QUERY FORM

BMT	
Manuscript ID	**BMT-2012-658 [Art. Id: bmt2012269]**
Author	
Editor	
Publisher	

Journal: BMT

Author :- The following queries have arisen during the editing of your manuscript. Please answer queries by making the requisite corrections at the appropriate positions in the text.

Query No.	Description	Author's Response
Q1	Please confirm if the edit to the sentence 'Furthermore, patients planned..' are ok.	
Q2	Please check the sentence 'On the other hand..' for clarity.	
Q3	Please provide volume number and page range for reference [28].	
Q4	Please provide the expansion of 'VGPR'.	

Bone Marrow Transplantation (2012), 1–7

www.nature.com/bmt

ORIGINAL ARTICLE

Intermediate-dose Ara-C plus G-CSF for stem cell mobilization in patients with lymphoid malignancies, including predicted poor mobilizers

S Giebel[1], T Kruzel[1], T Czerw[1], M Sadus-Wojciechowska[1], J Najda[1], E Chmielowska[2,3], S Grosicki[4], A Jurczyszyn[5], M Pasiarski[6], E Nowara[7], M Glowala-Kosinka[1], A Chwieduk[1], I Mitrus[1], A Smagur[1] and J Holowiecki[1]

The optimal protocol for mobilization of hematopoietic stem cells in patients with lymphoid malignancies has not been determined so far. We retrospectively analyzed the efficacy and safety of Ara-C at a dose of 1.6 g/m^2 compared with CY at a dose of 4.0 g/m^2, both combined with filgrastim. Seventy and fourty-five patients, respectively, were included, among whom 60% were defined as 'predicted poor mobilizers'. The use of Ara-C was associated with significantly higher peak number of circulating CD34$^+$ cells compared with CY ($P<0.0001$). In the Ara-C group, 95% of patients with multiple myeloma (MM) collected at least 5×10^6 CD34$^+$ cells/kg required for tandem transplantation, and 97% of lymphoma patients collected at least 2×10^6 CD34$^+$ cells/kg, needed for a single autologous hematopoietic SCT (autoHSCT), which was achieved with a single leukapheresis in 91% of cases. Results for the CY group were significantly inferior ($P<0.0001$). No patient mobilized with Ara-C experienced febrile neutropenia, whereas 35% required platelet transfusions. Among patients who proceeded to autoHSCT, the time of both neutrophil and platelet recovery was significantly shorter for those mobilized with Ara-C than CY. We conclude that intermediate-dose Ara-C + filgrastim is a very effective and relatively safe mobilization protocol for patients with lymphoid malignancies.

Bone Marrow Transplantation (2012) **0,** 000–000. doi:10.1038/bmt.2012.269

Keywords: SCT; mobilization; Ara-C; CY

INTRODUCTION

Autologous hematopoietic SCT (autoHSCT) is a standard treatment of patients with multiple myeloma (MM) and selected patients with Hodgkin's (HL) or non-Hodgkin's lymphoma (NHL). Currently, 99% of the procedures are performed using peripheral blood as a source of stem cells.[1] The minimal number of CD34$^+$ cells required for neutrophil and platelet recovery after autoHSCT is 2×10^6/kg. However, some data indicate that higher levels are associated with less need for blood product transfusions and administration of antibiotics, as well as prolonged survival in both MM and lymphoma settings.[2–7] Furthermore, patients planned for double autoHSCT, as used in MM, require relatively higher CD34$^+$ cell yield. Therefore, 5×10^6/kg is considered the optimal level.[8–10]

Mobilization protocols may either be based on the use of cytokines alone, most frequently G-CSF, or cytokines in combination with chemotherapy. Chemomobilization was demonstrated to increase CD34$^+$ cell yield.[11] On the other hand, it may produce severe toxicity and the need for transfusions.[11] In patients with MM, CY at wide dose range 1.5–7 g/m^2 is most commonly used for mobilization.[12] For patients with lymphomas, mobilization is often a part of salvage multiagent chemotherapy. Unfortunately, 5–40% of patients fail to mobilize sufficient number of CD34$^+$ cells.[6,13–15] Several factors were identified to predict poor mobilization, including increasing patients' age, thrombocytopenia and long-term antecedent chemotherapy.[16–22] New mobilization strategies have been investigated including the use of plerixafor, CXCR4 inhibitor, in combination with G-CSF, with or without chemotherapy. This agent enabled effective CD34$^+$ cell harvest in 64.8–81.6% of the 'proven' or 'predicted poor mobilizers'.[23,24] However, the optimal, cost-effective first-line regimen for procurement of autoHSCTs remains unknown.

Most patients are referred to our transplant center from other ones after completion of their first- or subsequent-line therapies and are frequently heavily pre-treated. Therefore, our mobilization strategy aiming to collect the optimal number of CD34$^+$ cells is oriented toward efficacy, and based on chemotherapy in combination with G-CSF. CY 4 g/m^2 + G-CSF was initially used as a first-line attempt. In case of mobilization failure, we introduced Ara-C at the dose of 2.4 g/m^2 + G-CSF following Montillo *et al.*,[25] who used this combination in a setting of 14 patients with CLL among whom eight had failed previous mobilization with G-CSF alone. We further decreased the dose of Ara-C to 1.6 g/m^2 to minimize hematologic toxicity. Both doses appeared highly effective allowing adequate CD34$^+$ cell harvest in 14 consecutive cases.[26] As a consequence, we decided to change our standard operating procedures and introduce intermediate-dose Ara-C +

[1]Department of Bone Marrow Transplantation, Maria Sklodowska–Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Gliwice, Poland; [2]Department of Brachytherapy and Clinical Oncology, Mikolaj Kopernik Medical University, Torun, Poland; [3]Department of Chemotherapy, Oncology Center, Bydgoszcz, Poland; [4]Department of Hematology, Municipal Hospital, Chorzow, Poland; [5]Department of Hematology, University Hospital, Krakow, Poland; [6]Department of Hematology, Holycross Cancer Center, Kielce, Poland and [7]Department of Clinical and Experimental Oncology, Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Gliwice, Poland. Correspondence: Dr S Giebel, Department of Bone Marrow Transplantation, Maria Sklodowska–Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Wybrzeze Armii Krajowej 15 Street, Gliwice 44-101, Poland.
E-mail: sgiebel@io.gliwice.pl
Received 18 September 2012; revised 6 November 2012; accepted 28 November 2012

G-CSF as the first-line mobilization for patients with lymphoid malignancies. In this study, we retrospectively analyzed the efficacy and toxicity of Ara-C + G-CSF in comparison with CY + G-CSF.

