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Collection of peripheral blood progenitor cells for autografting with low-dose cyclophosphamide plus granulocyte colony-stimulating factor

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Abstract

Background and Objective. **The combination of high or intermediate-dose cyclophosphamide (CY) plus granulocyte colony-stimulating factor (G-CSF) is useful to mobilize hematopoietic progenitor cells to peripheral blood, but the patients require hospitalization. The aim of this study was to evaluate the efficiency of low-dose CY plus G-CSF (5 ug/kg/day sc) as an outpatient treatment in order to collect enough progenitor cells for hematopoietic rescue in autologous peripheral blood transplantation (APBSCT).**

Design and Methods. **We analyzed twenty-eight consecutively treated patients with lymphoma or multiple myeloma. The number of $CD34^+$ cells in blood samples was determined from day +7. Leukapheresis (LKP) began when the absolute number of $CD34^+$ cells in peripheral blood was > 2500/mL and the apheresis product was assayed for mononuclear cells (MNC), granulocyte-macrophage colony-forming units (CFU-GM), total nucleated cells (tNC) and $CD34^+$ cells.**

Results. **Twenty-eight outpatients with advanced hematologic malignancies (13 non-Hodgkin lymphoma, NHL; 10 Hodgkin's disease, HD; and 5 multiple myeloma, MM), median age 44 years (range 23-65) received a single dose of CY (1.5 g/m² iv day 0) followed by G-CSF (5 ug/kg/day sc) from day +1 to the end of LKP. Considering patients who had successful mobilization (64%), a median of 7.1×10^6/kg $CD34^+$ cells (range 3.5-11.9), 5.7×10^5/kg CFU-GM (range 1.5-9.2), 4.4×10^8/kg MNC (range 1.9-7.9) were collected. Treatment was well tolerated and none of these patients was hospitalized due to neutropenic fever. Only one patient received two packed red blood cells following chemotherapy. Autologous peripheral blood stem cell transplantation (APBSCT) has been performed in 18 patients (64%). The mean number of days to achieve $>0.5\times10^9$ PMN/L and $> 20\times10^9$ PLT/L was 12 (10-17) and 12.6 (8-24), respectively.**

Interpretation and Conclusions. **Considering a preestablished threshold of 2.5×10^6/kg $CD34^+$ cells to proceed to APBSCT, the mobilization therapy was successful in 64% of the patients but was unsuccessful in 10 patients (5 NHL, 4 HD and 1 MM). Hematopoietic recovery was complete and stable in all patients. Low-dose CY plus G-CSF is efficient to collect enough PBSC for hematopoietic rescue after myeloablative therapy in patients with lymphoproliferative disorders or multiple myeloma.**

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The mobilization of stem cells from bone marrow to peripheral blood (PBSC) during hematopoietic recovery after high dose chemotherapy and G-CSF administration leads to the collection of enough SC for hematopoietic rescue after myeloablative chemoradiotherapy.[1,2]

Different mobilization regimens have been developed to successfully collect PBSC for hematopoietic rescue after high-dose chemoradiotherapy. High or intermediate doses of CY (7-4 g/m²/day iv) followed by granulocyte-macrophage/granulocyte-colony stimulating factor (GM/G-CSF) (10-5 ug/kg/day sc) or both, have demonstrated their efficiency in the mobilization of PBSC.[3] However, high doses of CY induce severe neutropenia of 10 to 12 days duration, generally associated with infectious episodes and need for hospitalization. Low doses of CY (1.5 g/m²/day iv), especially when associated with G-CSF, should produce less neutropenia, thus allowing a safe outpatient treatment.

In this study, we have prospectively monitored a group of 28 patients with HD or NHL and MM for mobilization of PBSC with low-dose CY (1.5 g/m²/1 day iv) plus G-CSF (5 ug/kg/day sc).

