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Plerixafor Added to Chemotherapy Plus G-CSF Is Safe and Allows Adequate PBSC Collection in Predicted Poor Mobilizer Patients with Multiple Myeloma or Lymphoma

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We evaluated the safety and efficacy of plerixafor, subsequent to disease-specific chemotherapy followed by granulocyte-colony stimulating factor (G-CSF), in 37 multiple myeloma (MM) or lymphoma patients, who were candidates for autologous stem cell transplantation (ASCT) predicted as poor mobilizers (PMs). Patients were identified as predicted PMs according to the history of a previously failed mobilization attempt or the presence of ≥1 factors predicting an unsuccessful harvest, such as advanced disease, prior extensive radiotherapy, or prolonged treatment, with stem cell poisons, advanced age, or extensive bone marrow involvement. Plerixafor (0.24 mg/kg) was administered subcutaneously for up to 3 consecutive days while continuing G-CSF for 9 to 11 hours before the planned apheresis. Plerixafor administration was safe and no significant adverse events were recorded. We observed a median 4-fold increase (range: 1.4-32) in the number of circulating $CD34^+$ cells following plerixafor compared with baseline $CD34^+$ cell concentration (from a median of 5 cells/μL, range: 1-32, to a median of 32 cells/μL, range: 6-201). Twenty-seven of the 37 patients (14 of 17 with MM and 13 of 20 with lymphoma) had $\geq 2\times10^6$ $CD34^+$ cells/kg collected in 1-3 apheretic procedures. Of the 27 patients rescued with plerixafor, 24 (13 MM, 11 lymphoma) have been transplanted with plerixafor-mobilized peripheral blood stem cells, showing a rapid and durable hematologic recovery. Our results suggest that the addition of plerixafor to G-CSF after disease-oriented chemotherapy is safe and allows for a satisfactory harvest in order to perform a safe ASCT, in a relevant proportion of lymphoma and MM patients considered to be PMs.

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INTRODUCTION

Autologous stem cell transplantation (ASCT) is a mainstream therapy for patients with lymphoma or multiple myeloma (MM); however, 5% to 40% of MM or lymphoma patients fail to mobilize adequate numbers of peripheral blood stem cells (PBSCs), and thus cannot undergo a planned ASCT [1]. Over the past decade, different criteria have been proposed to define a successful $CD34^+$ cell mobilization, leading to an adequate apheresis yield. The current minimal threshold $CD34^+$ cell dose needed for the achievement of a fast, complete, and stable long-term engraftment, has been determined as $\geq 2\text{-}2.5\times10^6$ $CD34^+$ cells/kg for a single ASCT [2-8]. Reinfusion of higher doses of $CD\ 34^+$ cells has been associated with reductions in the duration of hospital stay and transfusion support requirements [9]. Moreover, in some studies, lymphoma patients transplanted with $>2\times10^6$ $CD34^+$

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cells/kg showed significantly better survival rates [10]. The 2 most commonly used mobilization regimens are the cytokine granulocyte-colony stimulating factor (G-CSF) and chemotherapy followed by G-CSF. The latter is reportedly associated with better harvests [11-14], even though it did not seem to reduce the percentage of mobilization failures [4].

Plerixafor, a novel CXCR4 inhibitor, is effective in mobilizing PBSCs, particularly when used in combination with G-CSF [15], and it reportedly augments the mobilization of the $CD34^+$ cells from bone marrow (BM) into peripheral blood (PB) when given after 4 days of G-CSF. Studies in non-Hodgkin lymphoma (NHL) and MM patients showed that the combination of G-CSF and plerixafor resulted in a significant increase in the $CD34^+$ cell yield after apheresis compared with the administration of G-CSF alone [16,17]. Moreover, plerixafor administration combined with G-CSF allowed for the progression to ASCT in a relevant proportion of lymphoma and MM patients, and for the achievement of rapid and sustained neutrophil (PMN) and platelet (PLT) engraftment of the mobilized PBSCs [18].

Consequently, plerixafor represents a valuable option for MM or lymphoma patients who mobilize poorly. Unfortunately, there are still some controversies concerning the identification of poor mobilizers (PMs). Data regarding the identification of PMs and the main factors affecting mobilization ability in MM and lymphoma patients derive from retrospective studies and are often difficult to analyze [1,4]. Nevertheless, early identification of PMs is an important issue that can prevent mobilization failures and designate these subjects for "ad hoc" mobilization strategies.

Over the past decade, some retrospective studies have confirmed the efficacy of plerixafor administration in combination with G-CSF in MM and lymphoma patients, although scarce information is available regarding the efficacy of plerixafor when associated with chemotherapy plus G-CSF. Therefore, we addressed this issue and assessed the safety and efficacy of plerixafor administered after chemotherapy followed by G-CSF in a population of MM and lymphoma patients identified as PMs, using a set of standardized criteria.

PATIENTS AND METHODS

Study Design

MM and lymphoma patients, according to the local policy of 5 italian centers, were enrolled in this prospective observational cohort study. Patients received plerixafor as compassionate use, on the basis of the presence of at least 1 of the following standardized criteria devised to identify patients as predicted PMs:

1. Mobilization failure, defined as evidence of a previously failed attempt to collect $\geq 2\times10^6$ $CD34^+$ cells/kg after both G-CSF alone and chemotherapy followed by G-CSF. This criterion included both patients who did not undergo apheresis, because of an unsatisfactory peak of circulating $CD34^+$ cells (eg, $<10/\mu L$) and those who underwent at least 3 consecutive apheresis procedures with total yields $\leq 2\times10^6$ $CD34^+$ cells/kg [1,4].
2. Presence of ≥ 1 adverse factors for PBSC mobilization, such as advanced disease, prior treatment with extensive radiotherapy (including BM-bearing tissues), prolonged chemotherapy (≥ 2 courses), past exposure to stem cell poisons (SCP) (eg, fludarabine, lenalidomide and alkylating agents such as melphalan), advanced age (>65 years old), or extensive BM involvement (>30%) before mobilization.