SUBJECTS AND METHODS

Patients

We analyzed results of 70 consecutive patients with lymphoid malignancies treated with Ara-C + G-CSF between July 2011 and March 2012, and 45 patients mobilized with CY + G-CSF between April 2010 and June 2011 in Maria Sklodowska–Curie Memorial Cancer Center and Institute of Oncology, Gliwice, Poland. Only patients with at least PR of their disease and proven chemosensitivity were accepted for autoHSCT in our center. The median age was 57 years (20–69 years) and did not differ between the treatment groups. As well, other clinical characteristics were comparable except for more frequent thrombocytopenia found among patients treated with CY than Ara-C (Table 1). In respective groups, 18(41%) and 28(40%) patients were classified as 'predicted poor mobilizers', according to criteria recently proposed by Gruppo Italiano Trapianto di Midollo Osseo.[27]

Mobilization regimens

Ara-C was administered as a 2 h i.v. infusion at a dose of 0.4 g/m^2 twice daily on days 1 and 2 (total dose 1.6 g/m^2). CY was given at a dose of 2 g/m^2 on days 1 and 2 (total dose 4 g/m^2), with adequate hydration (3 L) and administration of mesna. G-CSF (filgrastim) (7–10 μg/kg) was started on day 5 and continued until last leukapheresis. Anti-emetic prophylaxis consisted of ondansetron 2 × 8 mg per day. Patients were hospitalized either during the whole mobilization period or only during the chemotherapy administration and leukaphereses. The choice was dependent mainly on logistic aspects, for example, the distance from the patients' place of living to the hospital. No anti-infectious prophylaxis was routinely used. Platelet transfusions were indicated when the platelet level dropped below 20 × 10^9/L or below 50 × 10^9/L with concomitant need for introduction of the central venous catheter. Packed RBC transfusions were administered to maintain Hb level >8 g/dL. Toxicity of both procedures was assessed using Common Terminology Criteria for Adverse Events Version 4.0.

Leukaphereses

The number of circulating CD34$^+$ cells was first evaluated on the second day of neutrophil recovery >1 × 10^9/L in patients who experienced grade 3 or 4 neutropenia or, in remaining patients, on the first day with increase of neutrophil count. The analysis was done with the use of flow cytometry, as previously described.[27] Leukapheresis was started when the CD34$^+$ blood level was at least 10/μL. If the level was not achieved, G-CSF administration was continued and CD34$^+$ cell counted until CD34$^+$ level decreased compared to the preceding day.

Leukaphereses were performed using Spectra-Optia Apheresis System (CaridianBCT Inc, Lakewood, CO, USA) according to the manufacturers'

Table 1. Patients characteristics

	Ara-C + G-CSF	*CY + G-CSF*	P
N	70	45	
Median age, years	57 (22–69)	58 (20–68)	0.55
Age >60 years	23 (33%)	19 (42%)	0.33
Sex: male/female	36(51%)/34(51%)	21(47%)/24 (53%)	0.85
Diagnosis/disease phase			
MM	39 (56%)	24 (53%)	0.85
CR	8	4	
PR	10	4	
VGPR	21	16	
HL	9 (13%)	5 (11%)	1.0
Primary resistance, PR	5	3	
Relapse, PR	4	2	
NHL	22 (31%)	16 (36%)	0.69
Diffuse large B-cell lymphoma	10	9	
Mantle cell lymphoma	7	2	
Follicular lymphoma	2	2	
Other subtypes	3	3	
CR1	8	5	
Primary resistance, PR	4	6	
Relapse, CR	9	1	
Relapse, PR	1	4	
Interval diagnosis-mobilization (months)	10 (3–84)	12 (4–291)	0.2
Lines of preceding chemotherapy	2 (1–6)	1 (1–5)	0.7
1	33 (47%)	23 (51%)	0.71
2	28 (40%)	17 (38%)	0.7
⩾3	9 (13%)	5 (11%)	1.0
Cycles of preceding chemotherapy[a]	8 (3–36)	8 (3–24)	0.74
Preceding radiotherapy	25 (36%)	16 (36%)	1.0
Preceding pelvic radiotherapy	4 (6%)	2 (4%)	1.0
Previous autoHSCT	1 (1%)	1 (2%)	1.0
Thrombocytopenia at mobilization	1 (1%)	5 (11%)	0.03
BM involvement at mobilization[b]	2 (3%)	2 (4%)	1.0
High risk of mobilization failure[c]	28 (40%)	18 (41%)	1.0

Abbreviation: autoHSCT = autologous hematopoietic SCT; HL = Hodgkin's lymphoma; MM = multiple myeloma; NHL = non-Hodgkin's lymphoma. [a]Among all patients with MM, 53 were treated with thalidomide, 9 with bortezomib, none with lenalidomide; intial therapy of HL was most frequently either ABVD (doxorubicin, bleomycin, vinblastin, dacarbazin; *N* = 8) or BEACOPP (bleomycin, etoposide, doxorubicin, CY, vincristin, procarbazine, prednisne; *N* = 4); first line treatment of NHL was usually R-CHOP (rituximab, CY, vincristin, prednisone; *N* = 31). [b]BM involvement at mobilization was restricted to patients with MM, and defined as >5% plasma cells. [c]'Predicted poor mobilizers' were defined as the presence of one major criterion (previous extensive radiotherapy to marrow bearing tissue or full courses of previous therapy potentially affecting stem cell mobilization), or at least two minor criteria (advanced phase disease, refractory disease, BM involvement at mobilization, BM cellularity <30%, or age >65 years).[27]

protocols for mononuclear cell harvesting, processing two blood volumes. The target $CD34^+$ cell yield was $>2\times10^6$/kg for patients with HL and NHL (planned for a single autoHSCT), whereas $>5\times10^6$/kg for patients with MM (all planned for tandem autoHSCT).