Materials and Methods

Patients

Patient characteristics are summarized in Table 1. Twenty-eight patients (23 males and 5 females) who were considered candidates for APBSCT have been included in this study. Median age was 44 years (range 26-65) and diagnoses were HD (n=10), NHL (n=13) and MM (n=5). Sixteen patients (57%) had

Table 1. Patient characteristics.

	NHL	*HD*	*MM*
Number	13	10	5
Sex (M/F)	11/2	8/2	5/0
Age, median (range)	45 (29-65)	37 (23-53)	54 (45-64)
BMI at diagnosis	5 (38%)	2 (20%)	5 (100%)
Prior cycles of CHT, median (range)	9 (5-15)	9 (6-15)	7 (5-13)
Status at mobilization			
CR	2	2	–
VGPR	6	2	–
OR/PR	3	2	3
RD	2	4	2
BMI at mobilization	2	2	5
Venous access			
Central catheter	7	6	2
Peripheral veins	6	4	3
Median time from last cycle of CHT to mobilization (range)	4.4 m (1-20)	2.2 m (1-3)	2.6 m (2-3)

NHL: non Hodgkin's lymphoma; HD: Hodgkin's disease; MM: multiple myeloma; BMI: bone marrow involvement; CHT: chemotherapy; CR: complete remission; VGPR: very good partial remission; PR: partial remission; OR: objective response; RD: resistant/relapse disease.

Table 2. Results of CY (1.5 g/m² iv) plus G-CSF (5 ug/kg/day sc) mobilization.

Variables	*Mean (range)*
LKP patients	3.8 (1-6)
$CD34^+ \times 10^3$ cells/mL the first day of LKP	50.4 (4.2-277.5)
Leukocytes ($\times 10^9$/L) the first day of LKP	11.9 (1.8-27.7)
First day of LKP	8 (7-10)
Days on G-CSF	10.4 (8-14)
$CD34^+$ cells$\times 10^6$/kg	7.1 (3.5-11.9)
MNC$\times 10^8$/kg	4.4 (1.9-7.9)
CFU-GM$\times 10^5$/kg	5.7 (1.5-9.2)

LKP: leukapheresis; MNC: mononuclear cells.

BM involvement at diagnosis. Fourteen patients (50%) had received second-line chemotherapy before mobilization, but only one had received a third line of treatment. No patient had previously received pelvic irradiation.

Two NHL patients were, at mobilization, in complete remission (CR), 6 in very good partial response (VGPR), 3 in partial response (PR) and 2 patients in first relapse without BM involvement. Three HD patients were in CR at mobilization, two in VGPR, two in PR, one had progressive disease and the last two had chemotherapy-resistant disease. Three patients with MM were in PR and 2 had progressive disease at mobilization. Nine patients (32%) (2 HD, 2 NHL and 5.MM) had BM involment at mobilization.

Median time elapsed from the last cycle of chemotherapy to mobilization treatment was 3.4 months (range, 1-20). Fifteen patients (54%) required the insertion of a central venous catheter, but 13 (46%) had adequate peripheral venous access for LKP.

Treatment regimen

All 28 patients were given low dose CY (1.5 g/m²$\times$ 1 day) as an intravenous infusion over 1 hour (day 0) at the outpatient department. Subcutaneous G-CSF (5 ug/kg/day) (Amgen, Thousand Oaks, Ca, USA) was given from day 1 after chemotherapy to the end of LKP. Patients received 4 doses of MESNA for hemorrhagic cystitis prophylaxis, the first two as iv administration and the last two orally.

Monitorization of PBSC and LKP

An estimated number of >1$\times 10^9$/L leukocytes was thought to be present on day +7 after CY administration. Blood samples were obtained daily from day +7 to determine the number of circulating $CD34^+$ cells.[4] LKPs began when the absolute number of $CD34^+$ cells in PB was >2,500 per mL.[5] The target cell dose harvested for a successful autologous transplant was set at $\geq 2.5\times 10^6$/L $CD34^+$ cells per kg of the patient's body weight.

