to construct multivariate regression models, which allowed for the length of prior therapy to influence the strength and statistical significance of the age effect on dichotomized stem cell yield.

Except for the use of chemotherapy in the mobilization, all prognostic factors considered in the analyses were continuous variables. To develop clinically useful models of collection in elderly patients, we evaluated prognostic factors dichotomized at levels of historical interest to our institution for patients more than 70 years of age ($n = 106$), and subjected them to univariate and multivariate analysis

using logistic regression, according to the procedures described above. For patients ≥ 70 years, additional parameters evaluated were: age ≥ 75 , haemoglobin, serum and urine monoclonal protein levels, LDH, GOT, GPT, alkaline phosphatase, and bilirubin.

Two of the three factors from the final multivariate models (platelets $\leq 200 \times 10^9/l$ and prior therapy ≥ 12 months) were defined as risk factors for collection, and added together to stratify the patients into three groups by number of risk factors. The effect of the third multivariate factor (mobilization with chemotherapy) on the average number of CD34⁺ cells per day of collection was then assessed in each stratum by means of the Kruskal–Wallis test.

To account for the possible confounding effect of the different mobilization regimens, each regimen was defined as a stratum and analysed by conditional logistic regression across the strata (Stokes *et al*, 2000). This enabled the identification of the multivariately significant prognostic factors while controlling for the different mobilization regimens. Mobilization regimens that did not include at least 20 patients or had less than 5% elderly patients (aged > 65 years) were not included; the total number of patients in this analysis was 905.

To determine how the length of prior standard chemotherapy affected the influence of patient age on CD34⁺ cell yield, the patients were divided into multiple groups defined by length of prior therapy. The significant factors on multivariate analysis were modelled for each group in order to obtain odds ratios within each window of prior therapy duration.

To determine the effect of age on post-transplant platelet recovery with infusion of $< 2 \times 10^6/kg$ CD 34 cells, the recovery of the youngest (< 50 years) and oldest (> 65 years) patients was compared. The older age group was defined as > 65 years and not ≥ 70 , because of the number of patients ≥ 70 years with $< 2 \times 10^6/kg$ CD34 cells infused was too low. Cumulative times to platelet recovery were estimated using the method of Kaplan and Meier (1958) and differences between the groups were assessed with the log-rank test.

RESULTS

Stem cell yields in patients aged over 70 years

Among all patients ≥ 70 years at the time of stem cell collection, the fraction of patients collecting ≥ 10 , 4–10, 2–4 and $< 2 \times 10^6$ CD34⁺ cells/kg is shown in Table II, together

with the median number (range) of apheresis procedures required. Considering 2 and 4×10^6 CD34⁺/kg as the minimum and preferred number of stem cells required to safely perform a single transplant, 85% of patients were able to collect the minimum and 70% the preferred number of stem cells for a single transplant. The median number of days to collect 2 and 4×10^6 CD34⁺ cells/kg was 2 d and 3 d respectively. However, all patients continued apheresis with intent to collect 10 (previously treated patients) or 20×10^6 CD34⁺/kg (newly diagnosed patients) to support at least two cycles of HDT (see *Patients and methods*). This resulted in a median of five collections among all patients, and 70% of patients collected at least the minimum number of stem cells to support two cycles of HDT (4×10^6 CD34⁺ cells/kg) in a single cycle of mobilization. Among patients collecting $< 10 \times 10^6$ CD34⁺ cells/kg, 20 patients had a second ($n = 12$), third ($n = 7$), or fourth ($n = 1$) stem cell collection attempt. These additional collections increased the fraction of elderly patients with enough stem cells for a single HDT to 92% and for two cycles of HDT to 80% (data not shown).

Toxicity of stem cell mobilization in elderly patients

A chemotherapy regimen was used to mobilize stem cells in 47 patients, and 59 patients were mobilized with growth factors only. The incidence of adverse events requiring treatment (\geq grade 3 using NCI toxicity criteria) was 5% for patients receiving growth factors only and 30% for patients receiving chemotherapy ($P = 0.0001$). All events were grade 3 with the most common events being confusion, constitutional symptoms and neutropenic fever; none were fatal. No patient was unable to proceed to transplant as a result of the toxicities experienced during stem cell mobilization.

Variables affecting stem cell yield

Variables were tested for their impact on the probability of collecting ≥ 2 (good) and < 1 (poor) $\times 10^6$ CD34⁺ cells/kg/d. In univariate analysis, pre-mobilization platelet count, length of prior therapy, mobilization regimen, B2M, albumin and the percentage of marrow plasma cells were significant for good, poor or both collection variables (Table III). These variables were entered into a multivariate model with stepwise selection. The variables that remained independently significant for both good and poor collection included the pre-mobilization platelet count, length of prior therapy, type of mobilization and patient age (Table IV). Expressing the CD34⁺ cell yield as a continuous variable

Table II. Total CD34⁺ cells collected on patients ≥ 70 years during first mobilization.