The main endpoint of the study was to assess whether the use of plerixafor after disease-specific chemotherapy followed by G-CSF would be safe and would allow adequate PBSC collection in MM and lymphoma patients considered to be predicted PMs according to the previously mentioned criteria. Secondary endpoints evaluated were: the increase in $CD34^+$ cell count in PB after plerixafor in the different groups of patients; median number of apheresis days needed to collect the target dose of $CD34^+$ cells; percentage of patients able to undergo ASCT; engraftment kinetics after reinfusion of plerixafor-mobilized PBSC; and the overall outcome of the autografted patients.

The enrollment of a minimum of 24 and a maximum of 42 patients was planned to allow the evaluation of safety. The study was conducted in compliance with the Declaration of Helsinki and Good Clinical Practice guidelines and was approved by the institutional review board of each center. All patients provided written informed consent.

Study Patients

Between April 2009 and May 2010, a total of 37 (17 MM, 20 lymphoma) patients were enrolled (22 males, 15 females). The median age was 58 (range: 20-74). The demographic details are reported in Table 1a,b. All 37 patients fulfilled ≥ 1 criteria for the identification of predicted PMs: Most (30) had advanced-stage disease, and 28 had received at least 2: prior chemotherapy courses (median 2, range: 2-4). A previous administration of SCP was documented in 17 patients and, most important, 25 of the 37 patients had previously failed at least 1 mobilization attempt (in 3 cases ≥ 2 attempts). Twelve patients (5 MM, 7 lymphoma) did not fail a previous mobilization attempt, but were included in the study on the basis of meeting 1 or more of the remaining criteria. Specifically, of the 5 MM patients, 1 had received previous extensive spinal radiotherapy, 2 lenalidomide (for 9 and 4 months, respectively), 1 ASCT with high-dose

Table 1 (a,b). Main Characteristics of MM (a) and Lymphoma (b) Patients before Mobilization

(a)

MM Patients	Age (years)	Sex	Disease Stage at Diagnosis	Number of Prior CHT	Previous SCP	Previous RX Ther	Failure of Previous Mobilization	Disease Status at PBSC Mobilization
1	62	F	IIIA	2	Y	N	Y	nCR
2	60	M	IIIA	1	N	N	Y	PR
3	61	F	IIIA	1	N	N	Y	PR
4	66	M	IIIA	1	Y	N	Y	PR
5	57	F	IIIA	3	Y	N	Y	PR
6	67	F	IIIB	4	Y	N	Y	PD
7	63	M	IIA	1	N	N	Y	PR
8	58	F	IIIA	2	N	Y	N	CR
9	59	M	IIIB	2	Y	N	Y	PR
10	59	M	IIIB	2	Y	N	Y	PR
11	52	F	IIIA	2	Y	N	N	VGPR
12	64	F	IIA	3	Y	N	Y	CR
13	65	M	IIIA	1	N	N	N	VGPR
14	57	F	IIIA	2	Y	N	Y	PR
15	57	F	IIIA	3	Y	N	N	PD
16	53	M	IIA	3	Y	N	N	PR
17	53	F	IIIA	2	N	N	Y	VGPR

(b)

Lymphoma Patients	Age (years)	Sex	Disease Stage at Diagnosis	BM Infiltrate ≥30%	Number of Prior CHT	Previous SCP	Previous RX Ther	Failure of Previous Mobilization	Disease Status at PBSC Mobilization
1	47	M	IV	Y	1	N	N	Y	PR
2	52	F	IV	Y	3	N	N	Y	RD
3	58	M	IV	N	2	N	N	Y	REL
4	50	M	IV	Y	2	N	N	N	CR
5	53	M	III	N	2	Y	N	N	REL
6	54	M	IV	Y	3	Y	N	N	REL
7	68	M	IV	Y	2	N	N	Y	PR
8	71	M	IVA	Y	2	N	N	Y	SD
9	50	M	IVA	Y	2	Y	N	Y	CR
10	52	M	IVE	N	1	Y	N	Y	CR
11	52	F	IIA	Y	3	Y	Y	Y	CR
12	54	F	IVA	Y	1	Y	N	Y	CR
13	74	M	IIIA	Y	2	N	N	Y	PR
14	54	M	IVB	Y	2	N	N	N	RD
15	60	M	IVA	Y	3	N	N	N	VGPR
16	54	M	IIB	N	1	N	Y	Y	CR
17	64	F	IIA	N	3	N	N	N	REL
18	20	M	IIIA	N	3	N	N	N	REL
19	72	F	II_SB	N	3	N	N	Y	PR
20	58	M	IV_SB	Y	2	N	N	Y	PR

MM indicates multiple myeloma; BM, bone marrow; N, number; CHT, chemotherapy; SCP, stem cell poison; RX Ther, radiotherapy; PBSC, peripheral blood stem cell; CR, complete remission; PR, partial response; VGPR, very good partial response; SD, stable disease; RD, resistant disease; REL, relapse.

melphalan 200 mg/m^2, and the remaining had an advanced disease with extensive BM involvement and advanced age. Among the 7 lymphoma patients, 2 had Hodgkin's lymphoma (HL) and were heavily pretreated (≥2 full courses of chemotherapy), whereas 5 had NHL: 2 had received ≥6 cycles of chemotherapy including fludarabine (1 also had an extensive BM lymphoma involvement), and 3 had indolent lymphoma with an extensive (>30%) BM involvement (1 had also received 3 full courses of chemotherapy).

