Bone Marrow Transplantation (2009), 1–6
© 2009 Macmillan Publishers Limited All rights reserved 0268-3369/09 $32.00
www.nature.com/bmt

ORIGINAL ARTICLE

Safety and efficacy assessment of plerixafor in patients with multiple myeloma proven or predicted to be poor mobilizers, including assessment of tumor cell mobilization

G Tricot[1], MH Cottler-Fox[2] and G Calandra[3]

[1]*Department of Medicine, University of Utah, Salt Lake City, UT, USA;* [2]*Department of Pathology and Myeloma, Institute for Research and Therapy, University of Arkansas for Medical Sciences, Little Rock, AR, USA and* [3]*Formerly AnorMED, Inc., now Genzyme Corporation, Cambridge, MA, USA*

This was an open-label, single-center, phase II study of 20 patients with multiple myeloma who were either proven poor mobilizers ($n = 10$; group A) or predicted poor mobilizers ($n = 10$; group B) and were planned for autologous hematopoietic SCT. The aim was to assess the safety and efficacy of plerixafor for stem cell mobilization and tumor cell contamination. The peripheral blood (PB) CD34+ cell count was generally very low pre-plerixafor and increased significantly post-plerixafor administration. Cumulative apheresis yields of $\geqslant 2 \times 10^6$ CD34+ cells/kg were observed in 7 of 10 patients (group A) and 8 of 10 patients (group B). Among the proven poor mobilizers, there was no evidence of tumor cell mobilization in the PB after G-CSF plus plerixafor treatment. Seventeen of 20 (85%) patients underwent transplantation. Neutrophil engraftment occurred at a median of 13 days for all patients. Platelet engraftment occurred at a median of 16 days and 19 days for all proven and predicted poor mobilizers, respectively. At 12 months, 12 of 17 patients had documented durable grafts, 3 of 17 patients died and 2 of 17 patients were lost to follow-up; but they had documented graft durability at the previous 3- and 6-month visit. The safety profile of plerixafor in all patients was consistent with previous reports.

Bone Marrow Transplantation advance online publication, 22 June 2009; doi:10.1038/bmt.2009.130

Keywords: AMD3100; mobilization; multiple myeloma; autologous transplant; tumor cell contamination; plerixafor

Introduction

High-dose chemotherapy with autologous hematopoietic SCT has been shown in clinical trials to improve response rate and overall survival in patients with multiple myeloma (MM) compared with conventional chemotherapy.[1–3] However, to use this strategy, sufficient cells must be mobilized and collected for transplantation and there is a potential risk of contamination by residual tumor cells in the apheresis product. Ideally, there should not be any tumor cells in the apheresis product. Tumor cell contamination does occur and there is no consensus on the acceptable and safe limit of tumor cells in the apheresis product.[4–11] There are various assays to quantify tumor cell contamination with sensitivities ranging from 1/100 cells using flow cytometry to 1/100 000 cells using PCR.[12] Although PCR testing is the superior method in terms of sensitivity, it is impractical for clinical practice as it is expensive, time consuming and requires patient-specific primers to be produced from myeloma cell samples.[12] Compared with PCR testing, assessment by flow cytometry has the advantages of being less expensive, less time consuming, easier to perform, and providing confirmation about 'potentially' clinically relevant contamination.

Plerixafor, a reversible CXCR4 inhibitor, has been reported to improve stem cell collection in poor mobilizers who require autologous hematopoietic SCT for various indications.[13,14] Although initial experience with plerixafor seemed to be safe in poor mobilizers, there is lack of information on tumor cell mobilization with plerixafor in this population.

This is the first study that specifically addresses tumor cell mobilization in MM patients treated with plerixafor.

The purpose of this phase II study was to evaluate the safety and efficacy of plerixafor in patients with MM who were proven or predicted poor mobilizers and specifically to assess tumor cell mobilization in proven poor mobilizers using flow cytometry.

Patients and methods

Study design

This was an open-label single-center phase II study. Patients with MM who were scheduled for treatment with autologous peripheral blood (PB) hematopoietic stem cell transplantation and who were proven or predicted poor

Correspondence: Dr G Tricot, Department of Medicine, Utah Blood and Marrow Transplant and Myeloma Program, University of Utah, Huntsman Cancer Institute, 2000 Circle of Hope, SOM Room 5C402, Salt Lake City, UT 84112, USA.
E-mail: guido.tricot@hsc.utah.edu
Received 15 December 2008; revised 24 April 2009; accepted 24 April 2009

mobilizers were eligible. The study was IRB approved and consisted of two phases: the first phase included patients who were proven to be poor mobilizers (group A) and the second phase included patients who were predicted to be poor mobilizers (group B). Enrollment in group A was completed before enrollment in group B began.