	CD34 ⁺ cells collected, $\times 10^6/kg$			
	< 2	$\geq 2-4$	$\geq 4-10$	≥ 10
Number of patients (%)	16 (15)	16 (15)	31 (29)	43 (41)
Number of collection days, median (range)	5 (2–7)	6 (5–9)	6 (1–15)	4 (1–8)

Table III. Univariate analyses for predictors of CD34⁺ cell yield for patients ≥70 years and all 984 patients (first mobilization attempt).

Variable name	Good collection CD34 ⁺ cells ≥ 2 × 10 ⁶ /kg/d		Poor collection CD34 ⁺ cells ≤ 1 × 10 ⁶ /kg/d	
	≥ 70 years	All patients	≥ 70 years	All patients
	P-value	P-value	P-value	P-value
Pre-mobilization platelet count, ≥ 200 × 10 ⁹ /l*	0.0001	< 0.0001	0.01	< 0.0001
Age	Not applicable	< 0.0001	Not applicable	< 0.0001
Prior therapy ≤ 12 months*	0.003	< 0.0001	0.006	< 0.0001
CRP ≤ 7.0 mg/l†	0.015	ns	0.037	ns
Urine M Protein g/day	0.028	Not tested	0.032	Not tested
Mobilization, chemotherapy versus growth factors alone	0.033	< 0.0001	0.052	< 0.0001
B2M > 2.9 mg/l*	0.096	0.029	0.089	ns
Serum albumin	ns	0.040	ns	ns
Percentage of bone marrow plasma cells	ns	0.027	ns	ns

*Dichotomized ≤ or > 12 months prior therapy.

†Dichotomized on group median, all other variables continuous.

Creatinine, haemoglobin, Age > 75 years, ALT, AST, alkaline phosphatase, bilirubin, serum M protein, LDH had *P*-values > 0.1.

Table IV. Multivariate logistic regression results for patient ≥ 70 years and all 984 patients, first mobilization.

Variable Label	Final multivariate models			
	Age ≥ 70 years (<i>n</i> = 106)		All patients (<i>n</i> = 984)	
	P-value	Odds ratio (95% CI)	P-value	Odds ratio (95% CI)
Good collection: CD34 ⁺ ≥ 2 × 10 ⁶ cells/kg/d Platelets ≥ 200 × 10 ⁹ /l	0.0001	6.6 (2.5–17.3)	< 0.0001	2.99 (2.1–4.2)
Prior Therapy ≤ 12 months	0.006	3.8 (1.5–9.6)	< 0.0001	1.5 (1.3–1.7)
Chemotherapy mobilization	0.049	2.5 (1.0–6.3)	< 0.0001	3.0 (2.2–4.1)
Age in decades	–	–	0.0086	0.83 (0.73–0.95)
Poor collection: CD34 ⁺ < 1 × 10 ⁶ cells/kg/d Platelets ≥ 200 × 10 ⁹ /l	0.011	0.34 (0.14–0.78)	< 0.0001	0.26 (0.18–0.39)
Prior therapy ≤ 12 months	0.012	0.34 (0.15–0.79)	< 0.0001	0.71 (0.67–0.83)
Chemotherapy mobilization	0.075	0.46 (0.20–1.08)	< 0.0001	0.47 (0.34–0.66)
Age in decades	–	–	0.0004	1.3 (1.1–1.5)

provided the same results (data not shown). A separate analysis of patients ≥ 70 years showed differences in univariate analysis (Table III), but nearly identical results in multivariate analysis (Table IV).

To account for the possible confounding effect of the different mobilization regimens, we performed a series of analyses on subsets of patients who received the same mobilization regimen. The groups examined included: CTX (6 g/m²) plus GM-CSF (*n* = 202), CAD plus G-CSF (*n* = 60), CTX and G-CSF + GM-CSF (*n* = 74), G-CSF alone (*n* = 299), CTX plus G-CSF (*n* = 187), DCEP plus G-CSF (*n* = 51), DT-PACE plus G-CSF (*n* = 33). The first two groups include only newly diagnosed patients and the last three groups include only previously treated patients. Six of seven groups showed a decreased chance of a good collection and increased chance of a poor collection with

increasing age. The group that was the exception, CTX + G-CSF + GM-CSF, had the smallest number of older patients (seven of 74 were ≥ 65 years) and the narrowest age distribution of any group. A composite *P*-value was calculated by conditioning the results of the seven homogeneously treated groups. Patient age remained a significant adverse risk factor for stem cell yield, independent of prior therapy and premobilization platelet count (*P* = 0.01 for good and *P* = 0.0005 for poor collection; Table V). The effect was apparent for groups mobilized with chemotherapy or G-CSF alone and for previously treated or newly diagnosed patients with identical amounts of chemotherapy prior to mobilization.

The independent effect of age on the frequency of collecting ≥ 2 vs < 1 × 10⁶ CD34⁺/kg/d is shown in Fig 1. The decline of CD34⁺ cell yield was incremental between 30

Table V. Subset analysis of age within specific mobilization regimens.

