Effect of chemotherapy with alkylating agents on the yield of CD34+ cells in patients with multiple myeloma. Results of the Spanish Myeloma Group (GEM) Study

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Background and Objectives. Although alkylating agents are clearly beneficial in multiple myeloma (MM), their deleterious effect on bone marrow hematopoietic progenitor cells usually precludes their use as front-line therapy in patients scheduled to undergo autologous stem cell transplantation (ASCT). We analyzed the impact of first-line chemotherapy with alkylating agents on stem cell collection in MM patients.

Design and Methods. Seven hundred and eighty-nine patients included in the Spanish multicenter protocol GEM-2000 underwent mobilization therapy after four courses of alternating VBMCP/VBAD chemotherapy.

Results. The mobilization regimens consisted of standard or high-dose granulocyte colony-stimulating factor (G-CSF) in 551 (70%) patients, and chemotherapy and G-CSF in 206 (26%) patients. The CD34+ cell yield was lower than 4×10^6/kg in 388 patients (49%), and equal or greater than 4×10^6/kg in 401 patients (51%). Multivariate analysis indicated that advanced age ($p<0.0001$) and longer interval between diagnosis and mobilization ($p=0.012$) were the two variables associated with a lower CD34+ cell yield. Significant differences in CD34+ cell yield were not observed between the mobilization regimens. Of the 789 patients included in the protocol, 726 (92%) underwent the planned ASCT, whereas 25 (3%) patients did not because of the low number of CD34+ cells collected. Following ASCT, 0.5×10^9 neutrophils/L could be recovered after 11 days (median time; range, 5-71 days) and 20×10^9 platelets/L could be recovered after 12 days (median time; range, 6-69 days).

Interpretation and Conclusions. A short-course of therapy with alkylating agents according to the GEM-2000 protocol was associated with an appropriate CD34+ cell collection, and allowed the planned ASCT to be performed in the majority of MM patients.

Key words: multiple myeloma, peripheral blood stem cell collection, alkylating agents, CD34+ cells.

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Several recent studies have shown that high-dose therapy followed by autologous blood stem cell transplantation (ASCT) significantly improves the outcome of patients with newly diagnosed symptomatic multiple myeloma (MM).[1,2] The high-dose programs usually include two pretransplant therapeutic phases: the induction phase and the mobilization phase. VAD or VAD-like combinations are commonly used as first-line induction therapy in patients with MM.[1,3-5] This regimen produces high response rates, is generally well tolerated, and because there is no damage to stem cells, it does not jeopardize the subsequent peripheral blood stem cell (PBSC) collection.[5,6]

Previous studies by the Spanish PETHEMA cooperative group have shown that the VCMP/VBAD combination, which includes alkylating agents, is a highly effective standard regimen of conventional chemotherapy for MM patients up to 70 years old.[7-9] Moreover, in two recent randomized trials comparing ASCT and alkylating agent-based regimens, significant differences in survival were not observed, which may be due, at least in part, to the higher efficacy of these regimens over VAD.[10,11] A new trial (referred to as GEM-2000) to increase the rate of response to ASCT and prolong its duration was designed within the Spanish Myeloma Group (GEM) for patients with previously untreated MM younger than 70 years. The therapeutic regimen included conventional chemotherapy with VBMCP/VBAD followed by high-dose therapy and ASCT. Here, we report on the impact of this alkylating agent-based induction on PBSC mobilization (measured by the number of CD34+ cells collected), analyzing the influence of the mobilization regimen (granulocyte colony-stimulating factor [G-CSF] alone vs. G-CSF plus cyclophosphamide) on the number of CD34+ cells collected and on the final feasibility of performing the planned autologous transplantation.

Design and Methods

Patients aged less than 70 years with Durie-Salmon stage II or III or symptomatic stage I MM, were included in the study.

Chemotherapy before PBSC collection

Patients with serum creatinine <4 mg/dL received two courses of VBMCP and two courses of VBAD chemotherapy, in alternating fashion, administered every 4-5 weeks, before PBSC mobilization. VBMCP chemotherapy consisted of vincristine, 0.03 mg/kg intravenously (IV) on day 1; carmustine (BCNU), 0.5 mg/kg IV on day 1; cyclophosphamide, 10 mg/kg IV on day 1; melphalan, 0.25 mg/kg orally on days 1-4; prednisone, 1 mg/kg on days 1-4. Patients with serum creatinine levels higher than 2 mg/dL received half the melphalan dose until creatinine values had decreased to less than 2 mg/dL. VBAD chemotherapy consisted of vincristine, 1 mg/m^2 IV on day 1; BCNU, 30 mg/m^2 IV on day 1; adriamycin, 40 mg/m^2 IV on day 1; dexamethasone, 40 mg, orally on days 1-4, 9-12, and 17-20.