The main objectives of the study were to determine whether the addition of plerixafor to a mobilizing regimen of granulocyte-CSF (G-CSF) would improve the yield of CD34+ cells obtained in the apheresis product and whether the cells collected would engraft after autologous transplantation. An additional objective was to determine the presence of tumor cells in the apheresis product.

Study population

All patients who entered the study signed an informed consent and were between 18 and 75 years of age with a diagnosis of MM, previously treated or untreated irrespective of BM plasmacytosis. Other inclusion criteria included: plt count $\geqslant 100\,000/\mu l$ and serum creatinine <3 mg per 100 ml, normal liver function tests, left ventricle ejection fraction $>45\%$ and carbon monoxide diffusing capacity $>45\%$ with forced expiratory volume in the first second $>50\%$ predicted, WBC count >2000 cells/μl, hematocrit $>26\%$ and negative test for the HIV. Women of childbearing potential were required to be on an approved form of contraception.

In addition to the study inclusion criteria, patients enrolled into groups A and B had to meet group-specific criteria. Patients enrolled in group A were required to have received a previous mobilization from which $<2\times10^6$ cells/kg of CD34+ cells were collected for transplantation. Patients enrolled in group B were required to have received previous chemotherapy and G-CSF treatment as a mobilization regimen and, after recovery of their WBC count (>2000 cells/μl for at least 2 days), had a PB CD34+ count of 5–12 cells/μl or were required to have received extensive earlier chemotherapy and a pre-mobilization plt count of 100 000–150 000/μl (two measurements obtained within 1 week). Patients fulfilling either of the criteria were predicted to yield total apheresis collections of $<1\times10^6$ CD34+ cells/kg, which would be insufficient for transplantation.

Patients were excluded if they had any one of the following: a significant comorbid condition that rendered them at high risk for treatment complications; a residual acute medical condition resulting from an earlier chemotherapy; cardiovascular disease that included proof of or predisposition to ventricular arrhythmias, brain metastases or carcinomatous meningitis, active infection, fever and hypercalcemia; body weight exceeding 150% of their ideal body weight; earlier treatment with an experimental therapy within 4 weeks of enrolling in this study; or enrollment in another experimental protocol.

Study treatment

There was a minimum of 4 weeks' recovery time from the last cytotoxic chemotherapy to the start of G-CSF for mobilization for patients in group A. All patients received a mobilization regimen of G-CSF (administered according to the standard procedures at the study center) plus plerixafor. The G-CSF (10 μg/kg) was administered daily, pre-apheresis, for up to 7 consecutive days or until a total of $\geqslant5\times10^6$ CD34+ cells/kg, but $<15\times10^6$ CD34+ cells/kg were collected. Plerixafor (0.24 mg/kg) was administered subcutaneously daily in the evening, ~10–11 h before each apheresis, starting on Day 1 and for up to 7 consecutive days or until $\geqslant5\times10^6$ $CD34^+$ cells/kg were collected.

Efficacy and safety assessment

The criteria used for efficacy evaluation were PB CD34+ cell count, apheresis yield, neutrophil and plt engraftment and graft durability. Within-patient changes in PB CD34+ cells/μl from pre-plerixafor administration to immediately before the next apheresis session were evaluated using a fold increase calculation. Follow-up data were gathered for neutrophil and plt engraftment and graft durability up to 12 months post transplant. The day of neutrophil engraftment was defined as the first of 3 consecutive days with an ANC of $>500/\mu l$; plt engraftment day was defined as the first of 3 consecutive days with an untransfused plt count of $>20\,000/\mu l$. In the event of a tandem transplant, follow-up data were gathered up to 12 months after the second transplant. The criteria used for safety evaluation, included adverse event monitoring, clinical laboratory evaluations, physical examination, vital signs and injection site assessments. Graft durability was determined by the investigator. As a guideline, the protocol defined graft durability as the maintenance of normal (acceptable) blood counts at 3, 6 and 12 months post transplantation.