LKP were performed using a blood cell separator (Fenwall CS-3000, Baxter) and whole blood was processed at a rate of 50-60 mL per minute. The average amount of total blood processed during LKP was 15 liters, and the procedure ordinarily required about 4-5 hours. Each LKP product was assayed for MNC, tNC, CFU-GM and $CD34^+$ cells, following previously described standard techniques. Briefly, CFU-GM were measured according to the Pike and Robinson agar technique[6] and total numbers of colonies (> 50 cells) were counted under an inverted microscope after 14 days. The number of $CD34^+$ cells present in the circulation and harvested product was assessed by direct immunofluorescence (PE-8G12; Becton Dickinson, San José, CA, USA). Collected PBSC were frozen in homologous ABO group-compatible fresh plasma with the addition of 10% dimethyl sulfoxide.

Results

The median number of LKP per patient was 3.8 (range, 1-6). Seventeen patients (61%) underwent LKP on one or two consecutive days, whereas eleven patients (39%) underwent LKP on 3 or 4 consecutive days. Patients began LKP on day +8 after chemotherapy (range 7-10), with numbers of leukocytes and $CD34^+\times 10^3$ cells in PB of 11.9$\times 10^9$/L (range 1.8-27.7) and 50.4/mL (range 4.2-244.5), respectively. G-CSF was administered for 10 days (range, 8-14). Taking into account patients with a good mobilization, the median collected CFU-GM $\times 10^4$/kg, MNC $\times 10^8$/kg and $CD34^+$ cells $\times 10^6$/kg were 5.7 (range 1.5-9.2), 4.4 (range 1.9-7.9) and 7.1 (range 3.5-

Table 3. Characteristics of the patients who mobilized poorly.

UPN	Sex/Age	Dx	Previous treatment	Status/BMI premobilization*	Time from CHT to mobilization°	CD34⁺ cells ×10⁶/kg	Central catheter
1	M/30	NHL-T	CHOP×9 DHAP×2	VGPR/NOT	2 months	1.04	yes
2	M/46	MM	VCMP/VBAD×6	prog/yes	3 months	–	yes
3	M/45	NHL-B	CHOP×12 VIA×3	PR/not	3 months	1.86	yes
4	M/65	NHL-T	CHOP×8 MINE×8	VGPR/not	2 months	0.8	yes
5	F/52	NHL-B	CHOP×8 CHOP-BLEO ESHAP COPP×6	VGPR/yes	20 months	1.68	yes
6	M/20	HD	CVPP/ABVD×6 RT (nodal) CEP×6	relapse/yes	3 months	1.95	yes
7	M/23	HD	CMVPP×6 DHAP×3 RT mantle ABVD×4	prog/not	3 months	1.64	yes
8	M/40	HD	ABVD×5 RT mantle	CR/not	1 month	–	no
9	M/53	NHL-B	CNOP×6 CHOP×4	VGPR/yes	2 months	2.4	no
10	M/51	HD	ABVD×6 CEP×3	PR/yes	1 month	1.3°	yes

11.9), respectively. Considering a threshold value of 2.5×10^6 CD34⁺ cells/kg to warrant a successful APBSCT, the mobilization therapy was successful in 64% of patients. Mobilization failed in 10 patients, 5 NHL, 4 HD and 1 MM (Table 3).

There were no febrile episodes, and hospitalization was not necessary in any case. One patient received transfusion of two packed red blood cells, but platelet transfusions were not required. Fifty-seven percent of the patients had joint pain due to G-CSF, but no patient discontinued growth factor treatment due to toxicity.