Mobilization regimen and PBSC collection

Although the optimal number of CD34$^+$ cells for ASCT has not been established,[12] the accepted minimum recommended dose is ≥2×10^6 cells/kg.[3] In our study, however, a double ASCT was planned for patients who did not achieve complete remission (CR) after the first transplant. Accordingly, we fixed a limit of 4×10^6 cells/kg as the minimum CD34$^+$ yield and this was the target dose used for the analysis.

We collected the PBSC after the fourth course of chemotherapy in every case. The mobilization regimen consisted of G-CSF administered subcutaneously (SC) at conventional (10-12 μg/kg per day, 351 patients) or high (16-24 μg/kg per day, 200 patients) doses. We started PBSC collection on the fifth day of G-CSF administration. An alternative mobilization regimen included cyclophosphamide (1.5 g/m^2) administered IV on day 1, followed by G-CSF (5 μg/kg per day) administered SC 24 hours later and daily thereafter until the end of the PBSC collection (206 patients). PBSC collection was started when the CD34$^+$ cell count in peripheral blood was greater than 10 cells/μL. Patients could undergo more than one round of PBSC collection if the yield of CD34$^+$ cells after the first cycle did not reach the target dose for ASCT. As mentioned above, patients who had not attained a CD34$^+$ cell count ≥4×10^6 cells/kg after one or two mobilization attempts were considered poor mobilizers.

Chemotherapy after PBSC collection

Two additional courses of VBMCP and VBAD chemotherapy were administered after PBSC collection and before transplantation. Patients were assessed for disease response before PBSC collection and immediately before transplantation according to European Bone Marrow Transplantation criteria.[13]

Conditioning regimen and ASCT

In the first 233 patients, the conditioning regimen prior to ASCT consisted of busulfan (3 mg/kg given orally every 8 hours from day –6 to day –3) followed by melphalan (140 mg/m^2 administered IV on day –2). Because of the high incidence of sinusoidal obstruction syndrome,[14] the conditioning regimen from November 2001 onwards consisted of high-dose melphalan (200 mg/m^2 administered IV on day -1). For patients who did not achieve complete remission after the ASCT, we planned a second ASCT using CBV as the conditioning regimen or an allogeneic transplant with a less intense conditioning regimen if an HLA-identical sibling donor was available. Figure 1 shows the schema of the Spanish multicenter trial GEM-2000.

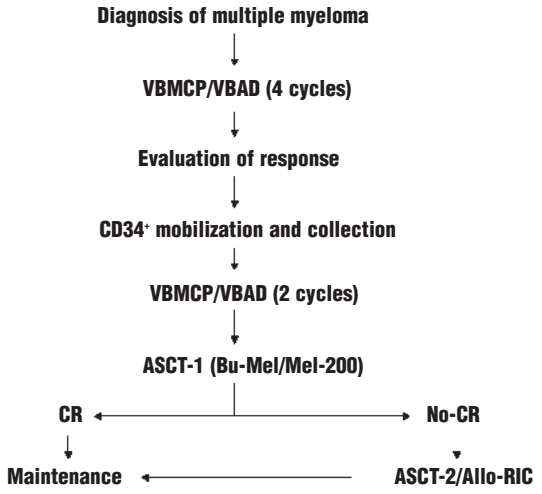

Figure 1. Schema of the GEM-2000 protocol. ASCT: autologous stem cell transplantation; CR: complete remission; Allo-RIC: allogeneic transplantation with reduced intensity conditioning.