Tumor cell mobilization

Peripheral blood samples were analyzed for the presence of aneuploid myeloma cells by flow cytometry measuring the specific cytoplasmic Ig light chain content vs DNA content as has been described previously.[15] This method can detect the presence of $<1\%$ abnormal plasma cells in BM or PB.

Statistical methods

Categorical data were summarized using frequency tables, presenting the patient counts and the percentage of patients falling into the category. Continuous data were summarized and reported as mean, s.d., median, minimum and maximum. SAS software was used to perform all analyses.

Results

Patient demographics and characteristics

Patient demographics and baseline characteristics are summarized in Table 1. The mean age was 60.7 years in group A (proven poor mobilizers) and 55.9 years in group B (predicted poor mobilizers). All 20 patients were Caucasian. A total of 7 patients in group A (70.0%) and 4 patients in group B (40.0%) were female. The median time from confirmed diagnosis to study entry was 10.5 months in group A and 5.5 months in group B. All patients had received earlier chemotherapy. A total of 3 patients in group A (30.0%) and 1 patient in group B (10.0%) had previously received radiotherapy.

Table 1 Patient demographics and baseline characteristics

	Group A (N = *10*)	*Group B* (N = *10*)	*All patients* (N = *20*)
Gender			
Female (%)	7 (70.0)	4 (40.0)	11 (55.0)
Male (%)	3 (30.0)	6 (60.0)	9 (45.0)
Age (years)	60.7 ± 6.6	55.9 ± 11.4	58.3 ± 9.4
Ethnic group			
Caucasian, *N* (%)	10 (100.0)	10 (100.0)	20 (100.0)
Pre-treatment weight (kg)	75.3 ± 16.1	78.4 ± 21.1	76.9 ± 18.3
Stage at study entry, N *(%)*			
I	1 (10.0)	1 (10.0)	2 (10.0)
II	0 (0.0)	2 (20.0)	2 (10.0)
III	8 (80.0)	6 (60.0)	14 (70.0)
IV	0 (0.0)	0 (0.0)	0 (0.0)
Months since diagnosis	17.4 ± 18.4	19.0 ± 35.5	18.2 ± 27.5
Mean (s.d.)	17.4 (18.4)	19.0 (35.5)	18.2 (27.5)
Median	10.5	5.5	8.0
Minimum, maximum	(4.0,63.0)	(1.0,117.0)	(1.0,117.0)
Earlier chemotherapy, *N* (%)	10 (100.0)	10 (100.0)	20 (100.0)
Earlier radiotherapy, *N* (%)	3 (30.0)	1 (10.0)	4 (20.0)

Table 2 Pre-plerixafor and post-plerixafor absolute peripheral blood CD34+ cell count and fold increase summary (cells/μl)

	Group A (N = *10*)	*Group B* (N = *10*)	*All patients* (N = *20*)
Pre-plerixafor injection			
Mean (s.d.)	1.9 (1.1)	2.6 (1.6)	2.3 (1.4)
Median	1.7	3.2	2.1
Minimum, maximum	(0.8, 4.5)	(0.4, 4.9)	(0.4, 4.9)
Post-plerixafor (pre-first apheresis)			
Mean (s.d.)	8.3 (8.0)	7.0 (4.9)	7.6 (6.5)
Median	5.8	7.7	6.0
Minimum, maximum	(2.3, 29.7)	(1.3, 14.7)	(1.3, 29.7)
Fold increase from baseline			
Mean (s.d.)	4.2 (1.9)	2.9 (1.0)	3.6 (1.6)
Median	4.0	3.0	3.0
Minimum, maximum	(1.8, 6.6)	(0.5, 4.7)	(0.5, 6.6)

Peripheral blood CD34+ cells and apheresis yield

The PB CD34+ cell count was generally very low pre-plerixafor treatment, median 2.1 (range 0.4–4.9 cells/ul) (Table 2). These low PB CD34+ cell counts indicated that all patients in both groups had mobilized poorly with either G-CSF alone or G-CSF and chemotherapy before plerixafor treatment. Peripheral blood CD34+ cell counts increased significantly post-plerixafor administration from a median 2.1 (range 0.4–3.9) cells/μl to 6 (range 1.3–29.7) CD34+ cells/μl. The median fold increase in PB CD34+ cell count was 3.0 for the entire study population, 4.0-fold for group A and 3.0-fold for group B.