Up to now, 18 patients (64%) have undergone APBSCT. Conditioning regimen consisted of TBI (13.25 Gy, hyperfractionated in 3 days) and high-dose melphalan (140 mg/m²/day iv, bolus on day –2) for MM patients, TBI plus high-dose CY (60 mg/kg/day iv, on days -6,-5) for patients with lymphoproliferative disorders and CY (1.5 mg/m²/days -6-3 iv), BCNU (300 mg/m²/day -6 iv) and etoposide (150 mg/m²/12h days -6-4 iv) for HD patients. Number of viable nucleated cells infused was 4.4×10^8/kg (range, 1.9-7.9). Hematopoietic recovery after infusion has been complete and stable in all patients with a median number of days to achieve $>0.5\times10^9$/L neutrophils and $>20\times10^9$/L of 12 (range, 10-17), 12.6 (range 8-24) and $>1\times10^9$/L neutrophils and $>50\times10^9$/L platelets of 16 (range 11-26) and 19 (range 11-27), respectively.

Discussion

Several previous reports have demonstrated that high or intermediate doses of CY (7-4 g/m²/day iv) plus GM-CSF or G-CSF are good therapeutic strategies to mobilize progenitor cells into PB.[1,2] However, patients generally require hospitalization because of the severe aplastic period secondary to the administration of chemotherapy. The use of CY at lower doses with the addition of G-CSF would produce a shorter neutropenic period and the whole collection program could be carried out on an outpatient basis. The above findings indicate that 1.5 g/m² of CY plus G-CSF seems to be sufficient to mobilize around 64% of patients with lymphoma or MM with a minimum threshold of 2.5×10^6/kg CD34⁺ cells. This finding contrasts with previous reports,[5] where hematopoietic progenitor collections were suboptimal using lower CY dose. The reason(s) for the relatively low

yields may be the use of a single agent (CY) for mobilization, since the combination of chemotherapy plus G-CSF is known to be more effective.[7-9] Siena *et al.*[7] showed that the combination of chemotherapy and growth factors to mobilize progenitor cells is superior to G-CSF alone. Although transplantation of G/GM-CSF-mobilized peripheral stem cells can result in rapid marrow recovery and sustained hematopoiesis, this technique presents difficulties when mobilizing heavily pretreated patients or patients with bone marrow involvement. The administration of low-dose CY plus G-CSF at a dose of 10 ug/kg/d sc or CY at a dose of 3 g/m^2 ev followed by growth factor could also be recommendable, as has been showed by Haynes *et al.*[10]

Low-dose CY is adequate for mobilization of hematopoietic progenitor cells to peripheral blood avoiding hospital admission and the use of antimicrobians and transfusions. However, in comparison with other protocols that use higher doses of chemotherapeutic agents,[1-3,11,12] this regimen had not antitumor effect and did not offer the possibility of treating the underlying malignant disease. This effect was achieved with the use of high dose therapy previously used for peripheral blood stem cell rescue.

Patients with successful mobilization have undergone APBSCT and all of them engrafted. Median time to recover stable neutrophil (>0.5 $\times 10^9$/L) and platelet (>20$\times 10^9$/L) counts have not been significantly different from that of patients transplanted with cells collected after more myeloablative regimens.[3]

This mobilization regimen, however, may not be appropriate for the collection of a minimum threshold of 5$\times 10^6$/kg CD34$^+$ cells needed for patients who require *ex vivo* treatment of the apheresis product, due to the cellular loss associated with the purging procedure. In this subgroup of patients, other mobilization regimens with other drugs at higher doses[10] or higher doses of growth factors (G-CSF at 10 µg/kg/day sc) can be considered as first line mobilization therapy to warrant a successful collection.

Contributions and Acknowledgments

AS was responsible for the conception of the study, its design and data handling. MB collected and analyzed the data and prepared the first draft of the manuscript. AS and RM helped MB in data analysis and corrected the different versions of the manuscript. AS, RM and SB were the clinicians involved in the follow-up of the patients. PM was the responsible of the Apheresis Section of the Blood Bank and directly supervised all the procedures. JG was the responsible for peripheral blood stem cell mobilization follow-up and nanalysis of the apheresis products. The last name indicates the senior author.

Disclosures

Conflict of interest: none.

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Manuscript processing

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