Subgroup	$\geq 2 \times 10^6$ CD34 ⁺ /kg/d		$< 1 \times 10^6$ CD34 ⁺ /kg/d	
	n	n ≥ 65 years	Odds ratio	Odds ratio
CTX* + GM-CSF	202	14	0.90	1.27
CAD + G-CSF	60	16	0.26	4.76
HDCTX + G/G-CSF	74	7	1.8	0.78
G-CSF alone	299	108	0.80	1.41
HDCTX + G-CSF	187	18	0.70	1.37
DCEP + G-CSF	51	15	0.74	1.41
DTPACE + G-CSF	33	18	0.83	1.61
All groups	906	196	0.82, P = 0.01	1.35, P = 0.0005

*Cyclophosphamide 6 g/m².

Multivariate model included age, prior therapy and premobilization platelet count. Odds ratios are for increasing age by decade.

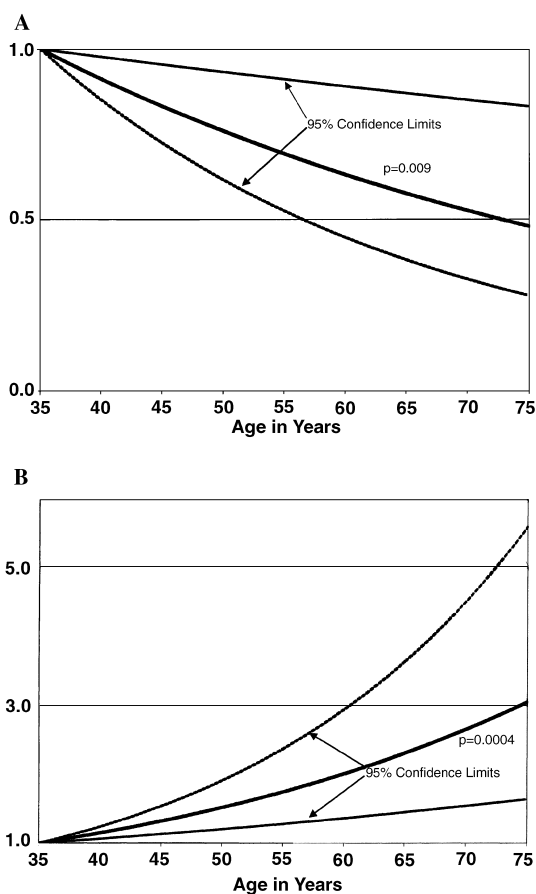


Fig 1. The effect of age on probability of good [$\geq 2 \times 10^6$ CD34⁺ cells/kg/d (A)] or poor stem cell collection [$< 1 \times 10^6$ CD34⁺ cells/kg/d (B)], after adjusting for covariates through multivariate logistic regression. The upper and lower curves show the 75% confidence intervals.

and 80 years, with no evidence for a specific age threshold showing acceleration in the fall of CD34⁺ yield. Furthermore, the effect of age was only apparent when analysed

over the entire age span. The reduction in mobilization of CD34⁺ cells in patients ≥ 70 years was not significant when compared with patients in the 50–60 or 60–70 year age groups, and only became apparent when tested against patients below age 50 years (when controlled for age differences in the prevalence of the prognostically significant variables).

The improvement in CD34⁺ cell yield with chemotherapy-based mobilization compared with growth factors alone depended on the presence of other prognostically significant variables. As shown in Fig 2, the greatest benefit was seen in patients with ≤ 12 months prior standard therapy and premobilization platelet counts $\geq 200 \times 10^9/l$ ($P < 0.0001$). When either risk variable was present (prior therapy > 12 months or platelets $< 200 \times 10^9/l$), chemotherapy-based mobilization had a reduced, but still highly significant, beneficial effect on CD34⁺ cell yield ($P = 0.0001$). For patients with a single risk variable, the premobilization platelet count was a stronger favourable predictor than ≤ 12 months prior therapy. For patients with both risk factors, there was no improvement in CD34⁺ cell yield with chemotherapy versus growth factors only ($P = 0.35$). Among patients ≥ 70 years of age similar results were seen. This is important because the prevalence of \geq grade 3 toxicity among elderly patients receiving chemotherapy increased with the number of risk factors. Although this trend did not achieve statistical significance, 17% (three of 18) of elderly patients with neither risk factor experienced \geq grade 3 toxicity compared with 37% (seven of 19) of patients with one risk factor and 40% (four of 10) for patients with both risk factors.

Age was an independent predictor of CD34⁺ cell yield, but was also strongly correlated with type of mobilization regimen, length of prior therapy and premobilization platelet count (Fig 3). The frequency of each of the independent favourable variables for stem cell collection declined significantly from age < 40 to ≥ 70 years. Comparing the length of prior therapy before and after referral to our Institution, we found that 77% of the age-related delay occurred prior to referral (data not shown). The fraction of