Collected cells were cryopreserved using a controlled-rate freezer in 10% DMSO, and stored in liquid nitrogen. Additionally, in randomly selected 13 patients, 1 mL of the product was cryopreserved separately and further tested in a clonogenic assay, for the presence of burst-forming unit-erythroid (BFU-E), CFU-erythroid (CFU-E); CFU-granulocyte/macrophage (CFU-GM) and CFU-erythroid, granulocyte, macrophage, megakaryocyte (CFU-GEMM), as previously described.[28]

Statistical methods

The peak number of circulating $CD34^+$ cells, number of collected $CD34^+$ cells and the ratio of patients who collected sufficient $CD34^+$ cell yield for autoHSCT were primary study end points. The differences between Ara-C and CY groups were evaluated with the use of U Mann–Whitney test for quantitative variables and Fisher's exact test, two sided, for qualitative variables. Time to neutrophil and platelet recovery was estimated using the Kaplan–Meier method. The probabilities were compared using log-rank test. Differences with P values <0.05 were considered statistically significant. Statistical analysis was performed using Statistica software version 10 (StatSoft Inc., Tulsa, OK, USA).

RESULTS

Analysis of circulating $CD34^+$ cells

The peak number of circulating $CD34^+$ cells/μL was significantly higher after Ara-C + G-CSF (median 120 (range, 0–523)) compared with CY + G-CSF [33 (1–240), $P<0.0001$). As well, the proportion of patients who reached at least 10 $CD34^+$ cells/μL was higher for Ara-C than CY (97 versus 76%, $P=0.0005$). Significant differences in favor of Ara-C + G-CSF were found in separate analysis of patients with MM or lymphomas (Table 2). In addition, 93% of 'predicted poor mobilizers' reached at least 10 $CD34^+$ cells/μL after Ara-C + G-CSF compared with 67% in the CY cohort ($P=0.04$).

Analysis of stem cell harvest

Altogether 68 patients proceeded to leukaphereses in the Ara-C group, and 37 patients in the CY group (three patients despite $CD34^+$ cell blood level <10/μL, that is, 9/μL, 9/μL and 8/μL). Leukaphereses were started significantly later in patients receiving Ara-C + G-CSF than CY + G-CSF (day 14 (11–27) versus day 12 (11–19), $P<0.0001$). In the Ara-C group, 57 patients (84%) were started stem cell harvest between day 13 and 15, most frequently on day 14 (28 patients, 41%). s.d. equaled 1.1 day. In the CY group leukaphereses were started between day 12 and 14 in 32 (86%) patients and s.d. was 1.6. Number of collected $CD34^+$ cells was significantly higher for patients mobilized with Ara-C + G-CSF (14.5×10^6/kg (2.2–54.6)) than after CY + G-CSF (5.1×10^6/kg (0.9–14.3), $P<0.0001$. The difference remained significant when three patients with $CD34^+$ cell yield <10/μL were excluded from the analysis ($P<0.0001$). A single apheresis was sufficient to collect adequate number of $CD34^+$ cells in 91% patients in the Ara-C group compared with 24% in the CY group ($P<0.0001$). Similar differences were observed in subgroup of patients with MM and lymphomas, analyzed separately. As well, among 'predicted poor mobilizers' the $CD34^+$ cell yield was significantly higher after Ara-C + G-CSF than after CY + G-CSF (8.3×10^6/kg (2.2–52.1) versus 4.4×10^6/kg (0.9–13.9), $P=0.01$), which was achieved with a single apheresis in 85 and 20% patients, respectively ($P=0.0001$).

The efficacy of mobilization and harvest

Altogether 68 out of 70 patients (97%) treated with Ara-C + G-CSF collected at least 2×10^6 $CD34^+$ cells/kg, and 58 patients (83%) collected at least 5×10^6 $CD34^+$ cells/kg (Table 3). Among patients with high risk of mobilization failure, respective proportions were 95 and 72%. In particular, 95% patients with MM achieved sufficient $CD34^+$ yield for double autoHSCT and 97% of patients with lymphomas—for a planned single procedure. The efficacy of the mobilization and harvest procedure was significantly lower for CY + G-CSF with 62% of patients collecting

Table 2. Efficacy of mobilization

	Ara-C + G-CSF	*CY + G-CSF*	P
Whole group			
N	70	45	
Peak level of $CD34^+$ in peripheral blood (/μL)	120 (0–523)	33 (1–240)	<0.0001[a]
$\geqslant10$ $CD34^+$ cells/μL in peripheral blood	68 (97%)	34 (76%)	0.0005[a]
MM			
N	39	24	
Peak level of $CD34^+$ in peripheral blood (/μL)	136 (3–523)	44 (3–240)	<0.0001
$\geqslant10$ $CD34^+$ cells/μL in peripheral blood	38 (97%)	19 (79%)	0.03
Lymphomas (all Hodgkin and non-Hodgkin)			
N	31	21	
Peak level of $CD34^+$ in peripheral blood (/μL)	78 (0–492)	20 (1–170)	0.002
$\geqslant10$ $CD34^+$ cells/μL in peripheral blood	30 (97%)	15 (71%)	0.01
Diffuse large B-cell lymphoma			
N	10	9	
Peak level of $CD34^+$ in peripheral blood (/μL)	87 (30–492)	20 (1–80)	0.002
$\geqslant10$ $CD34^+$ cells/μL in peripheral blood	10 (100%)	6 (67%)	0.09
Patients with high risk of mobilization failure			
N	28	18	
Peak level of $CD34^+$ in peripheral blood (/μL)	79 (0–523)	23.5 (2–240)	0.01
$\geqslant10$ $CD34^+$ cells/μL in peripheral blood	26 (93%)	12 (67%)	0.04

[a]The differences remained statistically significant after excluding patients with thrombocytopenia at mobilization ($P<0.0001$ for the peak level of circulating $CD34^+$ cells and $P=0.004$ for proportion of patients with peak level $\geqslant10$ $CD34^+$ cells/μL).