Mobilization Regimens

Disease-specific mobilization regimens were planned according to the local institutional guidelines. The most frequent mobilization regimens were cytoxan at an intermediate dose in 12 MM patients and DHAP [19] in 13 lymphoma patients; a minority of patients received VP16 at a high dose [20] or HyperC-VAD [21]. The details of the mobilizing chemotherapy are shown in Table 2. G-CSF at 5 to 10 μg/kg/day was administered subcutaneously, starting at 48 to 96 hours after the end of chemotherapy and continued until the last apheresis day.

Plerixafor administration was planned in order to reach at least 2-2.5×10^6 CD34$^+$ cells/kg (within ≤3 consecutive apheresis days) for patients scheduled for a single ASCT. On the other hand, a minimum threshold of 4-5×10^6 CD34$^+$ cells to be collected was planned for the MM patients who were candidates

Table 2. Details of Mobilization Schedules, White Blood Cells Count, and CD34$^+$ Cells Kinetics and Collections in MM and Lymphoma Patients

Characteristics	MM	Lymphoma
CHT mobilizing regimen	HD-CTX: 12 VP16: 3 Others: 2	DHAP: 13 HyperCVAD: 2 VP16: 2 Others: 3
Plerixafor injections:, median (range)	2 (1-3)	1 (1-2)
WBC before plerixafor ($\times 10^3/\mu L$):, median (range)	17 (2.1-68)	8.15 (1.4-61)
WBC 11 hours after plerixafor ($\times 10^3/\mu L$), median (range)	26.5 (3.5-79)	16.1 (7.2-65)
CD34$^+$ before plerixafor ($\times 10^3/\mu L$):, median (range)	6 (2-32)	5 (0-26)
CD34$^+$ 11 hours after plerixafor ($\times 10^3/\mu L$): median (range)	33 (6-201)	29 (0-116)
Fold increase CD34$^+$ count:, median (range)	4 (2-25)	3 (0-32)
Total number of CD34$^+$ cells collected ($\times 10^6$/kg):, median (range)	4.9 (0-15.2)	2.65 (0-8.2)
Total number of apheresis: median (range)	2 (0-3)	1 (0-2)

MM indicates multiple myeloma; CHT, chemotherapy; HD-CTX, high-dose cytoxan; DHAP, dihydroxyacetone phosphate; CVAD, cyclophosphamide, vincristine, doxorubicin; WBC, white blood cell.

for double ASCT. Plerixafor (0.24 mg/kg; Genzyme Europe BV, Naarden, the Netherlands) was added to G-CSF under a compassionate use program. The drug was administered subcutaneously at 0.24 mg/kg/day for up to 3 days the evening before the planned leukapheresis (from 9-11 hours before starting the procedure). The patients received a median of 2 plerixafor administrations (range: 1-3) after mobilization.

PBCS Collection and Transplantation

The start of PBSC collections was generally planned when the CD34$^+$ cell count in the PB was $\geq 5/\mu L$ after plerixafor administration. The mobilization attempts never reaching the threshold of CD34$^+$ $\geq 10/\mu L$ in the PB, after at least 3 consecutive days of plerixafor administration, or failing to yield a total of $\geq 2\times 10^6$ CD34$^+$ cells/kg, were considered failures. CD34$^+$ cell count was determined using the single platform as recommended by the International Society of Hematotherapy and Graft Engineering (ISHAGE) protocol, combined with a viability test performed with 7-actinomycin D [22]. Double-volume leukapheresis or large-volume leukapheresis (ie, 3-blood volume $\pm 15\%$) was used according to institutional guidelines. A maximum of 3 apheresis days was performed for each patient.

Table 3. Comparison of Characteristics Influencing the Mobilization Ability in MM and Lymphoma Patients

Characteristics	MM	Lymphoma	*P*
Age (median)	59 (SD 4.556)	54 (SD 7.865)	.123
Sex (M/F)	7/10	15/5	.08
Previous chemotherapy courses (median)	2 (SD 0.899)	2 (SD 0.745)	.637
SCP (Y/N)	11/6	6/14	.075
RX ther (Y/N)	1/16	2/18	1
Previous mobilization failure (Y/N)	12/5	13/7	.99
PB CD34$^+$ cells* before plerixafor (median)	6 (SD 11.425)	5.5 (SD 7.048)	.718

MM indicates multiple myeloma; SCP, stem cell poison; RX ther, radiotherapy; PB, peripheral blood; SD, Standard deviation.
*CD34$^+$ cells/μL in PB before the first plerixafor administration.

The collected PBSCs were reinfused after myeloablative conditioning consisting of high-dose melphalan 200 mg/m^2 for MM patients and of diverse chemotherapy-based regimens for lymphoma patients (Table 5). Posttransplantation G-CSF was administered at 5 μg/kg/day, starting 3 to 7 days after PBSC reinfusion, according to the local institutional policy, up to PMN recovery. All patients were hospitalized in single rooms with HEPA filters and positive air pressure until neutrophil engraftment. The antimicrobial prophylaxis consisted of the administration of oral quinolones and fluconazole at 400 mg/day. All patients received empirical antibiotic therapy in case of fever $>38^\circ$C and the transfusion support consisted of irradiated blood products. Time to PMN and PLT recovery were defined as the number of days needed to achieve an absolute neutrophil count (ANC) higher than $0.5\times 10^3/\mu L$ (first of 3 consecutive days) and an unsupported PLT count higher than $20\times 10^3/\mu L$ and $50\times 10^3/\mu L$.

