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Stem cell transplantation

Successful peripheral blood stem cell harvesting with granulocyte colony-stimulating factor alone after previous mobilization failure

A total of 138 patients whose stem cell mobilization failed following chemotherapy and granulocyte colony--stimulating factor (G-CSF) at a dose of 5 μg/kg/d were given a higher dose of G-CSF (10 μg/kg/d) for 5 days after a 7-day resting period. Stem cell mobilization was successful in 90 patients, who yielded a median of 3.5×10⁶ CD34+ cells/kg, partially successful in 17 patients (1-2.4×10⁶ CD34+ cells/kg) and failed in the remaining 31 patients.

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Autologous peripheral blood stem cells (PBSC) are currently mobilized by administering either chemotherapy (CT) plus granulocyte colony-stimulating factor G-CSF or G-CSF alone.[1,2] The most important problem, occurring in 10%-20% of cases, especially in heavily pretreated patients, is failure to obtain a sufficient number of CD34+ cells after apheresis.[2-4] In 1995, at the 37th annual meeting of the American Society of Hematology, we presented the first successful regimen for PBSC harvesting rescue with G-CSF alone (10 μg/kg/d) a few days after mobilization by CT plus G-CSF had failed.[5] Here we report further results from a large cohort of patients which confirm the relevance and safety of this procedure.

From 1997 to 2002, 1292 patients, referred to St-Louis and Necker hospitals, received CT plus G-CSF (5 μg/kg/d) in order to mobilize PBSC. The peripheral blood (PB) CD34+ cells were quantified when the white blood cell (WBC) count had recovered to 1000/μL after CT. Leukapheresis was initiated when the PB CD34+ cell count reached 10/μL. If PB CD34+ counts remained negative (<10 CD34+ cells/μL) for 6 consecutive days despite continued administration of G-CSF and a high level of WBC (> 20,000/μL), the PBSC harvest was not performed. Successful mobilization was defined by a harvest of at least 2.5×10⁶ CD34+ cells/kg. Partially successful mobilization was defined by a harvest of between 1 and 2.4×10⁶ CD34+ cells/kg. Failure was defined by three consecutive negative PB CD34+ cell counts or by a harvest of fewer than 1×10⁶ CD34+ cells/kg.

PBSC mobilization was unsuccessful in 138 patients (Table 1). After a 7-day resting period, a once daily subcutaneous administration of G-CSF at the dose of 10 μg/kg/d for 5 to 6 days was offered to all these patients. On the fifth and the sixth days of G-CSF administration, PB CD34+ cells were counted. If the count exceeded 10/μL, leukapheresis was initiated on the fifth or sixth day. Cumulative chemotherapy-induced toxicity to the bone marrow was calculated for each patient using a scoring system devised by Drake *et al.*[6] Data were compared using the χ^2 test or Fisher's exact test when indicated. Paired sample data were compared using Wilcoxon's signed rank test. Mobilization was successful in 90 patients (65.2%) from whom a median of 3.5×10⁶ CD34+ cells/kg (range 2.5 to 28) were harvested with a median of 2 leukaphereses (range, 1-4). Mobi-

Table 1. Patients' characteristics.

N. of patients	138
Sex (male/female)	68/70
Median age, years (range)	49 (16-68)
Diagnosis, N. of patients	
Non-Hodgkin's lymphoma	48
Hodgkin's disease	16
Multiple myeloma	28
Acute leukemia	27
Solid tumor	19
Previous therapy scoring system*	
Median Pre PBSC harvesting score (range)	50 (0-270)
Median n. of drugs used (range)	3 (0-9)
Median n. of drug exposure to toxicity factor 4 (range)	0 (2-27)
Median n. of cycles of chemotherapy (range)	6 (0-64)
Previous extensive radiotherapy (n. of patients)	21
Median time elapsed between last cycle of CT prior to the mobilization regimen by CT plus G-CSF (months, range)	0 (0-48)
Mobilization regimen	
Cyclophosphamide (120 mg/kg) + G-CSF (5 μg/kg/d)	79
DHAP + G-CSF (5 μg/kg/d)	9
CHOP-regimen + G-CSF (5 μg/kg/d)	10
Anthracycline + Arac + G-CSF (5 μg/kg/d)	26
Others + G-CSF (5 μg/kg/d)	14

**Chemotherapy scoring system by Drake et al.;[6] CT: chemotherapy.*

Table 2. Previous experience of PBSC mobilization using different rescue strategies.