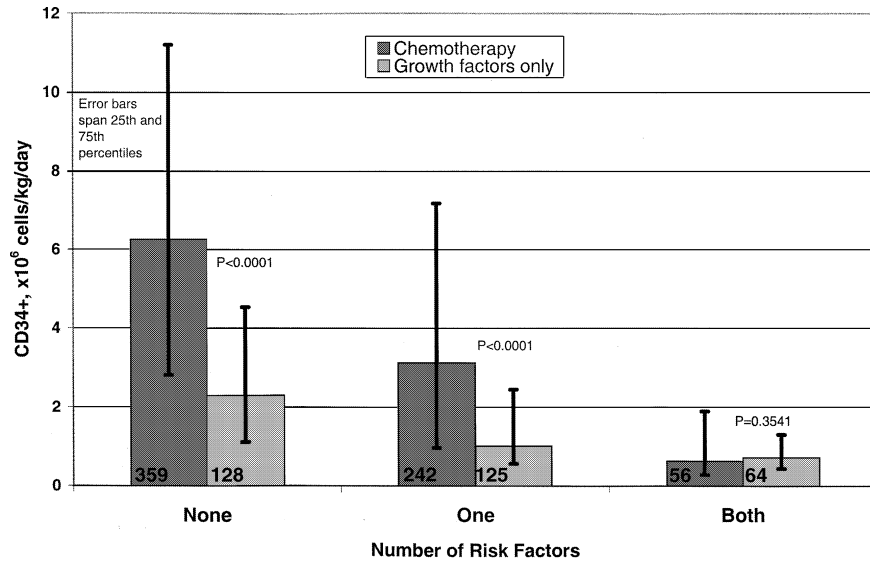


Fig 2. Comparison of the CD34⁺ cell yield for mobilization with chemotherapy versus growth factors alone, according to the number of risk factors. Each pair of columns shows the median CD34⁺ cell yield within risk groups 0, 1, or with both risk factors. The risk factors are platelets < 200 × 10⁹/l and prior therapy > 12 months.

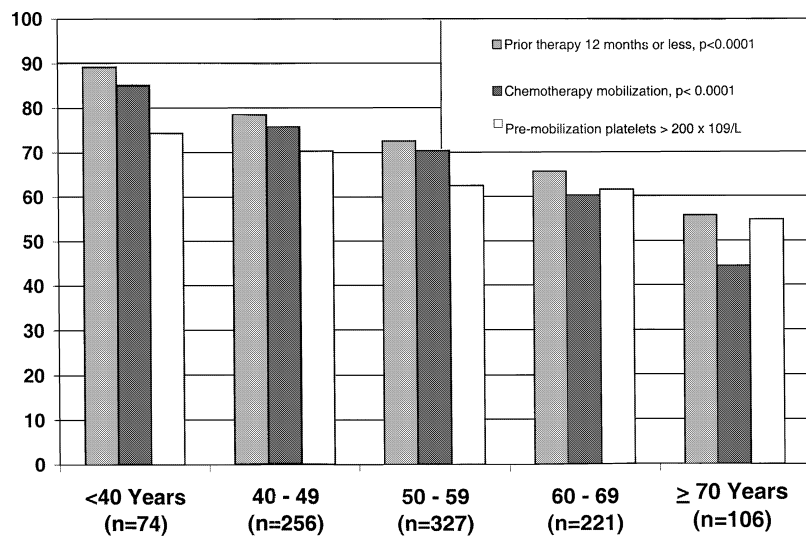


Fig 3. The prevalence of unfavourable variables for CD34⁺ cell yield increased with increasing patient age. The three columns above each age decade from left to right show the fraction of patients with ≤ 12 months prior chemotherapy, pre-mobilization platelet count ≥ 200 × 10⁹/l and those mobilized with chemotherapy.

patients with >12 months prior therapy at the time of referral increased from 8.1% for patients < 40 years to 16% for patients 40–49 years old and to 27% for patients ≥ 60 years at the time of stem cell harvest.

Influence of age on CD34⁺ mobilization in patients with minimal prior cytotoxic therapy

The presence of an age bias in referral patterns, noted above, raised the question of whether the age-related decline in stem cell mobilization was present before any chemotherapy or resulted from age-related differences in the sensitivity of haematopoietic stem cells to chemotherapy. We approached this question in two ways: first by evaluating the effect of age on CD34⁺ cell yield in a group of 98

patients treated with our front-line protocol, Total Therapy II (Barlogie *et al.*, 1997). These patients had received only a single course of VAD and DCEP chemotherapy prior to CD34⁺ cell harvesting with CAD chemotherapy. None had chemotherapy or radiation therapy prior to study entry and at the time of CD34⁺ cell collection the blood volumes processed per day were comparable in all patients. The CD34⁺ cell yield per day of collection showed a weak but significant negative correlation with age ($r = -0.23$, $P = 0.03$). This result suggests that there is already a small but real influence of age on stem cell yield after minimal prior chemotherapy. Second, we examined the relationship between age and stem cell yield within groups defined by length of prior therapy, and adjusted by multivariate