Statistical analysis

We used both univariate and multivariate analyses to evaluate factors influencing the number of CD34$^+$ cells collected/kg of patient's body weight. The independent variables analyzed for their effect on CD34$^+$ cell collection included sex, age, Durie-Salmon stage, International Staging System stage, M component, type of light chain, status of the disease at mobilization, mobilization regimen (chemotherapy plus G-CSF, standard dose G-CSF, and high-dose G-CSF), and interval from diagnosis to mobilization. In the univariate analysis, statistical differences between groups were determined by the χ^2 test or Fisher's exact test for categorical variables and by the non-parametric Mann-Whitney test for continuous variables. All the variables were included in the multivariate analysis, which was performed using a stepwise logistic regression model. The relationship between age and CD34$^+$ cell numbers in apheresis products was assessed by linear regression and correlation analysis. p values lower than 0.05 were considered statistically significant. Neutrophil and platelet recovery after ASCT was plotted according to the Kaplan-Meier method. The significance of differences between curves was estimated by the log-rank test. Statistical analyses were performed with statistical software (BMDP, University of California, Berkeley, CA, USA).[15]

Table 1. Patients' characteristics.

Characteristic	*Median (range)*	*No. of cases (%)*
Sex		
Men		430 (55)
Women		359 (45)
Age (years)	59 (29-72)	
M component		
IgG		413 (52)
IgA		218 (28)
Bence-Jones		143 (18)
Other		14 (2)
Light chain		
κ		479 (60)
λ		299 (39)
Non-secretory		11 (1)
Stage (Durie-Salmon)		
I		70 (9)
II		303 (38)
III		415 (53)
ISS stage†		
I		270 (38)
II		290 (41)
III		147 (21)
Serum albumin (g/dL)	3.7 (1.1-6.4)	
Serum creatinine (mg/dL)	1 (0.2-14)	
β2 microglobulin (mg/L)	3.1 (0.2-36)	
Serum lactate dehydrogenase		
Normal		669 (88)
Elevated		94 (12)

†*ISS: International Staging System.*

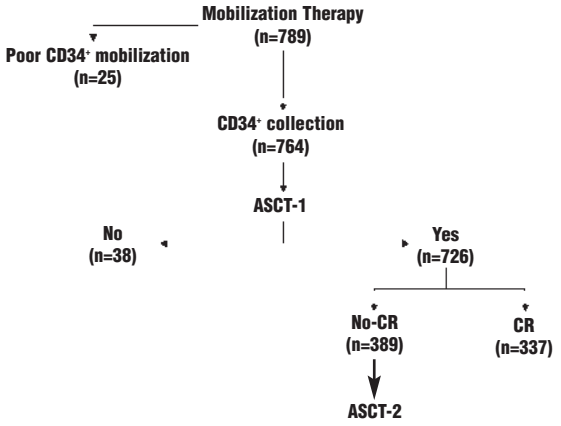

Figure 2. Feasibility of ASCT. The numbers refer to patients at each treatment step. ASCT: autologous stem cell transplantation; CR: complete remission.

Results

Patients' characteristics

From January 2000 to December 2004, 789 consecutive untreated MM patients from 66 Spanish centers entered the protocol and underwent mobilization therapy. The median age of the series was 59 years (range, 29-72 years), and there were 430 male and 359 female patients. The patients' characteristics are shown in Table 1. After first-line chemotherapy, 175 patients (22%) had achieved complete remission (85 of them (11%) with negative immunofixation predictive of a good outcome), 463 patients (58%) had achieved partial remission, 92 patients (11%) showed minimal response to the therapy, and 30 patients (4%) had progressive disease. In 36 patients (5%), the evaluation of the response was still pending at the time of mobilization therapy.

CD34+ cells collected

Following first-line chemotherapy, patients entered the mobilization phase of the protocol. The median time between diagnosis and mobilization was 5 months (range, 4-7 months). Only 25 patients (3%) did not undergo apheresis because of poor mobilization of the CD34+ cells (less than 10 CD34+ cells /μL in peripheral blood) (Figure 2). Hence, 764 patients underwent PBSC collection. The median number of CD34+ cells harvested for the whole series was 4.1×10^6/kg (range, 0-23.26×10^6/kg) in two apheresis procedures (median; range, 1-9). As previously mentioned, a CD34+ cell dose of 4×10^6 CD34+ cells/kg was the target in this study. This target was reached in 401 patients, but the CD34+ cell yield was below 4×10^6 cells/kg in 363 patients. These, together with the 25 patients considered to be poor mobilizers, made up group A (49% of the total number of patients), whereas the remaining 401 patients with a yield of CD34+ cells greater than 4×10^6/kg made up group B (51% of the total number of patients).