References	*Number of patients*	*Median Age*	*First round of PBSC mobilizaton*	*Second round G-CSF μg/kg*	*Median interval between the 2 mobilizations*	*Median number of previous chemotherapy*	*Median CD34⁺ cell/kg harvested*	*Median number of leukaphereses*	*Proportion of success*
Fraipnt *et al.*[7]	20	51	CT + G-CSF 5 μg/kg	10	34 days	13	1.9	3	45%
Weaver *et al.*[8]	50	50	CT + G-CSF 6 μg/kg	10 to 32	12 to 174 days	2	0.16	4.5	22%
Gazitt *et al.*[9]	18	52	CT + G-CSF 10 - 16 μg/kg	32	0	≥ 3	2.9	5	80%
Voralia *et al.*[10]	21	NI	CT + G-CSF 10 μg/kg	32	NI	NI	0.61	NI	28%
Present study	138	49	CT + G-CSF 5 μg/kg	10	7	6	3.5	2	65%

NI: not indicated; CT: chemotherapy.

lization was partially successful in 17 patients (12.3%) (median of 3 leukapheresis, range, 1-4). The remaining 31 patients (22.5%) failed to mobilize a sufficient number of CD34⁺ cells into the peripheral blood in order to allow leukapheresis. The patients who had partially successful PBSC rescue mobilization by the procedure reached a sufficient yield of CD34⁺ cells following a third PBSC mobilization procedure using the same regimen. Thus, a total of 77.5% of patients succeeded in mobilizing a sufficient number of CD34⁺ cells despite initial failure.

Age, different diagnosis, score of previous CT, time between last cycle of CT and PBSC mobilization by CT plus G-CSF, extensive radiotherapy, WBC count and initial PB CD34⁺ cell count (0 to 9 CD34⁺ cells/μL) noted after the first mobilization attempt were not identified by statistical analysis to influence the result of the rescue procedure. No severe side effects were noted with the rescue procedure.

Seventy patients were evaluable for hematologic reconstitution following their autograft. The median number of days to reach a neutrophil count > 0.5×10^9/L and a platelet count > 20×10^9/L was 13 and 14, respectively.

Our procedure is safe and the PBSC were harvested by a reasonable number of leukephereses. Moreover, the short CT-free period (7 days) before attempting the second mobilization procedure does not usually delay the schedule of subsequent CT.

Similar procedures have been experimented by other researchers who report on limited series with great heterogeneity in the delay and doses at which G-CSF was reintroduced for PBSC rescue mobilization after initial failure following CT and/or G-CSF administration (Table 2).[7-10] Our findings contrast with the published results of others who did not find such an approach to be effective despite probably similar populations of patients with respect to age, diagnosis and chemotherapy schedules for the first round of PBSC mobilization. Our procedure seems to produce a higher yield of CD34⁺ cells with fewer leukaphereses than do other strategies.

The specific sequence and short interval between the two steps of our procedure may constitute a stronger stimulation for PBSC mobilization than other procedures. In conclusion, this strategy of PBSC rescue mobilization is easy and may be prescribed for a large proportion of patients who fail to mobilize stem cells in response to CT plus G-CSF. This procedure allows the CT schedule necessary to treat the malignant disease to be administered without difficulty.

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Stem Cell Transplantation

A reduced intensity conditioning regimen for allografting following autografting is feasible and has strong anti-myeloma activity

Sixteen patients with stage III multiple myeloma (MM) and a median age of 51 years were treated with autografting followed by reduced intensity conditioning allotransplantation (RICT). Nine patients are alive in remission at a median of 30 months after their transplants, one patient is alive in relapse and 6 patients died of progressive disease (5) or extensive chronic graft-versus-host disease, infections and progressive disease (1). We suggest that this two-step approach is feasible and it has strong anti-myeloma activity.