Table 3. Summary of hematopoietic stem cell mobilization and harvest

	Ara-C + G-CSF	*CY + G-CSF*	P
Whole group			
N	70	45	
$\geqslant 2 \times 10^6$/kg collected CD34$^+$ cells	68 (97%)	28 (62%)	<0.0001
$\geqslant 5 \times 10^6$/kg collected CD34$^+$ cells	58 (83%)	22 (49%)	0.0002
MM			
N	39	24	
$\geqslant 2 \times 10^6$/kg collected CD34$^+$ cells	38 (97%)	18 (75%)	0.01
$\geqslant 5 \times 10^6$/kg collected CD34$^+$ cells	37 (95%)	16 (67%)	0.005
Lymphomas (all Hodgkin and non-Hodgkin)			
N	31	21	
$\geqslant 2 \times 10^6$/kg collected CD34$^+$ cells	30 (97%)	10 (48%)	<0.0001
$\geqslant 5 \times 10^6$/kg collected CD34$^+$ cells	21 (68%)	6 (29%)	0.01
Diffuse large B-cell pymphoma			
N	10	9	
$\geqslant 2 \times 10^6$/kg collected CD34$^+$ cells	10 (100%)	4 (44%)	0.01
$\geqslant 5 \times 10^6$/kg collected CD34$^+$ cells	7 (70%)	2 (22%)	0.07
Patients with high risk of mobilization failure			
N	28	18	
$\geqslant 2 \times 10^6$/kg collected CD34$^+$ cells	26 (93%)	9 (50%)	0.001
$\geqslant 5 \times 10^6$/kg collected CD34$^+$ cells	17 (61%)	7 (39%)	0.23

Abbreviation: MM = multiple myeloma

at least 2×10^6 CD34$^+$ cells/kg ($P<0.0001$) and 49% collecting at least 5×10^6 CD34$^+$ cells/kg ($P=0.0002$). The CD34$^+$ yield was adequate for transplantation in 67% of patients with MM ($P=0.005$, compared to Ara-C) and 48% patients with HL or NHL ($P<0.0001$). Finally, among 'predicted poor mobilizers' 2×10^6 and 5×10^6 CD34+ cells/kg was achieved in 53% ($P<0.0001$) and 43% ($P=0.03$) of patients, respectively.

Toxicity

Mobilization with the use of Ara-C and G-CSF was associated with 17% incidence of grade 3 neutropenia and 36% rate of grade 4 neutropenia (Table 4). No episode of febrile neutropenia was observed, although 8 patients (12%) experienced grade 2 or 3 infections, mostly affecting upper respiratory tract and requiring oral antibiotics. In the CY + G-CSF group, the incidence of grade 4 neutropenia was significantly higher (70%, $P<0.0001$) and its duration longer (median 2 versus 0 in the Ara-C cohort, $P<0.0001$). One patient had febrile neutropenia ($P=0.39$), whereas six patients (14%) developed grade 2 or 3 infections ($P=0.37$).

Thirty-eight (54%) patients experienced grade 3 thrombocytopenia, and further 22 individuals (31%) had grade 4 thrombocytopenia after Ara-C + G-CSF mobilization. Altogether, 35% of patients required platelet transfusions. In the CY + G-CSF group, the incidence of grade 3 and grade 4 thrombocytopenia was significantly lower (16%, $P<0.0001$ and 14%, $P=0.04$, respectively). The need for platelet transfusions was less frequent (25%), however, the difference did not reach statistical significance ($P=0.22$). Requirement for packed RBC transfusions was comparable in two study cohorts.

Non-hematologic adverse events were more frequent among patients treated with CY and G-CSF compared with Ara-C + G-CSF. Altogether, grade 2 or 3 toxicities were observed in 50 and 23% of patients, respectively ($P=0.004$). In particular, administration of CY was associated with higher incidence of grade 2 nausea (18 versus 0%, $P=0.0003$). However, grade 3 adverse events were generally rare. No patient experienced grade 4 non-hematologic toxicity.

Clonogenic assay

Results of clonogenic assay were available for six patients treated with Ara-C + G-CSF (MM, $N=3$, NHL, $N=2$, HL, $N=1$) and 7 patients in the CY + G-CSF group (MM, $N=6$, DLBCL, $N=1$). The total number of colonies calculated per 10^5 of cryopreserved viable cells tended to be higher in the Ara-C cohort (1002 (64–2515) versus 255 (21–787), $P=0.2$). The difference was most pronounced for the number of CFU-GM (487 (22–1294) versus 112 (21–397), $P=0.2$) and BFU-E (387 (32–973) versus124 (9–262), $P=0.15$), whereas NS for CFU-E and CFU-GEMM.

Recovery after autoHSCT

Altogether, 67 patients (96%) proceeded to autoHSCT directly after Ara-C + G-CSF mobilization compared with 27 (60%) in the CY group ($P<0.0001$). In one patient from each group the transplantation procedure was postponed or canceled due to disease progression, despite collection of sufficient number of hematopoietic stem cells.