Statistical Methods

The 2 populations of MM and lymphoma patients were compared using the Mann-Whitney *U* test for the continuous variables and cross-tab tests for the discrete variables because of the small sample size. The Mantel-Haenszel Common Odds Ratio Test was employed for the dichotomized variables. Mobilization results in the 2 populations (CD34$^+$ peak, CD34$^+$ fold increase, CD34$^+$ cumulative harvest, and percentage of patients failing to achieve $\geq 2\times 10^6$ harvested CD34$^+$/kg) were compared using the Mann-Whitney *U* test for the continuous variables; cross-tab tests were used for the discrete variables. Similarly, the Mantel-Haenszel Common Odds Ratio Test was employed for the dichotomic variables. Engraftment kinetics in the 2 populations (eg, median number to achieve an ANC higher than $0.5\times 10^3/\mu L$, first of the 3 consecutive days, and an unsupported PLT count higher than $20\times 10^3/\mu L$ and $50\times 10^3/\mu L$) were compared using the Mann-Whitney *U* test [23].

Table 4. Comparison of Mobilization Ability, Harvest, and Engraftment in the Two Populations (MM and Lymphoma Patients)

	MM	Lymphoma	*P*
PB $CD34^+$ cells* after plerixafor (median)	33 (SD 45.499)	31 (SD 26.946)	.437
Fold increase (median)	4 (SD 5.985)	3 (SD 7.563)	.485
$CD34^+$ harvested ($\times 10^6$/kg) (mean)	6.36 (SE 1.121)	3.8 (SE 1.063)	**.03**
Number of leukapheresis (median)	2 (SD 0.845)	1 (SD 0.514)	.059
% of pts failing to harvest $\geq 2 \times 10^6$ CD34/kg	18	35	.24
Days for PMN >500 (median)	12 (SD 1.832)	14 (SD 3.795)	.076
Days for PLT >20,000 (median)	15 (SD 1.809)	18 (SD 22.033)	.037
Days for PLT >50,000 (median)	18 (SD 7.648)	30 (SD 50.904)	.011

MM indicates multiple myeloma; PB, peripheral blood; pts, patients; PMN, neutrophils; PLT, platelets; SD, standard deviation, SE, standard error.
*$CD34^+$ cells/μL 9 to 11 hours after plerixafor administration, before the first apheresis.

RESULTS

The 2 populations of MM and lymphoma patients were well matched for the main clinical characteristics influencing their mobilization ability (Table 3). Mobilization with chemotherapy followed by G-CSF and plerixafor was well tolerated, and we did not observe any grade 3-4 extrahematologic toxicities. Only 1 patient developed a fever of unknown origin during the neutropenic phase. We did not observe any significant laboratory abnormalities or any worsening of liver or renal function during plerixafor administration.

The median value of the white blood cell count before plerixafor administration was 9×10^3/μL (range: 1.4-68$\times10^3$/μL) and increased to 19×10^3/μL (range: 3.5-79$\times10^3$/μL) posttreatment.

Twenty-seven of the 37 patients who received plerixafor were successfully mobilized, collecting a median of 5.4×10^6 $CD34^+$ cells/kg (range: 2-15.2) over a maximum of 3 apheresis days. Ten patients failing the mobilization were considered not eligible for ASCT and received alternative treatments.

The median value of the circulating $CD34^+$ cells/μL before plerixafor administration was 5 (range: 0-32) and did not show any significant differences between MM and lymphoma patients ($P = .718$). After plerixafor administration and before the first apheresis, the median number of circulating $CD34^+$ cells was 32 (range: 0-201), with a median 4-fold increase (range: 1.4-32). A comparison of the mobilization results after plerixafor in MM and lymphoma patients is shown in Table 4. We did not observe statistical differences between the 2 populations in terms of $CD34^+$ peak, $CD34^+$ fold increase, or in the number of apheresis days, but we did find a significantly better harvest (in terms of total

Table 5. Disease Status after Mobilization and before ASCT and Outcome in the 24 MM and Lymphoma Patients

Transplanted Patients	Disease	Response after Chemomobilization	Conditioning Regimens	ANC >500/mL	PLT >20 x 10^3/mL	PLT >50 x 10^3/mL	Response at Day +90	Status at Day +90
1	HL	CR	FEAM [24]	12	17	19	CR	A
2	HL	SD	FEAM [24]	13	18	30	NE	NE
3	HL	PR	BEAM [25]	14	21	38	PR	A
4	NHL	PR	FEAM [24]	17	22	36	CR	A
5	NHL	CR	FEAM [24]	14	17	22	CR	A
6	NHL	PR	FEAM [24]	20	88	NR	PR	A
7	NHL	SD	FEAM [24]	14	34	NR	NE	NE
8	NHL	CR	BEAM [25]	10	9	26	CR	A
9	NHL	CR	TEAM [26]	23	10	24	CR	A
10	NHL	CR	Thio-Mel	16	30	180	CR	A
11	NHL	PR	BEAM [25]	12	15	33	PR	A
12	MM	nCR	Mel 200	11	15	18	nCR	A
13	MM	PR	Mel 200	11	11	16	PR	A
14	MM	VGPR	Mel 200	16	16	22	VGPR	A
15	MM	PR	Mel 200	13	13	16	PR	A
16	MM	PR	Mel 200	15	15	20	PR	A
17	MM	PR	Mel 200	13	15	15	PR	A
18	MM	CR	Mel 200	11	14	20	CR	A
19	MM	VGPR	Mel 200	11	13	30	VGPR	A
20	MM	CR	Mel 200	–	–	–	–	D
21	MM	VGPR	Mel 200	11	15	18	nCR	A
22	MM	PR	Mel 140	12	18	40	PR	A
23	MM	PR	Mel 200	12	NR	NR	NR	A
24	MM	PR	Mel 140	15	15	15	PR	A
Summary median (range)	3 HL/8 NHL/13 MM –	8 CR, nCR/14 PR, VGPR/2SD –	13 HDM/11 other –	– 13 (10-23)	– 15 (9-88)	– 22 (15-180)	9 CR, nCR/11 PR, VGPR	21 A/2 NE/1 D -