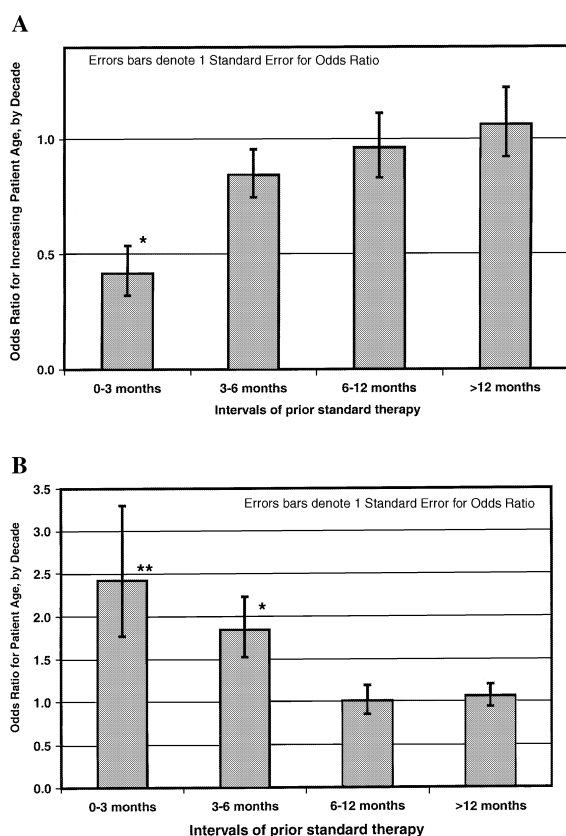


Fig 4. Impact of patient age on odds to collect well [$\geq 2 \times 10^6$ /kg CD34 cells/kg/d (A)] or poorly [10^6 CD34⁺ cells/kg/d (B)] within groups defined by length of prior therapy after adjusting for type of mobilization and premobilization platelet count by multivariate analysis. * $P < 0.05$ and ** $P < 0.01$.

analysis for type of mobilization (chemotherapy versus growth factors only) and premobilization platelet count. We found that the negative effect of age on stem cell yields occurred already in patients with ≤ 6 months of standard-dose therapy prior to stem cell mobilization. Age was the strongest predictor of good and poor stem cell yield in the group with 0–3 months, and the strongest predictor for poor stem cell yield in the group with 3–6 months of prior therapy. Beyond 6 months of prior therapy, the age effect declined and had no significant negative effect on stem cell yields after 12 months. (Fig 4). Given the small number of patients with < 1 month of prior therapy, we were unable to determine whether age influenced stem cell yields in patients with no prior chemotherapy.

Effect of age and stem cell dose on post-transplant neutrophil and platelet recovery

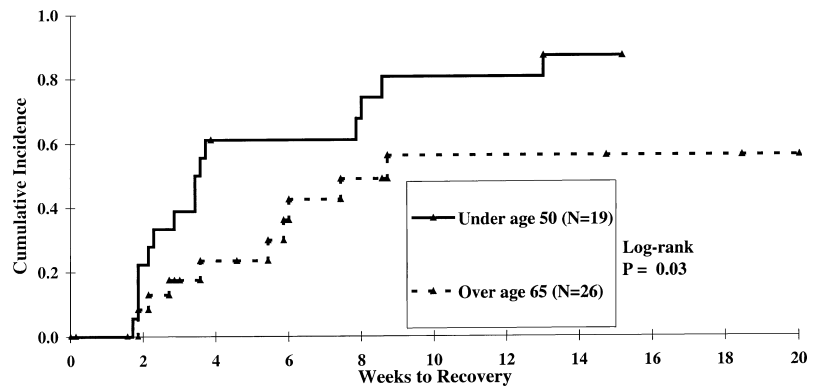
Neutrophil recovery was prompt for all age groups, with the attainment of an absolute neutrophil count $> 0.5 \times 10^9/l$ at a median of 11 d post transplant. All patients were given either G-CSF or GM-CSF post transplantation. No differences were seen between age groups. Recovery of platelets to $\geq 50 \times 10^9/l$ was analysed by adjusting for the CD34⁺ cell

dose infused with the transplant and only including patients aged < 50 years or ≥ 65 years at the time of CD34⁺ cell collection. The results indicate that platelet recovery was prompt for patients receiving $\geq 4 \times 10^6$ CD34⁺ cells/kg. The median day for platelet recovery was d 13 for patients < 50 years ($n = 131$) and d 15 for patients ≥ 65 years ($n = 54$, $P = 0.3$), and $> 95\%$ of patients in both age groups recovered platelets to $\geq 50 \times 10^9/l$ by d 100 after transplant. Patients receiving $< 2 \times 10^6$ /kg CD34⁺ cells recovered platelets to $\geq 50 \times 10^9/l$ at a median of 24 d if aged < 50 years vs 61 d if aged ≥ 65 years ($P = 0.03$). By 100 d post transplant, 19% of younger and 43% of older patients failed to recover platelets to $\geq 50 \times 10^9/l$, and by 150 d 13% of younger and 43% of elderly patients still had not recovered platelets to $\geq 50 \times 10^9/l$ (Fig 5). For patients receiving $2-4 \times 10^6$ CD34⁺/kg, platelet recovery was not significantly different between younger ($n = 39$) and older patients ($n = 43$, $P = 0.4$). However, within this intermediate group, 100% of younger patients recovered their platelets to $\geq 50 \times 10^9/l$ by d 150 while 22% of elderly patients still had not recovered at this time. The exaggerated delay in platelet recovery seen in elderly patients receiving a small CD34⁺ cell dose was not due to the administration of fewer CD34⁺ cells than their younger counterparts. Among all patients receiving $< 2 \times 10^6$ CD34⁺ cells/kg, patients ≥ 65 years received a median of 1.33 whereas patients < 50 years received 1.18×10^6 CD34⁺ cells/kg ($P =$ not significant).