The median number of CD34+ cells collected was 2.4×10^6/kg (range, 0.01-3.99×10^6/kg) in group A and 5.6×10^6/kg (range, 4-23.26×10^6/kg) in group B. As shown in Table 2, patients with a higher CD34+ cell yield needed fewer PBSC collections to reach the target dose for ASCT. Conversely, group A patients had lower yields of CD34+ cells despite having undergone more apheresis procedures (p=0.0002). Figure 2 shows the number of patients at different steps in the study.

Second mobilization and collection

Of the 796 patients included in the GEM-2000 protocol, 120 underwent more than one round of PBSC mobilization and collection to reach the target ASCT dose of 4×10^6 CD34+ cells/kg. In 46 of these patients, the additional mobilization was performed because of a very low CD34+ cell yield after the first mobilization attempt (<2×10^6 cells/kg). The mobilization regimens most commonly followed in the second cycle of mobilization and collection consisted of cyclophosphamide plus G-CSF in 53 patients and G-CSF alone in 61 patients (30 of these were treated with high-dose G-CSF). The median number of CD34+ cells collected in this second mobilization was 2.06×10^6/kg (range, 0.17-11.4×10^6/kg).

Table 2. Univariate analysis of associations between patients' variables and CD34⁺ cell yield.*

	Number of CD34⁺ cells collected		
	< 4×10⁶/kg	≥ 4×10⁶/kg	p value
Number of patients	388	401	
Sex			
Men	199 (51)	231 (58)	
Women	189 (49)	170 (42)	
Age (years)			0.003
≤60	183 (47)	231 (58)	
>60	205 (53)	170 (42)	
M component			
IgG	199 (51)	214 (53)	
IgA	119 (31)	99 (25)	
Bence-Jones	63 (16)	80 (20)	
Other	7 (2)	7 (2)	
Light chain			
κ	241 (63)	229 (58)	
λ	135 (35)	164 (41)	
Non-secretory	6 (2)	5 (1)	
Stage (Durie-Salmon)			
I	34 (9)	36 (9)	
II	164 (42)	139 (35)	
III	189 (46)	226 (56)	
ISS stage†			
I	141 (41)	129 (36)	
II	131 (38)	159 (44)	
III	73 (21)	74 (20)	
Status at mobilization			
Complete response	84 (23)	88 (23)	
Partial response	253 (68)	267 (70)	
Minimal response	5 (5)	50 (8)	
Stable disease	20 (5)	13 (3)	
Progression	16 (4)	14 (4)	
Mobilization regimen			0.04
Standard-dose G-CSF	187 (48)	164 (41)	
High-dose G-CSF	98 (25)	102 (25)	
Chemotherapy + G-CSF	85 (22)	121 (30)	
Other	18 (5)	14 (4)	
Diagnosis-mobilization interval (days)			0.02
<150	79 (20)	103 (26)	
≥150	309 (80)	298 (74)	
Number of apheresis procedures			0.0002
<2	207 (56)	275 (69)	
≥2	162 (44)	123 (31)	

Values are expressed as number of patients (%); †ISS, International Staging System.

Influence of the mobilization regimen on CD34⁺ cell yield

The mobilization regimen most frequently used was G-CSF alone, at standard (351 patients, 44%) or high (200 patients, 25%) doses, while cyclophosphamide plus G-CSF was used in 206 patients (26%). The remaining 32 patients (4%) received other regimens. The dose of G-CSF administered did not affect the yield of CD34⁺ cells. Thus, the median number of CD34⁺ cells collected did not differ significantly between patients receiving standard-dose G-CSF (4.17×10⁶/kg; range, 0-21.09×10⁶/kg) and patients receiving high-dose G-CSF, (4.38×10⁶/kg; range, 0-23.26×10⁶/kg). The median number of CD34⁺ cells collected after mobilization with cyclophosphamide plus G-CSF was 4.98×10⁶/kg (range, 0-17.1×10⁶/kg). Univariate analysis indicated that a higher proportion of patients in group B underwent the cyclophosphamide plus G-CSF mobilization regimen (p=0.04; Table 2).

Table 3. Variables associated with a lower CD34⁺ cell collection. Results of multivariate logistic regression.

	RR	95% CI	p value
Age	1.05	1.02-1.07	< 0.0001
Diagnosis-mobilization interval	1.04	0.99-1.08	0.012

RR: relative risk; CI: confidence interval.