ASCT indicates autologous stem cell transplantation; MM, multiple myeloma; HDM, high dose melphalan; ANC, absolute neutrophil count; PLT, platelets; HL, Hodgkin's lymphoma; NHL, non-Hodgkin lymphoma; CR, complete remission; PR, partial response; SD, stable disease; VGPR, very good partial response; nCR, near complete remission; NR, not reached; NE, not evaluable; A, alive; D, dead.

amount of the $CD34^+$ cells collected) in the MM patients (mean 6.36×10^6/kg vs 3.8×10^6/kg; $P = .03$).

The percentage of successful collections after mobilization with chemotherapy followed by G-CSF plus plerixafor was 73%: 65% in lymphoma patients and 82% in MM patients. At a confidence level of 95%, the percentage of patients failing to achieve $\geq2\times10^6$ $CD34^+$/kg was significantly higher in the lymphoma patients (data not shown).

Overall, of the 27 patients with satisfactory harvests ($\geq2\times10^6$/kg), 24 (65%) were autografted; the 3 remaining patients with satisfactory collections were not able to undergo ASCT because of rapid disease progression.

In detail, 14 of 17 MM patients had satisfactory harvests ($\geq2\times10^6$ $CD34^+$ cells/kg) and 13 received ASCT; 13 of 20 lymphoma patients (8 of 15 NHL and 5 of 5 HL) had satisfactory harvests and 11 underwent ASCT (8 of 8 NHL and 3 of 5 HL).

After mobilization, 9 of the 11 lymphoma patients who received ASCT were in complete remission or partial remission (PR), whereas all 13 MM patients achieved equal to or greater than PR before ASCT. All but 3 patients showed rapid and complete engraftment. Of the 3, 1 MM patient died of sepsis during the aplastic phase, 1 NHL patient showed both delayed PMN and PLT recovery reaching ANC $\geq$500/μL on day 20 and PLT $\geq$20,000 on day 88, without reaching PLT count $\geq$50,000, and the third patient reached ANC $\geq$500/μL on day 23 but showed a quick PLT recovery. The latter 2 patients are alive and in PR or complete remission, respectively, at last follow-up.

The differences in PMN engraftment kinetics were not significant between the 2 populations. A median of 12 days (range: 11-16, SD = 1.832) was observed in the MM patients and 14 days (range: 10-23, SD = 3.795) in the lymphoma patients ($P = .076$). In contrast, a significant difference was observed for PLT engraftment, with a median of 18 days to reach a PLT count $\geq$20,000/μL (range: 9-88, SD = 22.033) in the lymphoma patients versus 15 days (range: 11-18, SD = 1.809) in the MM patients ($P = .037$). It took 30 days to reach a PLT count $\geq$50,000/μL (range: 19-180, SD = 50.904) in the lymphoma patients versus 18 days (range: 15-40, SD = 7.648) in the MM patients ($P = .011$).

After a minimum follow-up of 90 days after ASCT, 21 patients (12 MM and 9 lymphoma patients) were alive and evaluable for response, with 11 MM patients considered responders (equal to or greater than PR) and 1 with refractory disease, whereas 6 lymphoma patients were in complete remission and 3 in PR (Table 5).

DISCUSSION

In the present study, we report the results of different mobilization regimens, based on chemotherapy, followed by G-CSF and plerixafor in a group of 37 MM and lymphoma patients who were candidates for ASCT. These patients were prospectively identified as predicted PMs based on uniformly standardized criteria. Most of these criteria were previously used in large studies with plerixafor [16,17,27], particularly in patients with a history of a previous mobilization failure, which is generally intended as the failure to collect at least 2×10^6 $CD34^+$ cells/kg [4,18,28] or to reach a peak $\geq$10-15 $CD34^+$ cells/μL in PB after mobilization [1].

Indeed, 25 of the 37 patients enrolled in our study had at least 1 previously failed mobilization attempt. In the remaining 12 patients, the identification of predicted PMs was based on a series of criteria that were demonstrated to negatively affect mobilization in large retrospective studies, such as: advanced age [29-31], advanced stage disease [32,33], extensive BM involvement, or previous heavy/prolonged treatment, including extensive radiotherapy or SCP [34-38].