In group A, 7 of 10 (70.0%) patients achieved cumulative apheresis yields of $\geqslant 2 \times 10^6$ CD34+ cells/kg. In group B, 8 of 10 (80.0%) patients achieved cumulative apheresis yields of $\geqslant 2 \times 10^6$ CD34+ cells/kg. Three patients in group B were apheresed before receiving plerixafor. After the first dose of plerixafor treatment, all three patients had an immediate increase in the number of CD34+ cells collected. One patient in group B underwent two series of apheresis with G-CSF and plerixafor ~1 month apart. A summary of the CD34+ cells collected by apheresis is shown in Tables 3 and 4 and Figure 1.

Table 3 Apheresis yields (CD34+ cells $\times 10^6$) by study day in proven poor mobilizers in group A

Patient	*Day 1*	*Day 2*	*Day 3*	*Day 4*	*Day 5*	*Day 6*	*Day 7*	*Total*
201	0.30	0.25	0.19	ND	ND	ND	ND	0.74
202	0.41	0.73	0.30	0.17	ND	ND	ND	1.61
203	1.94	2.16	1.52	0.96	0.57	ND	ND	7.15
204	1.06	1.16	1.05	0.93	0.86	0.90	ND	5.96
205[a]	0.33	0.30	0.38	0.37	0.37	0.27	0.53	2.55
206[a]	0.33	0.19	0.17	0.17	0.27	0.20	0.21	1.54
207[a]	0.78	0.64	0.47	0.50	0.50	0.26	0.46	3.61
208	1.73	2.57	1.26	0.64	0.93	0.51	0.40	8.04
209	0.13	0.20	0.60	0.54	0.20	0.30	0.23	2.20
210	2.83	4.10	3.21	2.66	ND	ND	ND	12.80

Abbreviation: ND = not done.
[a]Patients 01–205, 01–206 and 01–207 were treated with plerixafor but were not transplanted.

Transplantation and engraftment

Of the 20 patients enrolled, 17 patients (7 in group A and 10 in group B) proceeded to transplantation. A total of 11 patients (5 in group A and 6 in group B) received a second transplant. In group A, three patients did not receive a transplant. Of these three patients, only one patient achieved a cumulative yield that was insufficient for transplant (1.54×10^6 CD34+ cells/kg). The other two patients yielded a total of 2.55×10^6 CD34+ cells/kg and 3.61×10^6 CD34+ cells/kg, respectively, but decided not to proceed with transplantation immediately. Transplantation using pooled cells from other collections was allowed and occurred in eight patients.

Neutrophil engraftment occurred at a median of 13 days for all patients. The range was 10–19 days in group A and 11–23 days in group B. Plt engraftment was documented for all patients in group A (median 16 days; range 11–84) and for all patients in group B (median 19 days; range 14–98).

Follow-up assessments were completed up to 12 months post transplant for 12 of 17 (70.6%) patients (4 patients in group A and 8 patients in group B). All 12 patients assessed at 12 months post transplant were determined to have a durable graft. Of the remaining five patients, two patients died from disease progression and both had durable grafts, one patient died of cerebral infarction but had no durability assessment before death, and two patients did not have follow-up data at 12 months but had documentation of durable grafts at earlier follow-up study visits.

Tumor cell mobilization

Tumor cell mobilization was evaluated in 10 patients in group A. Evaluation of BM involvement at the time of

Table 4 Apheresis yields (CD34+ cells $\times 10^6$) by study day in predicted poor mobilizers in group B

Patient	*Day 1*	*Day 2*	*Day 3*	*Day 4*	*Day 5*	*Day 6*	*Day 7*	*Day 8*	*Day 9*	*Day 10*	*Total*
400	0.72	0.64	0.35	0.58	0.58	0.88	0.78	ND	ND	ND	4.53
401	0.46	0.27	0.17	0.13	0.08	ND	ND	ND	ND	ND	1.11
402	1.03	1.31	0.72	1.23	1.12	1.07	1.15	ND	ND	ND	7.63
403[a,b]	0.30*	0.40*	0.70*	1.70	0.27	ND	ND	ND	ND	ND	3.37
	0.20	0.40	0.20	0.23	0.21	0.20	0.21	ND	ND	ND	1.65
404[b]	1.77*	0.60*	0.60*	1.50	1.40	1.12	0.99	1.30	1.00	0.90	11.18
405[b]	1.16*	0.96*	3.45	2.71	3.37	2.33	2.22	2.14	1.60	ND	19.94
406	0.34	0.31	0.28	0.36	0.17	0.20	0.26	ND	ND	ND	1.92
407	0.68	0.46	0.79	0.96	0.30	0.27	0.28	ND	ND	ND	3.74
408	2.77	1.48	0.97	0.49	0.79	0.90	ND	ND	ND	ND	7.40
409	1.60	1.40	0.90	0.51	0.45	0.69	ND	ND	ND	ND	5.55