Conditioning regimen was based on TBR in 48 and 69% of patients in the Ara-C and CY group, respectively, and the number of transplanted CD34$^+$ cells was 8.2×10^6/kg (2.2–24.7) versus 3.9×10^6/kg (1.4–10.5), respectively ($P<0.0001$). All patients received G-CSF (filgrastim) 300 μg/d starting from day 7 after autoHSCT until engraftment.

Median time to neutrophil recovery $>0.5 \times 10^9$/L was significantly shorter among patients mobilized with Ara-C + G-CSF than with CY + G-CSF (median 11 days, range 9–13, mean 11.1, s.d. 0.7 versus median 12 days, range 10–21, mean 12, s.d. 2; $P=0.0004$) (Figure 1). As well, the time to platelet recovery $>20 \times 10^9$/L was shorter in the Ara-C cohort (median 9 days range 0–17, mean 8.3, s.d. 4.2 versus median 10 days, range 0–17, mean 10.6, s.d. 3.3; $P=0.01$) (Figure 2). No patient experienced secondary graft failure.

DISCUSSION

The search for optimal mobilization regimen was a subject of a series of prospective, randomized clinical trials, recently reviewed

Table 4. Toxicity and supportive treatment

N	*Ara-C + G-CSF* 70		*CY + G-CSF* 44[a]		P	P
Non-hematologic adverse events	*Grade 2*	*Grade 3*	*Grade 2*	*Grade 3*	*Grade 2 or 3*	*Grade 3*
Febrile neutropenia	—	—	—	1 (2%)	—	0.39
Infections	6 (9%)	2 (3%)	3 (7%)	3 (7%)	0.77	0.37
Allergy	3 (4%)	—	1 (2%)	—	1.0	—
Vein thrombosis	—	—	2 (5%)	—	0.15	—
Nausea	—	—	8 (18%)	—	0.0003	—
Vomiting	—	—	2 (5%)	—	0.15	—
Diarrhea	—	—	1 (2%)	—	0.39	—
Dyspepsia	—	—	1 (2%)	—	0.39	—
Mucositis	—	—	1 (2%)	—	0.39	—
Hypertensia	2 (3%)	3(4%)	—	—	0.08	0.28
Hypotensia	—	—	—	1 (2%)	—	0.39
Atrial fibrillation	—	—	1 (2%)	—	0.39	—
DIC	—	—	1 (2%)	—	0.39	—
Bone pain	1 (1%)	—	1 (2%)	—	1.0	—
Hypokalemia	1 (1%)	—	—	2(5%)	0.56	0.15
Elevated GGTP	—	—	—	1(2%)	—	0.39
Prolonged APTT	—	—	—	1(2%)	—	0.39
Any non-hematologic adverse event	13 (19%)	5 (7%)	19 (43%)	6 (14%)	0.004	0.33
Hematologic adverse events	*Grade 3*	*Grade 4*	*Grade 3*	*Grade 4*	*Grade 3 or 4*	*Grade 4*
Neutropenia	12 (17%)	25 (36%)	3 (7%)	31(70%)	0.01	0.0005
Thrombocytopenia	38 (54%)	22 (31%)	7 (16%)	6(14%)	<0.0001	0.04
Grade 3 or 4 neutropenia (days)	1 (0–7)		3 (0–13)		<0.0001	
Grade 4 neutropenia (days)	0 (0–5)		2 (0–10)		<0.0001	
Grade 3 or 4 thrombocytopenia (days)	2 (0–10)		0 (0–14)		<0.0001	
Grade 4 thrombocytopenia (days)	0 (0–2)		0 (0–6)		0.13	
RBC transfusions	12 (17%)		11 (25%)		0.35	
Platelet transfusions	26 (35%)		11 (25%)		0.22	

Abbreviations: APTT = activated partial thromboplastin time; DIC = disseminated intravascular coagulation; GGTP = gamma-glutamylotransferase. Toxicity was assessed using Common Terminology Criteria for Adverse Events Version 4.0. [a]data were available for 44 out of 45 patients treated with CY + G-CSF.

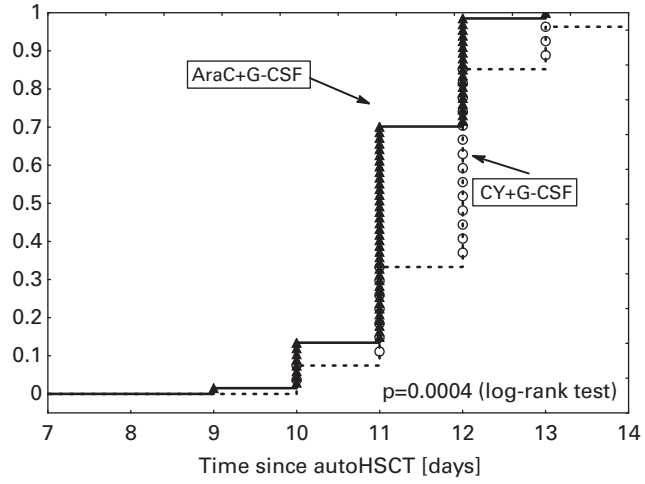

Figure 1. Recovery of neutrophils $>0.5 \times 10^9$/L after autoHSCT according to type of mobilization regimen. Only patients who proceeded to autoHSCT were analyzed that is, 67 subjects mobilized with Ara-C with filgrastim (Ara-C + G-CSF), and 26 patients treated with CY with filgrastim (CY + G-CSF).