Other factors affecting CD34⁺ cell collection

The median age of patients with a CD34⁺ cell yield lower than 4×10⁶/kg was significantly higher than that of patients with a CD34⁺ cell yield equal or greater than 4 ×10⁶/kg (59±8 years vs. 57±8 years; p<0.001). In the univariate analysis, the most prominent correlation among patients' variables was that between age and CD34⁺ cell yield (p=0.003) (Table 2). Interestingly, disease status at mobilization did not influence the number of CD34⁺ cells collected (Table 2).

Multivariate analysis showed that the most important factors associated with a lower CD34⁺ cell yield were the age of the patient and the time between diagnosis and mobilization, such that elderly patients and patients with a longer diagnosis-mobilization interval could be predicted to have a lower yield of CD34⁺ cells (Table 3). Correlation analysis of patients' age and CD34⁺ cell yield demonstrated an age-related decline in CD34⁺ cell numbers (r=-0.3; p=0.0001).

Hematopoietic recovery after ASCT

Although 63 of the 796 patients (8%) included in this study did not undergo the planned ASCT, this was due to a low CD34⁺ cell yield (<1×10⁶/kg) in only 25 of them (3%). At the time of the analysis, ASCT was still pending in 30 of the remaining 38 patients because they had not completed the two cycles of chemotherapy scheduled after PBSC collection; five other patients could not undergo ASCT because of chemotherapy-associated toxicity. In the remaining three patients, the transplant was not carried out because of rejection (one patient), progression before ASCT (one patient), and death (one patient). In the whole series, the median time to attain an absolute neutrophil count greater than 0.5×10⁹/L was 11 days (range, 5-71 days) and the median time to attain a platelet count of 20×10⁹/L was 12 days (range, 6-69 days). It should be noted that 74 of the 388 group A patients underwent ASCT despite a CD34⁺ cell yield lower than 2×10⁶/kg. After transplant, the median time for patients to achieve an absolute neutrophil count greater than 0.5×10⁹/L was 11 days (range, 5-44 days) for group A and 11 days (range, 5-71 days) for group B. Likewise, there was no difference in platelet recovery in the two groups of patients: the median time to reach 20×10⁹ platelets/L was 12 days (range, 6-65 days) in group A vs. 12 days (range, 7-69 days) in group B. Finally, the conditioning regimen (busulfan-melphalan vs. high-dose melphalan) did not affect hematopoietic recovery for patients undergoing the first ASCT (*data not shown*).

Discussion

Although not curative, ASCT increases the likelihood of complete remission, and in most studies prolongs event-free survival and overall survival, representing a major advance in myeloma therapy.[16,17] Whether complete remission is achieved before ASCT is an important predictor of the eventual outcome.[1] A more effective induction chemotherapy could increase the complete remission rate before transplant. In this regard, we and others have shown that a combination chemotherapy including alkylating agents is associated with high response rates.[7-9,18,19] However, the beneficial effect of these drugs could be counterbalanced by their risk of inducing stem cell damage, which would result in inadequate mobilization of the stem cells.[20] In this study, 401 patients (51%) attained the minimum cell yield set to perform the planned ASCT (4×10^6 CD34+ cells/kg). Nevertheless, the number of those actually transplanted was much higher (726 patients, 92%), including 74 patients with a CD34+ cell yield lower than 2×10^6/kg. This fact suggests that the administration of combination chemotherapy that included alkylating agents as a standard therapy before CD34+ mobilization has a minor clinical impact on the feasibility of the protocol. We did not observe differences in hemopoietic recovery related to the dose of progenitors administered. Overall, a poor CD34+ cell collection precluded transplant in only 25 patients (3%). Similar results were obtained in a previous study in which up to 15% of the patients did not undergo the planned transplant.[3]

The duration of standard therapy before mobilization seems to play an important role in its efficacy, especially when drugs that are toxic for stem cells are administered. Thus, extensive chemotherapy prior to mobilization has been reported to affect the yield of PBSC adversely;[20-22] accordingly, it has been recommended that mobilization and collection are performed early in the course of the disease in order to avoid poor CD34+ cell yields.[23,24] There is particular concern with the use of alkylating agents, and in this regard several groups have shown that long-term administration of melphalan before PBSC mobilization precludes successful harvest.[20-22] In our study, however, treatment with alkylating agents was administered during a short period of time before collection to minimize toxicity to stem cells, which probably explains the mobilization response observed, with the median number of CD34+ cells collected being $>4\times10^6$ CD34+ cells/kg.