Abbreviation: ND = not done.
[a]Patient 403 underwent two rounds of G-CSF/plerixafor treatment ~1 month apart. Apheresis yields after the first round of treatment are listed on line 1 and yields after the second round of treatment are listed on line 2.
[b]Aphereses before first dose of plerixafor are indicated with an asterisk.

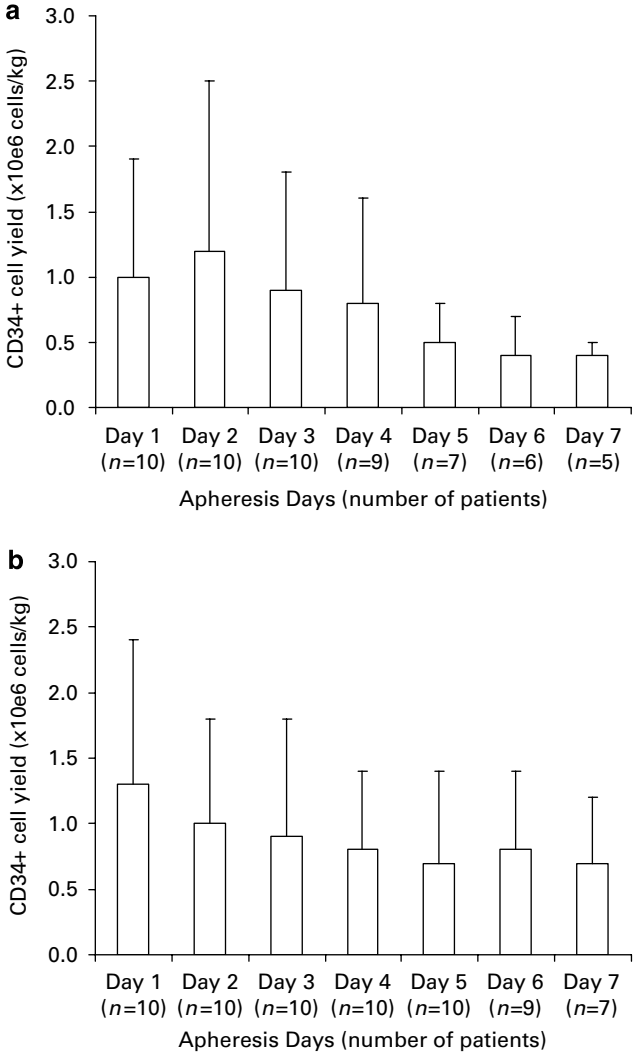

Figure 1 Apheresis yields by study day in (**a**) proven poor mobilizers (group A) and (**b**) predicted poor mobilizers (group B). Columns and error bars represent mean cell yield and 1 s.d., respectively.

mobilization showed no evidence of BM disease in four patients and minimal (5–10%) BM involvement in four other patients. Of the remaining two patients, one patient had moderate marrow involvement by myeloma (40% of cellularity was plasmacytosis), whereas the other had extensive marrow involvement by myeloma (70% of cellularity was plasmacytosis).

A total of 50 analyses of PB CD34+ cell collections were performed on samples drawn from 10 patients in group A at time points throughout the mobilization with plerixafor. Of the 50 samples analyzed, 46 were drawn at time points of interest or were not duplicates. Tumor cell mobilization was assessed from PB samples taken before the first dose of plerixafor and before the subsequent apheresis. Baseline samples were collected for all patients for comparison post-plerixafor treatment. A total of nine paired samples were available for analysis pre- and post first dose of plerixafor and nine paired samples (from the same set of patients) were available for analysis pre- and post second dose of plerixafor. As the frequency of apheresis is related to an increased risk of tumor contamination, the serial collections of paired samples allow for true assessment of tumor contamination. None of the PB samples showed >1% light-chain-restricted cells, except for the first collection product of patient 5, which had 2% kappa cells (the relevant light chain) with a DNA index of 1 and 1% lambda cells with a DNA index of 1. No plasma cells with aneuploid DNA content were observed in any of the samples.