DISCUSSION

The purpose of this study was to identify the parameters that were most predictive of the capacity to mobilize adequate numbers of CD34⁺ cells in patients with multiple myeloma and to investigate whether patient age had an independent effect on that capacity. Significant independent predictors were length of prior therapy, platelet count prior to the start of the mobilization regimen, type of mobilization regimen and patient age. The same results were obtained whether the CD34⁺ cell yield was expressed as a dichotomized (good or poor yield) or continuous variable, or when patients were analysed within homogeneously mobilized subgroups. Multivariate analysis confirmed the same prognostic variables for the whole patient population and the patients ≥ 70 years (except age). The effect of age was modest and incremental over the entire age range. Nevertheless, between the ages of 35 and 75 years, the age-specific deterioration in CD34⁺ cell yield resulted in a threefold increase in risk of a poor CD34⁺ cell yield ($< 1 \times 10^6$ /kg/d) and a twofold decrease in probability of good CD34⁺ cell yield ($\geq 2 \times 10^6$ /kg/d). The influence of patient age has been evaluated in numerous studies with contradictory results. The variation in results with regards to patient age is most probably due to confounding of age with other parameters that affect stem cell yield, and by combining multiple diagnoses with different age distributions and mobilization characteristics. In spite of the adverse effect of age on stem cell yield, 92% of elderly patients were able to collect enough CD34⁺ cells to support at least one

Fig 5. Proportion of patients recovering platelets to $\geq 50 \times 10^9/l$ who received $< 2 \times 10^6$ CD34⁺ cells/kg at the time of transplant.



cycle of HDT, although some required multiple collection attempts. Even among patients with both a poor platelet reserve and > 12 months of prior therapy, 87% and 56% were able to collect ≥ 2 and $\geq 4 \times 10^6$ CD34⁺/kg, respectively, with a single collection attempt. Thus, elderly patients should not be excluded from HDT trials based on perceived difficulties in collecting sufficient CD34⁺ cells.

We found that mobilization with chemotherapy increased the CD34⁺ cell yield compared with growth factors alone, but the benefit of chemotherapy did not extend to patients with both long duration of prior therapy (> 12 months) and low pre-mobilization platelet count ($< 200 \times 10^9/l$). When either risk factor was present, the beneficial effect of chemotherapy was present but diminished. We have reported that length of prior therapy was a significant predictor of CD34⁺ cell yield, following mobilization with cyclophosphamide (Guba *et al*, 1997) or growth factors alone (Desikan *et al*, 2001). Others have demonstrated similar findings for duration of prior therapy (Goldschmidt *et al*, 1997; Marit *et al*, 1998; Perea *et al*, 2001) or number of chemotherapy cycles (Demirer *et al*, 1996) in patients with multiple myeloma. However, to our knowledge, no study has examined the impact on CD34⁺ cell yield by chemotherapy relative to growth factors as a function of duration of prior therapy or pre-mobilization platelet count. This is important as toxicity remains a concern when using chemotherapy to mobilize stem cells, especially in the elderly. The use of chemotherapy, as opposed to growth factors alone, was associated with increased toxicity, from 5% to 30% in patients ≥ 70 years. Importantly, the prevalence of toxicity in elderly patients receiving chemotherapy was highest in those patients with long prior therapy and low platelets, the patient group least likely to benefit. Thus, targeting the use of chemotherapy-based mobilization to elderly patients with neither risk factor would maximize the beneficial effect of chemotherapy on stem cell yield with a minimal increase in toxicity.

Age had a significant impact on platelet recovery to $\geq 50 \times 10^9/l$. For patients ≥ 65 years receiving $< 2 \times 10^6$ CD34⁺ cells/kg, the time to platelet recovery was significantly delayed compared with patients < 50 years old (median of 61 d vs 24 d). Furthermore, a significantly higher fraction of elderly patients failed to recover their

platelet count by d 150 after transplant (43% vs 13%). With infusion of large numbers of CD34⁺ cells ($\geq 4 \times 10^6/kg$), the effect of age on platelet recovery was completely eliminated. The age-associated delay in platelet recovery could be explained by loss of engrafting capacity or other functional changes in CD34⁺ cells, similar to the loss of engraftment capacity that occurs in mice CD34⁺ cells with age (Chen *et al*, 1999). The lack of age-associated changes in neutrophil recovery could be due to the need for fewer stem cells to recover neutrophils than platelets. Patient age was strongly correlated with other prognostically adverse variables for CD34⁺ cell yield. The main cause of these associations appeared to be an age bias in referral for HDT to our centre. The length of therapy prior to referral increased with each age decade. Given the magnitude of the delays observed (> 1 years of prior therapy), it is unlikely that these delays are due to age-related delays in recovery from chemotherapy. The age bias we observed has broad implications for the interpretation of studies involving age, and influences the study size needed to compensate for confounding of age with other prognostic variables.