This is the fourth report on the use of plerixafor after chemotherapy followed by G-CSF. In the first report, 44 patients with lymphoma or MM received plerixafor after different kinds of chemotherapy schedules plus G-CSF [39]. This study, however, did not focus on the potential of plerixafor in PMs. In the second study, 13 patients received plerixafor after chemotherapy plus G-CSF, based on previous mobilization failure and evidence of a minimum number of circulating $CD34^+$ cells [40]. An extensive evaluation of the ASCT outcome after plerixafor-mobilized PBSC reinfusion was not reported in either of the 2 studies. In a recent German survey, 47 patients received plerixafor combined with G-CSF plus chemotherapy, yielding a median of 3.28×10^6 $CD34^+$ cells/kg. A good proportion of these patients (67%) were able to proceed to ASCT, achieving a timely and stable engraftment [41].

Our data confirm that the addition of plerixafor to G-CSF after chemotherapy is safe, suggesting that this strategy can effectively rescue most PMs candidates for ASCT and who previously failed a mobilization attempt, in a similar proportion to that observed in patients receiving plerixafor+ G-CSF without chemotherapy [27,42].

We observed a remarkable multiple-fold increase (median value: 4) in the number of circulating $CD34^+$ cells after plerixafor administration, both in MM and lymphoma patients. In addition, our results confirm the safety profile of plerixafor following chemotherapy. Of note, plerixafor administration did not induce any significant alterations in platelet values or hemoglobin levels during the postchemotherapy period before PBSC collection. Of note, 65% of PM patients with high-risk disease were rescued with ASCT and the outcome was good both in terms of engraftment and in terms of clinical response.

Historically, several strategies to collect PBSC have been reported, but chemotherapy plus G-CSF, or G-CSF alone, are the most widely used. Cytokine-only mobilization, with G-CSF instead of granulocyte macrophage-colony stimulating factor, is less toxic, easier to plan, and requires less time (5-7 days). Therefore, it is considered potentially more cost effective. Chemotherapy-based mobilization requires a longer period of time (10-15 days) and is less predictable, requiring additional monitoring and careful scheduling to ensure that the beginning of the collections coincides with peak $CD34^+$ cells levels. This strategy may be associated with relevant toxicities, such as infections, and requires greater resource utilization. Nevertheless, mobilization with chemotherapy remains an important option because of a greater yield of PBSCs for transplantations [43] and because of the additional cytoreductive effect described in several previous reports [44,45].

Several studies have investigated the effect of plerixafor added to G-CSF in cytokine-only mobilization strategies, especially in patients at the second mobilization challenge. However, very few studies have evaluated the effect of plerixafor after chemotherapy plus G-CSF. In a large study, remobilization strategy without plerixafor in MM and lymphoma patients, who previously failed to collect at least 2×10^6 $CD34^+$ cells/kg, resulted in rescues of no more than 23% patients, with 30% failing to pool sufficient numbers of stem cells from both collections [4].

Calandra et al. [18] mobilized, with plerixafor plus G-CSF, 115 patients defined as PMs by a previously failed attempt, low peripheral blood $CD34^+$ cell counts, or low apheresis yields (usually $<2\times10^6$ $CD34^+$ cells/kg). Tricot et al. [28] used plerixafor plus G-CSF in 20 patients identified as both proven PMs (in cases of previous mobilization failure) and predicted PMs, according to different criteria, such as history of extensive chemotherapy premobilization, PLT count $<100\times10^3/\mu L$, and $CD34^+$ peak $<12/\mu L$ after mobilization. Indeed, the use of plerixafor has been recently considered in patients without prior histories of mobilization failure, but with characteristics that adversely affect $CD34^+$ yield [46].

In our study, the combination of chemotherapy followed by G-CSF plus plerixafor allowed for successful harvests in 73% of patients. This highly successful mobilization rate in these heavily pretreated patients, associated with the very low toxicity of the mobilization procedure, suggests that chemotherapy followed by G-CSF plus plerixafor can represent a safe and effective strategy in this subset of patients. However, this study was not specifically designed to show any benefits of using plerixafor after chemotherapy plus G-CSF over the combination of G-CSF plus plerixafor. Our preliminary data suggest that, by pooling the total apheresis collections, MM patients collected significantly higher $CD34^+$ cell doses than the lymphoma patients. However, the $CD34^+$ increase rates after plerixafor did not significantly differ, suggesting that plerixafor is equally effective in the 2 populations. Moreover, the higher $CD34^+$ cell dose reinfused in the MM patients did not translate into faster PMN recovery, whereas a significantly faster PLT recovery was observed in the MM patients. Last, the administration of plerixafor after chemotherapy plus G-CSF can offer the potential advantage of better disease control, especially in patients with relapsed aggressive lymphoma. This can translate into a higher percentage of patients eligible for ASCT [47], compared with patients mobilized with G-CSF and plerixafor alone, in whom the lack of disease debulking could potentially lead to ASCT failures in some cases.

In conclusion, our data encourage the use of plerixafor after chemotherapy followed by G-CSF in lymphoma or MM patients identified as predicted PMs. The patients underwent this mobilization regimen without major toxicities, and most of them achieved minimum safe doses of $CD34^+$ cells for ASCT within a few days of apheresis and rapid engraftment. This strategy needs to be evaluated in a larger group of lymphoma and MM patients, who are identified as PMs according to well-standardized criteria and receiving homogeneous mobilizing protocols.