Safety assessment

All 20 patients experienced at least one adverse event (AE). One patient (5.0%) experienced AEs of mild intensity, seven patients (35.0%) experienced AEs of moderate intensity and 12 patients (60.0%) experienced AEs of severe intensity. The number of patients who experienced AEs considered possibly, probably or definitely related to the study drug were 2 (10.0%), 8 (40.0%) and 9 (45.0%), respectively. All drug-related AEs were mild-or-moderate in intensity.

Overall, the most common drug-related AEs that were experienced by ⩾2 patients were injection site erythema (17 patients), injection site reaction (3 patients), fatigue (2 patients), injection site pruritus (2 patients), injection site swelling (2 patients), diarrhea (3 patients) and hot

flushes (2 patients). It should be noted that one patient in group B had a serum creatinine level of 3.1 mg per 100 ml before study entry. This patient did not experience any AEs that were different from those experienced by the other patients.

A total of 25 serious AEs were experienced by eight patients in this study. All serious AEs were considered to be not related to the study drug. One patient discontinued the study because of the development of bundle branch block, bradycardia and hyperhydrosis, which were not related to the study drug.

Discussion

In this group of proven and predicted poor mobilizers, successful mobilization was achieved in 15 of 20 (75%) patients. This successful mobilization rate is similar to that of MM patients in the plerixafor compassionate use program.[14] Although most of the patients yielded sufficient CD34+ cells to proceed to transplant ($\geqslant 2 \times 10^6$ cells/kg), many required more than 5 days (up to 7 days) of apheresis. The fact that these patients had very low PB CD34+ cell counts before plerixafor treatment, and required so many aphereses, suggests that this group was even more severely impaired than those patients described in the compassionate use program. The safety profile of plerixafor in these two patient groups was consistent with previous reports.[13,14] Importantly, consistent with other studies, the cells collected after plerixafor treatment resulted in prompt and durable engraftment.[13,14]

Perhaps, the most important finding of this study was the absence of tumor cell mobilization after treatment with G-CSF and plerixafor. The assay used in this study to assess tumor cell contamination has a sensitivity of $<1/100$ cells. The results showed that none of the 10 patients with MM mobilized tumor cells after 2 days of apheresis. Importantly, serial samplings during consecutive apheresis procedures remained negative. One patient was analyzed for 3 days but showed no tumor cell mobilization.

The small number of patients tested in both studies limits the conclusions to be drawn; hence, the possibility of tumor cell mobilization in some MM patients cannot be excluded. It remains possible that there are differences in the kinetics of myeloma cell and CD34+ cell mobilization in the PB. Previous studies of tumor cell mobilization after plerixafor treatment showed no evidence of lymphoma cells in a small series of patients with non-Hodgkin's lymphoma.[16] Plerixafor has been reported to mobilize leukemia cells from patients with AML.[17] Thus, plerixafor should not be used for stem cell mobilization for autologous transplant in patients with AML and other leukemias.

Other methods used to mobilize stem cells, such as chemotherapy, and cytokines can also mobilize tumor cells and lead to significant contamination of stem cell products.[18–21] The kinetics of tumor cell mobilization, the underlying residual tumor burden in the patient, and the mobilization method used are important factors that affect the contamination of the apheresis product. Whether tumor cells are mobilized into PB at the same time as $CD34^+$ cells remains controversial. Several groups have reported that higher proportions of hematopoietic progenitor cells are observed early during apheresis (first 2 days), whereas peak levels of myeloma cells occur in the latter days of apheresis.[7,12,22] However, other studies have found no variation in the kinetics of tumor cell and $CD34^+$ stem cell mobilization.[7,20,23,24]

Studies investigating the effect of the mobilization regimen on tumor cell contamination of the apheresis product have also yielded conflicting results. In one study, mobilization with G-CSF alone resulted in tumor cell contamination in 25% patients, whereas chemomobilization resulted in tumor cell contamination in 51% patients.[18] In contrast, the study by Cremer *et al*.[25] suggests decreased tumor cell contamination after mobilization with high-dose CY plus G-CSF compared with G-CSF alone.