The effect of prior chemotherapy on age-related differences in CD34⁺ cell yield is an important issue we have attempted to address. When we grouped the 984 patients according to different lengths of prior therapy, we found significant differences in the importance of age as a predictor of stem cell yield. For patients with ≤ 6 months of prior therapy, age was the strongest predictor of stem cell yield, exceeding the importance of mobilization regimen and pre-mobilization platelet count. The influence of age diminished with > 6 months prior therapy and was non-existent with > 12 months of prior therapy. In these heavily treated patients, the pre-mobilization platelet count was important; chemotherapy improved mobilization yield when the platelet count was high and was no better than growth factors alone when platelet counts were low. The reduction in the importance of age in patients with > 12 months prior therapy may not be unexpected, as extensive prior therapy presumably makes differences in the CD34⁺ cell yield, based on any other parameter (including age), smaller. Elderly patients need to be mobilized earlier in the course of their disease than their younger counterparts.

ACKNOWLEDGMENTS

This work was supported in part by National Institutes of Health grant PO1-CA55819-06.

REFERENCES

- Attal, M., Harousseau, J.-L., Stoppa, A.-M., Sotto, J.-J., Fuzibet, J.-G., Rossi, J.-F., Casassus, P., Maisonneuve, H., Facon, T., Ifrah, N., Payen, C. & Bataille, R. (1996) A prospective, randomized trial of autologous bone marrow transplantation and chemotherapy in multiple myeloma. The Intergroupe Français du Myélome. *New England Journal of Medicine*, **335**, 91–93.
- Badros, A., Barlogie, B., Siegel, E., Morris, E., Desikan, R., Zangari, M., Fassas, A., Anaissie, E., Munshi, N. & Tricot, G. (2001) Autologous stem cell transplant in elderly multiple myeloma patients over age 70. *British Journal of Haematology*, **114**, 1–9.
- Bagnara, G.P., Bonsi, L., Strippoli, P., Bonifazi, F., Tonelli, R., D'Addato, S., Paganelli, R., Scala, E., Fagiolo, U., Monti, D., Cossarizza, A., Bonafe, M. & Franceschi, C. (2000) Hemopoiesis in healthy old people and centenarians. Well-maintained responsiveness of CD34⁺ cells to hemopoietic growth factors and remodeling of cytokine network. *Journal of Gerontological Biological Sciences*, **55A**, B61–B66.
- Barlogie, B. (2001) High-dose therapy and innovative approaches to treatment of multiple myeloma. *Seminars in Hematology*, **38**, 21–27.
- Barlogie, B., Jagannath, S., Vesole, D.H., Naucke, S., Cheson, B., Mattox, S., Bracy, D., Salmon, S., Jacobson, J., Crowley, J. & Tricot, G. (1997) Superiority of tandem autologous transplantation over standard therapy for previously untreated multiple myeloma. *Blood*, **89**, 789–793.
- Bensinger, W.I., Longin, K., Appelbaum, F., Rowley, S., Weaver, C., Lilleby, K., Gooley, T., Lynch, M., Higano, T., Klarnet, J., Chauncey, T., Storb, R. & Buckner, C.D. (1994) Peripheral blood stem cells (PBSCs) collected after recombinant granulocyte colony stimulating factor (rhG-CSF): an analysis of factors correlating with the tempo of engraftment after transplantation. *British Journal of Haematology*, **87**, 825.
- Bensinger, W., Appelbaum, F., Rowley, S., Storb, R., Sanders, J., Lilleby, K., Gooley, T., Demirer, T., Schiffman, K. & Weaver, C. (1995) Factors that influence collection and engraftment of autologous peripheral-blood stem cells. *Journal of Clinical Oncology*, **13**, 2547–2555.
- Canales, M.A., Arrieta, R., Hernandez-Garcia, M.C., Ojeda, E., Diez, J., Calero, F., Aguado, M.J., Bustos, J. & Hernandez-Navarro, F. (2000) Factors influencing collection and engraftment of CD34⁺ cells in patients with breast cancer following high-dose chemotherapy and autologous peripheral blood progenitor cell transplantation. *Journal of Hemotherapy and Stem Cell Research*, **9**, 103–109.
- Canales, M.A., Fernandez-Jimenez, M.C., Martin, A., Arrieta, R., Caballero, M.D., Diez, J., Quevedo, E., Garcia-Bustos, J., San Miguel, J.F. & Hernandez-Navarro, F. (2001) Identification of factors associated with poor peripheral blood progenitor cell mobilization in Hodgkin's disease. *Haematologica*, **86**, 494–498.
- Chen, J., Astle, B.A. & Harrison, D.E. (1999) Development and aging of primitive hematopoietic stem cells in BALB/cBy mice. *Experimental Hematology*, **27**, 928.