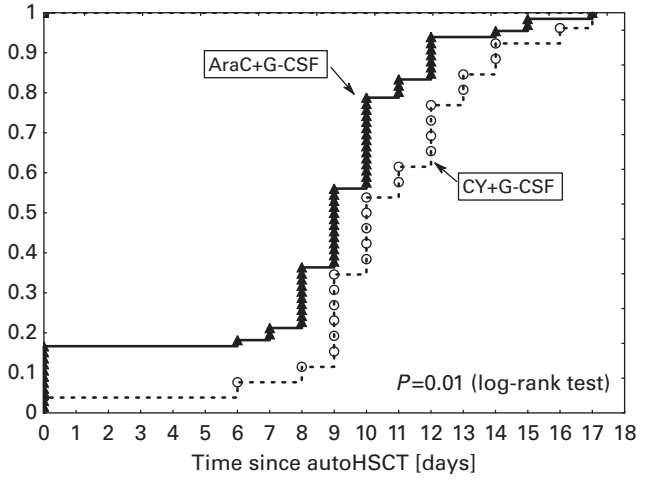

Figure 2. Recovery of platelets $>20 \times 10^9$/L after autoHSCT according to type of mobilization regimen. Only patients who proceeded to autoHSCT were analyzed that is, 67 subjects mobilized with Ara-C with filgrastim (Ara-C + G-CSF), and 26 patients treated with CY with filgrastim (CY + G-CSF).

by Sheppard *et al.*[29] So far, however, no definitive conclusions can be made with regard to the choice of optimal mobilization protocol.[30] Personalized approach, taking into account the presence of factors predicting for poor stem cell harvest together with status of the disease as well as economical and regulatory aspects, is postulated.[29] Therefore, G-CSF alone may be optimal for patients with low risk of mobilization failure and planned single autoHSCT. In contrast, chemomobilization may be

preferred in high-risk setting and if higher CD34$^+$ cell yield is required, for example, for double transplantation procedure. Administration of plerixafor may be considered good alternative to chemotherapy, however, its availability as a first-line approach is limited in many countries.[29] One of the arguments supporting the use of chemomobilization instead of G-CSF alone is the potential effect of *in vivo* purging, although the clonogenic potential of malignant cells present in the graft and their clinical significance is unclear.[10,12,31,32] Both Ara-C and CY used in our center are part of various multiagent treatment regimens, and both are expected to have activity against MM and lymphomas.

CY was most frequently used for chemomobilization in clinical trials.[29] Among patients with relapsed NHL, CY at a dose of 5 g/m^2 with G-CSF allowed the collection of a median 7.2×10^6/kg CD34$^+$ cells compared with 2.5×10^6/kg when G-CSF was used as a single agent.[11] Only one prospective study addressed the question of the optimal dosage comparing CY 4 g/m^2 with 1.5 g/m^2 in a mixed population of 27 patients with NHL and breast cancer.[33] Higher doses of CY were associated not only with two times higher CD34$^+$ cell yield, but also with increased toxicity. In contrast to CY, to the best of our knowledge, intermediate-dose Ara-C as a first-line stem cell mobilization has not been widely evaluated so far.

Efficacy of CY at a dose of 4 g/m^2 + G-CSF in the current analysis (62% success rate) appears inferior compared with other reports (77–94% success rate).[34–37] It must be noted, however, that high proportion of our patients had features associated with high risk of mobilization failure. Despite similar unfavorable characteristics, almost all patients could be successfully mobilized with Ara-C + G-CSF, with CD34$^+$ yield sufficient for either single or double autoHSCT, as needed. Importantly, in 62 out of the total 70 patients receiving Ara-C, the harvest could be completed with a single leukapheresis. This compares favorably with intermediate-dose CY, which in our study was associated with a median of two leukaphereses, whereas in other reports the median ranged from 2–4.[11,34–37] The advantage of Ara-C over CY in terms of the potency for stem cell mobilization is best reflected by the peak number of peripheral blood CD34$^+$, which was almost four times higher in the Ara-C group.

Safety issues and related pharmaco-economic aspects are important factors influencing the choice of mobilization regimen. In our center most patients stay in hospital during mobilization, which results from logistic rather than medical reasons. Therefore, the need for hospitalization could not be relevant end point of our analysis. On the other hand, we noted unexpectedly low rate of febrile neutropenia, with only 2% rate in the CY group and no event in the Ara-C cohort, which compares favorably with up to 70% in reports were CY at a dose of 4 g/m^2 was administered in an outpatient setting.[38,39] Non-hematologic toxicities were more frequent after CY than Ara-C but generally very few severe adverse events were noted regardless the choice of the protocol. Interestingly, the profile of hematologic toxicity was remarkably different for two regimens. Whereas Ara-C was associated with much higher rate of grade 3 and 4 thrombocytopenia, the use of CY resulted in two higher incidence of profound neutropenia. The differences may reflect diverse pattern of myelosuppression for the two drugs. If so, these findings could constitute the platform for further biological investigation to explain the striking advantage of Ara-C over CY in terms of the mobilization efficacy.

CD34 cells is a surrogate marker of hematopoietic stem cells, some of them, however, being CD34-negative.[40] Therefore, the number of CD34$^+$ cells in the product must not necessarily correspond to their clonogenic potential. In our study, there was a trend for higher number of colonies in the Ara-C compared with CY, although this comparison hampers due to the limited number of results. Furthermore, cells collected after Ara-C + G-CSF mobilization allowed significantly faster neutrophil and platelet recovery after autoHSCT, compared with the CY group. The difference resulted most probably from over two higher number of transplanted CD34$^+$ cells in the Ara-C group. Altogether, the above findings confirm high quality of the transplant material obtained using Ara-C-based mobilization.