In summary, this is the first report using serial monitoring of PB after a series of aphereses, in which plerixafor does not seem to mobilize tumor cells. Plerixafor was generally well-tolerated and effective in mobilizing CD34+ cells in patients with MM who were proven or predicted to not mobilize sufficient cells for transplant.

Conflict of interest

TG received research support from formerly AnorMed Inc. GC was an employee of AnorMed Inc. GC is currently a consultant for Genzyme Corporation.

Acknowledgements

The study was conducted at the University of Arkansas for Medical Sciences, Little Rock, AR 72205, USA. TG designed the study, recruited the patients, gathered and interpreted the data, and prepared the paper; MC designed the study, recruited the patients, gathered and interpreted the data, and prepared the paper; GC designed the study, interpreted the data, and prepared the paper.

References

1 Attal M, Harousseau J-L, Stoppa A-M, Sotto J-J, Fuzibet J-G, Rossi J-F *et al*. A prospective, randomized trial of autologous bone marrow transplantation and chemotherapy in multiple myeloma. *N Engl J Med* 1996; **335**: 91–97.
2 Child JA, Morgan GJ, Davies FE, Owen RG, Bell SE, Hawkins K *et al*. High-dose chemotherapy with hematopoietic stem-cell rescue for multiple myeloma. *N Engl J Med* 2003; **348**: 1875–1883.
3 Fermand J-P, Katsahian S, Divine M, Leblond V, Dreyfus F, Macro M *et al*. High-dose therapy and autologous blood stem-cell transplantation compared with conventional treatment in myeloma patients aged 55–65 years: long-term results of a randomized control trial from the group myelome-autogreffe. *J Clin Oncol* 2005; **23**: 9227–9233.
4 Craig JI, Langlands K, Parker AC, Anthony RS. Molecular detection of tumor contamination in peripheral blood stem cell harvests. *Exp Hematol* 1994; **22**: 898–902.
5 Gazitt Y, Reading CC, Hoffman R, Wickrema A, Vesole DH, Jagannath S *et al*. Purified CD34+ Lin− Thy+ stem cells do not contain clonal myeloma cells. *Blood* 1995; **86**: 381–389.