- Clement, A., Coffe, C., Adjizian, J.C., Villard, F., Jolly, D., Desbois, I. & Leon, A. (2000) Collection de cellules souches peripheriques, recherche de facteurs predictifs: une etude multicentrique. *Transfusion Clinique et Biologique*, **7**, 485–496.
- Corso, A., Caberlon, S., Pagnucco, G., Klersy, C., Zappasodi, P., Alessandrino, E.P., Vanelli, L., Mangiacavalli, S., Lazzarino, M. & Bernasconi, C. (2000) Blood stem cell collections in multiple myeloma: definition of a scoring system. *Bone Marrow Transplantation*, **26**, 283–286.
- Demirer, T., Buckner, C.D., Gooley, T., Rowley, S., Chauncey, T., Lilleby, K., Storb, R. & Bensinger, W.I. (1996) Factors influencing collection of peripheral blood stem cells in patients with multiple myeloma. *Bone Marrow Transplantation*, **17**, 937–941.
- Desikan, K.R., Tricot, G., Munshi, N.C., Anaissie, E., Spoon, D., Fassas, A., Toor, A., Zangari, M., Badros, A., Morris, C., Vesole, D.H., Siegel, D., Jagannath, S. & Barlogie, B. (2001) Preceding chemotherapy, tumour load and age influence engraftment in multiple myeloma patients mobilized with granulocyte colony-stimulating factor alone. *British Journal of Haematology*, **112**, 242–247.
- Fagiolo, U., Cossarizza, A., Scala, E., Fanales-Belasio, E., Ortolani, C., Cozzi, E., Monti, D., Franceschi, C. & Paganelli, R. (1993) Increased cytokine production in mononuclear cells of healthy elderly people. *European Journal of Immunology*, **23**, 2375–2378.
- Globerson, A. (1999) Hematopoietic stem cells and aging. *Experimental Gerontology*, **34**, 137–146.
- Goldschmidt, H., Hegenbart, U., Wallmeier, M., Hohaus, S. & Haas, R. (1997) Factors influencing collection of peripheral blood progenitor cells following high-dose cyclophosphamide and granulocyte colony-stimulating factor in patients with multiple myeloma. *British Journal of Haematology*, **98**, 736–744.
- Guba, S.C., Vesole, D.H., Jagannath, S., Bracy, D., Barlogie, B. & Tricot, G. (1997) Peripheral stem cell mobilization and engraftment in patients over age 60. *Bone Marrow Transplantation*, **20**, 1–3.
- Kaplan, E.L. & Meier, P. (1958) Nonparametric estimation from incomplete observations. *Journal of the American Statistical Association*, **53**, 457–481.
- Ketterer, N., Salles, G., Moullet, I., Dumontet, C.E.I., Jaafari-Corbin, A., Tremisi, P., Thieblemont, C., Durand, B., Neidhardt-Berard, E., Samaha, H., Rigal, D. & Coiffier, B. (1998) Factors associated with successful mobilization of peripheral blood progenitor cells in 200 patients with lymphoid malignancies. *British Journal of Haematology*, **103**, 235–242.
- Marit, G., Thiessard, F., Faberes, C., Cony-Makhoul, P., Boiron, J.M., Bernard, P., Pigneux, A., Puntous, M., Agape, P., Vezon, G., Broustet, A., Girault, D., Salmi, L.R. & Reiffers, J. (1998) Factors affecting both peripheral blood progenitor cell mobilization and hematopoietic recovery following autologous blood progenitor cell transplantation in multiple myeloma patients: a monocentric study. *Leukemia*, **12**, 1447–1456.
- Palumbo, A., Triolo, S., Argentino, C., Bringhen, S., Dominietto, A., Rus, C., Omede, P., Tarella, C., Pileri, A. & Boccadoro, M. (1999) Dose-intensive melphalan (MEL100) with stem cell support is superior to standard treatment in elderly myeloma patients. *Blood*, **94**, 1248–1253.
- Pecora, A.L., Preti, R.A., Gleim, G.W., Jennis, A., Zahos, K., Cantwell, S., Doria, L., Isaacs, R., Gillio, A.P., Michelis, M.A. & Brochstein, J.A. (1998) CD34⁺CD33⁻ cells influence days to engraftment and transfusion requirements in autologous blood stem-cell recipients. *Journal of Clinical Oncology*, **16**, 2093–2104.
- Perea, G., Sureda, A., Martino, R., Altes, A., Martinez, C., Cabezudo, E., Amill, B., Martin-Henao, G.A., Gonzalez, Y., Munoz, L., Peyret, M., Brunet, S. & Sierra, J. (2001) Predictive factors for a successful mobilization of peripheral blood CD34⁺ cells in multiple myeloma. *Annals of Hematology*, **80**, 592–597.
- Siegel, D.S., Desikan, K.R., Mehta, J., Singhal, S., Fassas, A., Munshi, N., Anaissie, E., Naucke, S., Ayers, D., Spoon, D., Vesole, D.,

- Tricot, G. & Barlogie, B. (1999) Age is not a prognostic variable with autotransplants for multiple myeloma. *Blood*, **93**, 51–54.
- Stokes, M.E., Davis, C.S. & Koch, G.G. (2000) Chapter 10. In: *Categorical Data Analysis using the SAS System*, 2nd edn, pp. 271–322. SAS Institute, Cary: NC.
- Tricot, G. (2001) Angiochemotherapy (ACT) for multiple myeloma (MM) with DT-PACE results in a high response rate, but in contrast to tandem transplants with melphalan does not affect durable disease control. *Blood*, **98**, 3531a.