In our series, age influenced the response to mobilization therapy, with increasing age being significantly associated with lower numbers of CD34+ cells collected. Although some groups did not observe an independent effect of age on CD34+ cell mobilization,[20,25-27] Morris *et al*. showed a clear association between these two parameters in a large series of patients.[23] These contradictory findings are probably due to the effect of other parameters on stem cell yield, such as prior radiotherapy and duration and type of chemotherapy before mobilization.[20,24,26] In contrast to previous reports, in our study all patients were treated similarly: they received only one chemotherapy regimen before mobilization and only a few patients underwent mobilization more than 6 months after diagnosis. Thus, the detrimental effect of increasing age on CD34+ cell yield was not influenced by other negative factors, suggesting that age is a strong predictor of good or poor stem cell yield. The reasons for this association are not known, but may be related to a lower stem cell reserve in the bone marrow of elderly patients. Additional studies are clearly warranted to further characterize the mechanisms of stem cell mobilization and develop better strategies for poor mobilizers. In this study, a longer interval between diagnosis and mobilization was the other factor significantly associated with a lower CD34+ cell yield. This might be a surrogate marker of a more pronounced myelosuppressive effect of the chemotherapy administered before PBSC mobilization. However, because CD34+ cells were collected within 7 months after diagnosis in every patient in our series, this finding seems to be of minor clinical relevance.

Mobilization with chemotherapy plus growth factors has been shown to be more effective than mobilization with growth factors alone.[26,27,29,30] In our series, we observed differences in the number of CD34+ cells collected upon mobilization with chemotherapy and growth factors by univariate, but not multivariate, analysis. Furthermore, the median number of CD34+ cells collected after mobilization with cyclophosphamide plus G-CSF was slightly higher than that obtained with G-CSF alone, but the difference was not statistically significant. This is probably because we administered a lower dose of cyclophosphamide than that generally used. Cyclophosphamide is the chemotherapeutic agent most commonly used for PBSC mobilization, but the optimal dose has not yet been established and doses used range from 2 to 7 g/m^2.[5,20,21,25-27,30] Although higher doses of cyclophosphamide may yield higher CD34+ cell numbers, they are associated with increased toxicity and morbidity.[20,27,30] Accordingly, it is currently difficult to reach a conclusion on the ideal dose of cyclophosphamide for mobilization in myeloma patients. Our results show that mobilization with G-CSF alone at the steady state is a valid alternative for PBSC collection in these patients.[24] In addition, because we did not observe differences in CD34+ cell yield related to the G-CSF dose used for mobilization, G-CSF could be an adequate mobilization regimen at the conventional dose of 10-12 μg/kg per day.

In contrast to other studies,[21,27,29,31] we did not find differences in hematopoietic recovery after transplant in relation to the number of CD34+ cells infused. One study suggested that 2×10^6 CD34+ cells/kg is clinically useful in myeloma patients for neutrophil recovery, but may not be sufficient for platelet recovery.[21] However, because patients were more heavily pretreated in that study than in the present one, it is difficult to make meaningful comparisons. On the other hand, it should be noted that our study was a multicenter trial, and interlaboratory differences in CD34+ cell enumeration may have contributed to this finding.

Several new therapeutic agents are currently available for patients with multiple myeloma.[6] Although results are still preliminary, these drugs do not seem to affect

PBSC mobilization and collection adversely and should play an essential role in the management of these patients in the near future. However, randomized trials comparing these new drugs with the conventional chemotherapy combinations are needed to define the optimal first-line treatment for patients with multiple myeloma.

In conclusion, the chemotherapy regimen used in the present study enabled us to test sensitivity not only to high-dose dexamethasone and doxorubicin (VAD regimen), but also to alkylating agents (melphalan, cyclophosphamide, and BCNU). This is important for defining further treatment strategies, but also for assessing the role of transplantation in primary refractory patients. Indeed, the response to high-dose melphalan could be better in patients who are primary refractory to VAD therapy (patients who have not been exposed to alkylating agents) than in patients refractory to VBMCP/VBAD therapy. Our study shows that advanced age is the main factor that significantly affects mobilization and collection of $CD34^+$ cells in myeloma patients undergoing ASCT as front-line therapy. Our study also show that although therapy with alkylating agents was associated with collection of the target number of $CD34^+$ cells in 51% of patients, the majority of patients included in the study actually received the planned ASCT. Thus, the risk of stem cell damage should not be a major reason to preclude short courses of this type of chemotherapy as front-line treatment in patients who are candidates for ASCT.

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