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REFERENCES

1. Wuchter P, Ran D, Bruckner T, et al. Poor mobilization of hematopoietic stem cells—definitions, incidence, risk factors, and impact on outcome of autologous transplantation. *Biol Blood Marrow Transplant*. 2010;16:490-499.
2. Bender JG, To LB, Williams S, et al. Defining a therapeutic dose of peripheral blood stem cells. *J Hematother*. 1992;1:329-341.
3. Klaus J, Herrmann D, Breitkreutz I, et al. Effect of CD34+ cell dose on hematopoietic reconstitution and outcome in 508 patients with multiple myeloma undergoing autologous peripheral blood stem cell transplantation. *Eur J Haematol*. 2007;78: 21-28.
4. Pusic I, Jiang SY, Landua S, et al. Impact of mobilization and remobilization strategies on achieving sufficient stem cell yields for autologus transplantation. *Biol Blood Marrow Transplant*. 2008;14:1045-1056.
5. Jillella AP, Ustun C. What is the optimum number of CD34+ peripheral blood stem cells for an autologous transplant? *Stem Cells Dev*. 2004;13:598-606.
6. Olivieri A, Offidani M, Montanari M, et al. Factors affecting hemopoietic recovery after high-dose therapy and autologous

peripheral blood progenitor cell transplantation: a single center experience. *Haematologica*. 1998;83:329-337.
7. Watts MJ, Sullivan AM, Jamieson E, et al. Progenitor-cell mobilization after low-dose cyclophosphamide and granulocyte colony-stimulating factor: an analysis of progenitor-cell quantity and quality and factors predicting for these parameters in 101 pretreated patients with malignant lymphoma. *J Clin Oncol*. 1997;15:535-546.
8. van der Wall E, Richel DJ, Holtkamp MJ, et al. Bone marrow reconstitution after high-dose chemotherapy and autologous peripheral blood progenitor cell transplantation: effect of graft size. *Ann Oncol*. 1994;5:795-802.
9. Scheid C, Draube A, Reiser M, et al. Using at least 5 × 10(6)/kg CD34+ cells for autologous stem cell transplantation significantly reduces febrile complications and use of antibiotics after transplantation. *Bone Marrow Transplant*. 1999;23:1177-1181.
10. Pavone V, Gaudio F, Console G, 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.
11. Koc ON, Gerson SL, Cooper BW, et al. Randomized cross-over trial of progenitor-cell mobilization: high dose cyclophosphamide plus granulocyte colony-stimulating factor (G-CSF) versus granulocyte-macrophage colony-stimulating factor plus G-CSF. *J Clin Oncol*. 2000;18:1824-1830.
12. Narayanasami U, Kanteti R, Morelli J, 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.
13. Spitzer G, Adkins D, Mathews M, et al. Randomized comparison of G-CSF + GM-CSF vs G-CSF alone for mobilization of peripheral blood stem cells: effects on hematopoietic recovery after high-dose chemotherapy. *Bone Marrow Transplant*. 1997; 20:921-930.
14. Demirer T, Ayli M, Ozcan M, et al. Mobilization of peripheral blood stem cells with chemotherapy and recombinant human granulocyte colony-stimulating factor (rhG-CSF): a randomized evaluation of different doses of rhG-CSF. *Br J Haematol*. 2002; 116:468-474.
15. Flomenberg N, Devine SM, DiPersio JF, 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.
16. DiPersio JF, Stadtmauer EA, Nademanee A, et al. Plerixafor and G-CSF versus placebo and G-CSF to mobilize hematopoietic stem cells for autologous stem cell transplantation in patients with multiple myeloma. *Blood*. 2009;113:5720-5726.
17. DiPersio JF, Micallef IN, Stiff PJ, et al. Phase III prospective randomized double-blind placebo-controlled trial of plerixafor plus granulocyte colony-stimulating factor compared with placebo plus granulocyte colony-stimulating factor for autologous stem-cell mobilization and transplantation for patients with non-Hodgkin's lymphoma. *J Clin Oncol*. 2009;27:4767-4773.
18. Calandra G, McCarty J, McGuirk J, 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*. 2008;41:331-338.
19. Velasquez WS, Cabanillas F, Salvador P, et al. Effective salvage therapy for lymphoma with cisplatin in combination with high-dose Ara-C and dexamethasone (DHAP). *Blood*. 1988;71:117-122.
20. Brugger W, Henschler R, Heimfeld S, Berenson RJ, Mertelsmann R, Kanz L. Positively selected autologous blood CD34+ cells and unseparated peripheral blood progenitor cells mediate identical hematopoietic engraftment after high-dose VP16, ifosfamide, carboplatin, and epirubicin. *Blood*. 1994;84: 1421-1426.
21. Kantarjian HM, O'Brien S, Smith TL, et al. Results of treatment with hyper-CVAD, a dose-intensive regimen, in adult acute lymphocytic leukemia. *J Clin Oncol*. 2000;18:547-561.
22. Keeney M, Brown W, Gratama J, et al. Single platform enumeration of viable CD34+ cells. *J Biol Regul Homeost Agents*. 2003; 17:247-253.
23. Riffenburgh Robert H. *Statistics for Medicine*, 2nd ed. New York: Elsevier; 2006.
24. Musso M, Scalone R, Marcacci G, et al. Fotemustine plus etoposide, cytarabine and melphalan (FEAM) as a new conditioning regimen for lymphoma patients undergoing auto-SCT: a multicenter feasibility study. *Bone Marrow Transplant*. 2010;45: 1147-1153.
25. Mills W, Chopra R, McMillan A, Pearce R, Linch DC, Goldstone AH. BEAM chemotherapy and autologous bone marrow transplantation for patients with relapsed or refractory non-Hodgkin's lymphoma. *J Clin Oncol*. 1995;13: 588-595.
26. Carella AM, Palumbo G, Greco M, et al. TEAM (thiotepa, etoposide, cytarabine, melphalan) as conditioning regimen for lymphoma treatment with autologous hematopoietic stem cell transplantation. *Bone Marrow Transplant*. 2009;43. Abstract 767.
27. Micallef IN, Stiff PJ, DiPersio JF, et al. Successful stem cell remobilization using plerixafor (Mozobil) plus granulocyte colony-stimulating factor in patients with non-Hodgkin lymphoma: results from the plerixafor NHL phase 3 study rescue protocol. *Biol Blood Marrow Transplant*. 2009;15:1578-1586.
28. Tricot G, Cottler-Fox MH, Calandra G. Safety and efficacy assessment of plerixafor in patients with multiple myeloma proven or predicted to be poor mobilizers, including assessment of tumor cell mobilization. *Bone Marrow Transplant*. 2010;45:63-68.
29. de la Rubia J, Blade J, Lahuerta JJ, et al. 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. *Haematologica*. 2006;91:621-627.
30. Morris CL, Siegel E, Barlogie B, et al. Mobilization of CD34+ cells in elderly patients (>/= 70 years) with multiple myeloma: influence of age, prior therapy, platelet count and mobilization regimen. *Br J Haematol*. 2003;120:413-423.
31. Hosing C, Saliba RM, Okoroji GJ, et al. High-dose chemotherapy and autologous hematopoietic progenitor cell transplantation for non-Hodgkin's lymphoma in patients >65 years of age. *Ann Oncol*. 2008;19:1166-1171.
32. Mendrone A Jr., Arrais CA, Saboya R, Chamone Dde A, Dulley FL. Factors affecting hematopoietic progenitor cell mobilization: an analysis of 307 patients. *Transfus Apher Sci*. 2008; 39:187-192.
33. Canales MA, Fernández-Jiménez MC, Martín A, et al. Identification of factors associated with poor peripheral blood progenitor cell mobilization in Hodgkin's disease. *Haematologica*. 2001; 86:494-498.
34. Haas R, Mohle R, Fruhauf S, et al. Patient characteristic associated with successful mobilizing and autografting of peripheral blood progenitor cells in malignant lymphoma. *Blood*. 1994;83: 3787-3794.
35. Dreger P, Klöss M, Petersen B, et al. Autologous progenitor cell transplantation: prior exposure to stem cell-toxic drugs determines yield and engraftment of peripheral blood progenitor cell but not of bone marrow grafts. *Blood*. 1995;86:3970-3978.
36. Desikan KR, Tricot G, Munshi NC, et al. Preceding chemotherapy, tumour load and age influence engraftment in multiple myeloma patients mobilized with granulocyte colony-stimulating factor alone. *Br J Haematol*. 2001;112:242-247.
37. 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.
38. Paripati H, Stewart AK, Cabou S, et al. Compromised stem cell mobilization following induction therapy with lenalidomide in myeloma. *Leukemia*. 2008;22:1282-1284.
39. Dugan MJ, Maziarz RT, Bensinger WI, et al. Safety and preliminary efficacy of plerixafor (Mozobil) in combination with chemotherapy and G-CSF: an open-label, multicenter, exploratory trial in patients with multiple myeloma and