In conclusion, we demonstrated that intermediate-dose Ara-C in combination with G-CSF is a very effective mobilization protocol allowing adequate stem cell harvest with a single leukapheresis in a vast majority of patients with MM and lymphomas, including 'predicted poor mobilizers'. The efficacy of Ara-C + G-CSF is significantly higher compared with CY at a dose of 4 g/m^2 + G-CSF, whereas non-hematologic toxicity—lower. Further investigation is warranted to prospectively evaluate the protocol.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1 Baldomero H, Gratwohl M, Gratwohl A, Tichelli A, Niederwieser D, Madrigal A *et al.* The EBMT activity survey 2009: trends over the past 5 years. *Bone Marrow Transplant* 2011; **46**: 485–501.
2 Siena S, Schiavo R, Pedrazzoli P, Carlo-Stella C. Therapeutic relevance of CD34 cell dose in blood cell transplantation for cancer therapy. *J Clin Oncol* 2000; **18**: 1360–1377.
3 Sola C, Maroto P, Salazar R, Mesia R, Mendoza L, Brunet J *et al.* Bone marrow transplantation: prognostic factors of peripheral blood stem cell mobilization with cyclophosphamide and filgrastim (r-metHuG-CSF): the CD34+ cell dose positively affects the time to hematopoietic recovery and supportive requirements after high-dose chemotherapy. *Hematology* 1999; **4**: 195–209.
4 Jantunen E, Fruehauf S. Importance of blood graft characteristics in auto-SCT: implications for optimizing mobilization regimens. *Bone Marrow Transplant* 2011; **46**: 627–635.
5 O'Shea D, Giles C, Terpos E, Perz J, Politou M, Sana V *et al.* Predictive factors for survival in myeloma patients who undergo autologous stem cell transplantation: a single-centre experience in 211 patients. *Bone Marrow Transplant* 2006; **37**: 731–737.
6 Pavone V, Gaudio F, Console G, Vitolo U, Iacopino P, Guarini A *et al.* Poor mobilization is an independent prognostic factor in patients with malignant lymphomas treated by peripheral blood stem cell transplantation. *Bone Marrow Transplant* 2006; **37**: 719–724.
7 Bolwell BJ, Pohlman B, Rybicki L, Sobecks R, Dean R, Curtis J *et al.* Patients mobilizing large numbers of CD34+ cells ('super mobilizers') have improved survival in autologous stem cell transplantation for lymphoid malignancies. *Bone Marrow Transplant* 2007; **40**: 437–441.
8 Goterris R, Hernández-Boluda JC, Teruel A, Gómez C, Lis MJ, Terol MJ *et al.* Impact of different strategies of second-line stem cell harvest on the outcome of autologous transplantation in poor peripheral blood stem cell mobilizers. *Bone Marrow Transplant* 2005; **36**: 847–853.
9 Weaver CH, Hazelton B, Birch R, Palmer P, Allen C, Schwartzberg L *et al.* An analysis of engraftment kinetics as a function of the CD34 content of peripheral blood progenitor cell collections in 692 patients after the administration of myeloablative chemotherapy. *Blood* 1995; **86**: 3961–3969.
10 Fu S, Liesveld J. Mobilization of hematopoietic stem cells. *Blood Rev* 2000; **14**: 205–218.
11 Narayanasami U, Kanteti R, Morelli J, Klekar A, Al-Olama A, Keating C *et al.* Randomized trial of filgrastim versus chemotherapy and filgrastim mobilization of hematopoietic progenitor cells for rescue in autologous transplantation. *Blood* 2001; **98**: 2059–2064.
12 Gertz MA. Current status of stem cell mobilization. *Br J Haematol* 2010; **150**: 647–662.
13 Demirer T, Buckner CD, Gooley T, Appelbaum FR, Rowley S, Chauncey T *et al.* Factors influencing collection of peripheral blood stem cells in patients with multiple myeloma. *Bone Marrow Transplant* 1996; **17**: 937–941.
14 Perea G, Sureda A, Martino R, Altés A, Martínez C, Cabezudo E *et al.* Predictive factors for a successful mobilization of peripheral blood CD34+ cells in multiple myeloma. *Ann Hematol* 2001; **80**: 592–597.
15 Kuittinen T, Nousiainen T, Halonen P, Mahlamäki E, Jantunen E. Prediction of mobilisation failure in patients with non-Hodgkin's lymphoma. *Bone Marrow Transplant* 2004; **33**: 907–912.
16 Fietz T, Rieger K, Dimeo F, Blau IW, Thiel E, Knauf WU. Stem cell mobilization in multiple myeloma patients: do we need an age-adjusted regimen for the elderly? *J Clin Apher* 2004; **19**: 202–207.