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The recent development of reduced intensity conditioning and allotransplantation (RICT) has opened a new way to assure engraftment of donor cells while reducing early transplant-related mortality (TRM). Taking advantage of this new approach we pionereed the combination of high-dose therapy and autologous stem cell transplantation (HDT/ASCT) followed by RICT to extend the benefit of allografting procedures.[1] Recently, we extended our experience to 16 patients with stage III multiple myeloma (MM) (Table 1). All patients received high dose melphalan (140 mg/m^2) followed by autologous peripheral blood progenitor cells previously collected after cyclophosphamide (3 g/m^2) and granulocyte colony-stimulating factor (G-CSF). At a median of 79 days after HTD/ASCT, the patients underwent RICT, consisting of fludarabine 30 mg/m^2 on days -4, -3, -2 and 2 Gy total body irradiation at 7cGy/min by a linear accelerator on day -1. Acute graft-versus-host disease (GVHD) prophylaxis consisted of mycophenolate mofetil (15 mg/Kg orally twice a day from day 0 until day +30) and cyclosporine 1 mg/kg i.v. from day -1 to day 35); subsequently, cyclosprorine A was administered at a dose of 5 mg/kg orally twice a day until day +90. Cytomegalovirus (CMV) reactivation was monitored and treated with ganciclovir. Donor chimerism was assessed on unfractionated bone marrow cells.[2] The evaluation of response was derived using ABMTR criteria.[3]

All patients were evaluated for response prior to and after transplants and then every 2-3 months (Table 2). Of 11 patients with responsive disease prior to HDT/ASCT, one patient maintained complete remission (CR) and one patient achieved CR from partial remission (PR) after ASCT; all other nine patients in PR maintained this state; 1/5 non-responsive patients achieved PR after ASCT. After RICT, 13 patients showed a complete donor chimerism at the time of engraftment; one patient with mixed chimerism received an infusion of donor lymphocytes (10^6 CD3$^+$ cells/kg) on day +60 and subsequently achieved full donor chimerism. Grade II-III acute GVHD occurred in 7 patients (43%) but no patient died of this complication. Six patients (37%) developed mild chronic GVHD and 3 patients (18%) developed an extensive form.

Three patients (18%) who were CMV seropositive or had CMV-seropositive donors developed CMV antigenemia and were treated with pre-emptively with ganciclovir.

Ten patients (1 after HDT/ASCT and 9 after RICT) (62%) of the 15 who were not in CR at our 2-step approach achieved CR and 1 (6%) achieved PR with an overall response rate of 68%. The patient who achieved CR after HDT/ASCT (patient #14 - Table 2) subsequently relapsed and died of MM 42 months after RICT. To date, nine patients are in continuing CR 11-36 months (median, 30) after RCT; 3 of them are still receiving immunosuppressive therapy for extensive chronic GVHD. In all patients the achievement of CR was gradual and a continued regression of monoclonal bands was observed with a median time to CR of 4 months. Eight out of 9 patients who developed acute/chronic GVHD achieved CR. Six patients did not achieve CR and died within 11 months: 5 patients of progressive disease and 1 patient of progressive disease, infections and extensive chronic GVHD. The overall survival and event-free survival following the 2-step procedure are shown in Figure 1.

Eight patients received a median number of 2 (range, 1-3) donor lymphocye infusions (DLI) with the median final dose infused being 2.4×10^7 CD3$^+$/Kg (range, 1×10^6-6×10^7). The median time from transplant to DLI was 80 days (range, 42-170). The indication for DLI was stable disease in 3 patients and mixed chimerism in 5 patients. None of 3 patients with

Table 1. Patients' charateristics at the time of autografting.

Patients	*16 (100%)*
Age, median (range)	51 (36-63)
Male/Female	11/5
Stage III	16 (100%)
β2 microglobulin >2.5 μg/mL	13 (81%)
Immunoglobulin class	
IgG	8(50%)
IgA	2 (12%)
Light chain	5 (31%)
Non-secretory	1 (6%)
Disease duration, months (range)	8 (5-40)
>12 months, n. (range)	4 (25%)
Prior chemotherapy	
VAD	16 (100%)
No cycles, median (range)	4 (3-9)
Response from last therapy before ASCT	
Complete remission	1 (6%)
Partial remission	10 (63%)
No response	5 (31%)