non-Hodgkin's lymphoma undergoing stem cell mobilization. *Bone Marrow Transplant*. 2010;45:39-47.
40. D'Addio A, Curti A, Worel N, et al. The addition of plerixafor is safe and allows adequate PBSC collection in multiple myeloma and lymphoma patients poor mobilizers after chemotherapy and G-CSF. *Bone Marrow Transplant*. 2011;46:356-363.
41. Hübel K, Fresen MM, Salwender H, et al. Plerixafor with and without chemotherapy in poor mobilizers: results from the German compassionate use program. *Bone Marrow Transplant*. 2011;46:1045-1052.
42. Worel N, Rosskopf K, Neumeister P, et al. Plerixafor and granulocyte-colony-stimulating factor (G-CSF) in patients with lymphoma and multiple myeloma previously failing mobilization with G-CSF with or without chemotherapy for autologous hematopoietic stem cell mobilization: the Austrian experience on a named patient program. *Transfusion*. 2011;51: 968-975.
43. Bensinger W, DiPersio JF, McCarty JM. Improving stem cell mobilization strategies: future directions. *Bone Marrow Transplant*. 2009;43:181-195.
44. Tarella C, Cuttica A, Vitolo U, et al. High-dose sequential chemotherapy and peripheral blood progenitor cell autografting in patients with refractory and/or recurrent Hodgkin lymphoma: a multicenter study of the Intergruppo Italiano Linfomi showing prolonged disease free survival in patients treated at first recurrence. *Cancer*. 2003;97:2748-2759.
45. Gianni AM, Bregni M, Siena S, et al. High-dose chemotherapy and autologous bone marrow transplantation compared with MACOP-B in aggressive B-cell lymphoma. *N Engl J Med*. 1997;336:1290-1297.
46. Stiff P, Micallef I, McCarthy P, et al. Treatment with plerixafor in non-Hodgkin's lymphoma and multiple myeloma patients to increase the number of peripheral blood stem cells when given a mobilizing regimen of G-CSF: implications for the heavily pretreated patient. *Biol Blood Marrow Transplant*. 2009;15: 249-256.
47. Olivieri A, Brunori M, Capelli D, et al. Salvage therapy with an outpatient DHAP schedule followed by PBSC transplantation in 79 lymphoma patients: an intention to mobilize and transplant analysis. *Eur J Haematol*. 2004;72:10-17.