17 Hosing C, Saliba RM, Ahlawat S, Körbling M, Kebriaei P, Alousi A *et al.* Poor hematopoietic stem cell mobilizers: a single institution study of incidence and risk factors in patients with recurrent or relapsed lymphoma. *Am J Hematol* 2009; **84**: 335–337.
18 Boccadoro M, Palumbo A, Bringhen S, Merletti F, Ciccone G, Richiardi L *et al.* Oral melphalan at diagnosis hampers adequate collection of peripheral blood progenitor cells in multiple myeloma. *Haematologica* 2002; **87**: 846–850.
19 Laszlo D, Galieni P, Raspadori D, Tozzi M, Lauria F, Martinelli G. Fludarabine combination regimen severely affected peripheral blood stem cell mobilization. *Acta Haematol* 2004; **111**: 228–229.
20 Ford CD, Green W, Warenski S, Petersen FB. Effect of prior chemotherapy on hematopoietic stem cell mobilization. *Bone Marrow Transplant* 2004; **33**: 901–905.
21 Ghobrial IM, Dispenzieri A, Bundy KL, Gastineau DA, Rajkumar SV, Therneau TM *et al.* Effect of thalidomide on stem cell collection and engraftment in patients with multiple myeloma. *Bone Marrow Transplant* 2003; **32**: 587–592.
22 Kumar S, Dispenzieri A, Lacy MQ, Hayman SR, Buadi FK, Gastineau DA *et al.* Impact of lenalidomide therapy on stem cell mobilization and engraftment post-peripheral blood stem cell transplantation in patients with newly diagnosed myeloma. *Leukemia* 2007; **21**: 2035–2042.
23 Hübel K, Fresen MM, Apperley JF, Basak GW, Douglas KW, Gabriel IH *et al.* European data on stem cell mobilization with plerixafor in non-Hodgkin's lymphoma, Hodgkin's lymphoma and multiple myeloma patients. A subgroup analysis of the European Consortium of stem cell mobilization. *Bone Marrow Transplant* 2012; **47**: 1046–1050.
24 Basak GW, Jaksic O, Koristek Z, Mikala G, Basic-Kinda S, Mayer J *et al.* Haematopoietic stem cell mobilization with plerixafor and G-CSF in patients with multiple myeloma transplanted with autologous stem cells. *Eur J Haematol* 2011; **86**: 488–495.
25 Montillo M, Tedeschi A, Rossi V, Cairoli R, Pungolino E, Intropido L *et al.* Successful CD34+ cell mobilization by intermediate-dose Ara-C in chronic lymphocytic leukemia patients treated with sequential fludarabine and Campath-1H. *Leukemia* 2004; **18**: 57–62.
26 Kruzel T, Sadus-Wojciechowska M, Najda J, Czerw T, Glowala-Kosinska M, Holowiecki J *et al.* Very high efficacy of intermediate-dose cytarabine in combination with G-CSF as a second-line mobilization of hematopoietic stem cells. *Int J Hematol* 2012; **96**: 287–289.
27 Olivieri A, Marchetti M, Lemoli R, Tarella C, Iacone A, Lanza F *et al.* Proposed definition of 'poor mobilizer' in lymphoma and multiple myeloma: an analytic hierarchy process by *ad hoc* working group Gruppo ItalianoTrapianto di Midollo Osseo. *Bone Marrow Transplant* 2012; **47**: 342–351.
28 Smagur A, Mitrus I, Giebel S, Sadus-Wojciechowska M, Najda J, Kruzel T *et al.* Impact of different dimethyl sulfoxide concentrations on cell recovery, viability and clonogenic potential of cryopreserved peripheral blood hematopoietic stem and progenitor cells. *Vox Sang* 2012doi:10.1111/j.1423-0410.2012.01657.x.
29 Sheppard D, Bredeson C, Allan D, Tay J. Systematic review of randomized controlled trials of hematopoietic stem cell mobilization strategies for autologous transplantation for hematologic malignancies. *Biol Blood Marrow Transplant* 2012; **18**: 1191–1203.
30 Bensinger W, DiPersio JF, McCarty JM. Improving stem cell mobilization strategies: future directions. *Bone Marrow Transplant* 2009; **43**: 181–195.
31 Dingli D, Nowakowski GS, Dispenzieri A, Lacy MQ, Hayman S, Litzow MR *et al.* Cyclophosphamide mobilization does not improve outcome in patients receiving stem cell transplantation for multiple myeloma. *Clin Lymphoma Myeloma* 2006; **6**: 384–388.
32 Bourhis JH, Bouko Y, Koscielny S, Bakkus M, Greinix H, Derigs G *et al.* Relapse risk after autologous transplantation in patients with newly diagnosed myeloma is not related with infused tumor cell load and the outcome is not improved by CD34+ cell selection: long term follow-up of an EBMT phase III randomized study. *Haematologica* 2007; **92**: 1083–1090.
33 Ahn JS, Park S, IM S-A, Yoon S-S, Lee J-S, Kim BK *et al.* High-dose versus low-dose cyclophosphamide in combination with G-CFS for peripheral blood progenitor cell mobilization. *Korean J Int Med* 2005; **20**: 224–231.
34 Pavone V, Gaudio F, Guarini A, Perrone T, Zonno A, Curci P *et al.* Mobilization of peripheral blood stem cells with high-dose cyclophosphamide or the DHAP regimen plus G-CSF in non-Hodgkin's lymphoma. *Bone Marrow Transplant* 2002; **29**: 285–290.
35 Facon T, Harousseau JL, Maloisel F, Attal M, Odriozola J, Alegre A *et al.* Stem cell factor in combination with filgrastim after chemotherapy improves peripheral blood progenitor cell yield and reduces apheresis requirements in multiple myeloma patients: a randomized, controlled trial. *Blood* 1999; **94**: 1218–1225.
36 Gazitt Y, Callander N, Freytes CO, Shaughnessy P, Liu Q, Tsai TW *et al.* Peripheral blood stem cell mobilization with cyclophosphamide in combination with G-CSF, GM-CSF, or sequential GM-CSF/G-CSF in non-Hodgkin's lymphoma patients: a randomized prospective study. *J Hematother Stem Cell Res* 2000; **9**: 737–748.
37 Quittet P, Ceballos P, Lopez E, Lu ZY, Latry P, Becht C *et al.* Low doses of GM-CSF (molgramostim) and G-CSF (filgrastim) after cyclophosphamide (4 g/m2) enhance the peripheral blood progenitor cell harvest: results of two randomized studies including 120 patients. *Bone Marrow Transplant* 2006; **38**: 275–284.
38 Fitoussi O, Perreau V, Boiron JM, Bouzigon E, Cony-Makhoul P, Pigneux A *et al.* A comparison of toxicity following two different doses of cyclophosphamide for mobilization of peripheral blood progenitor cells in 116 multiple myeloma patients. *Bone Marrow Transplant* 2001; **27**: 837–842.
39 Jantunen E, Putkonen M, Nousiainen T, Pelliniemi TT, Mahlamäki E, Remes K. Low-dose or intermediate-dose cyclophosphamide plus granulocyte colony-stimulating factor for progenitor cell mobilisation in patients with multiple myeloma. *Bone Marrow Transplant* 2003; **31**: 347–351.
40 Dao MA, Arevalo J, Nolta JA. Reversibility of CD34 expression on human hematopoietic stem cells that retain the capacity for secondary reconstitution. *Blood* 2003; **101**: 112–118.

Q3