6 Dreyfus F, Ribrag V, Leblond V, Ravaud P, Melle J, Quarre MC *et al*. Detection of malignant B cells in peripheral blood stem cell collections after chemotherapy in patients with multiple myeloma. *Bone Marrow Transplant* 1995; **15**: 707–711.
7 Lemoli RM, Fortuna A, Motta MR, Rizzi S, Giudice V, Nannetti A *et al*. Concomitant mobilization of plasma cells and hematopoietic progenitors into peripheral blood of multiple myeloma patients: positive selection and transplantation of enriched CD34+ cells to remove circulating tumor cells. *Blood* 1996; **87**: 1625–1634.
8 Boccadoro M, Omedé P, Dominietto A, Palumbo A, Bringhen S, Giaretta F *et al*. Multiple myeloma: the number of reinfused plasma cells does not influence outcome of patients treated with intensified chemotherapy and PBPC support. *Bone Marrow Transplant* 2000; **25**: 25–29.
9 Lopez-Perez R, Garcia-Sanz R, Gonzalez D, Balanzategui A, Chillon MC, Alaejos I *et al*. Gene scanning of VDJH-amplified segments is a clinically relevant technique to detect contaminating tumor cells in the apheresis products of multiple myeloma patients undergoing autologous peripheral blood stem cell transplantation. *Bone Marrow Transplant* 2001; **28**: 665–672.
10 Galimberti S, Morabito F, Guerrini F, Palumbo GA, Azzara A, Martino M *et al*. Peripheral blood stem cell contamination evaluated by a highly sensitive molecular method fails to predict outcome of autotransplanted multiple myeloma patients. *Br J Haematol* 2003; **120**: 405–412.
11 Vogel W, Kopp H, Kanz L, Einsele H. Myeloma cell contamination of peripheral blood stem-cell grafts can predict the outcome in multiple myeloma patients after high-dose chemotherapy and autologous stem-cell transplantation. *J Cancer Res Clin Oncol* 2005; **131**: 214–218.
12 Gazitt Y, Tian E, Barlogie B, Reading CL, Vesole DH, Jagannath S *et al*. Differential mobilization of myeloma cells and normal hematopoietic stem cells in multiple myeloma after treatment with cyclophosphamide and granulocyte-macrophage colony-stimulating factor. *Blood* 1996; **87**: 805–811.
13 Flomenberg N, Devine S, Dipersio J, Liesveld J, McCarty J, Rowley S *et al*. The use of AMD3100 plus G-CSF for autologous hematopoietic progenitor cell mobilization is superior to G-CSF alone. *Blood* 2005; **106**: 1867–1874.
14 Calandra G, McCarty J, McGuirk J, Tricot G, Crocker SA, Badel K *et al*. AMD3100 plus G-CSF can successfully mobilize CD34+ cells from non-Hodgkin's lymphoma, Hodgkin's disease and multiple myeloma patients previously failing mobilization with chemotherapy and/or cytokine treatment: compassionate use data. *Bone Marrow Transplant* 2007; **41**: 331–338.
15 Barlogie B, Alexanian R, Pershouse M, Smallwood L, Smith L. Cytoplasmic immunoglobulin content in multiple myeloma. *J Clin Invest* 1985; **76**: 765–769.
16 Gazitt Y, Freytes CO, Akay C, Badel K, Calandra G. Improved mobilization of peripheral blood CD34(+) cells and dendritic cells by AMD3100 plus granulocyte-colony-stimulating factor in non-Hodgkin's lymphoma patients. *Stem Cells Dev* 2007; **16**: 657–666.
17 Andreeff M, Konoplev S, Wang R-Y, Zeng Z, McQueen T, Shi Y-X *et al*. Massive mobilization of AML cells into circulation by disruption of leukemia/stroma cell interactions using CXCR4 antagonist AMD3100: first evidence in patients and potential for abolishing bone marrow microenvironment-mediated resistance. *Blood (ASH Annual Meeting Abstracts)* 2006; **108**: 568.
18 Anagnostopoulos A, Aleman A, Yang Y, Donato M, Weber D, Champlin R *et al*. Outcomes of autologous stem cell transplantation in patients with multiple myeloma who received dexamethasone-based nonmyelosuppressive induction therapy. *Bone Marrow Transplant* 2004; **33**: 623–628.
19 Kopp HG, Yildirim S, Weisel KC, Kanz L, Vogel W. Contamination of autologous peripheral blood progenitor cell grafts predicts overall survival after high-dose chemotherapy in multiple myeloma. *J Cancer Res Clin Oncol* 2009; **135**: 637–642.
20 Mateo G, Corral M, Almeida J, Lopez-Berges C, Nieto J, Garcia-Marcos A *et al*. Immunophenotypic analysis of peripheral blood stem cell harvests from patients with multiple myeloma. *Haematologica* 2003; **88**: 1013–1021.
21 Franklin WA, Glaspy J, Pflaumer SM, Jones RB, Hami L, Martinez C *et al*. Incidence of tumor-cell contamination in leukapheresis products of breast cancer patients mobilized with stem cell factor and granulocyte colony-stimulating factor (G-CSF) or with G-CSF alone. *Blood* 1999; **94**: 340–347.
22 Desikan KR, Jagannath S, Siegel D, Nelson J, Bracy D, Barlogie B *et al*. Collection of more hematopoietic progenitor cells with large volume leukapheresis in patients with multiple myeloma. *Leuk Lymphoma* 1998; **28**: 501–508.
23 Knudsen LM, Rasmussen T, Nikolaisen K, Johnsen HE. Mobilisation of tumour cells along with CD34+ cells to peripheral blood in multiple myeloma. *Eur J Haematol* 2001; **67**: 289–295.
24 Kiel K, Cremer FW, Ehrbrecht E, Wallmeier M, Hegenbart U, Goldschmidt H *et al*. First and second apheresis in patients with multiple myeloma: no differences in tumor load and hematopoietic stem cell yield. *Bone Marrow Transplant* 1998; **21**: 1109–1115.
25 Cremer FW, Kiel K, Wallmeier M, Haas R, Goldschmidt H, Moos M. Leukapheresis products in multiple myeloma: lower tumor load after mobilization with cyclophosphamide plus granulocyte colony-stimulating factor (G-CSF) compared with G-CSF alone. *Exp Hematol* 1998; **26**: 969–975.