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Allogeneic peripheral blood stem cell transplantation in patients with early-phase hematologic malignancy: a retrospective comparison of short-term outcome with bone marrow transplantation

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Abstract

Background and Objective. **Transplantation of mobilized allogeneic peripheral blood stem cells (PBSC) has recently been reported by several groups. However, few patients receiving an allograft in the early stage of their disease have been described so far.**

Design and Methods. **Fifteen patients with early stage hematologic malignancies were transplanted with cryopreserved allogeneic PBSC from HLA-identical siblings. PBSC were collected after priming with 10 µg/kg/day of glycosylated granulocyte colony-stimulating factor (G-CSF, lenograstim). Outcomes were compared to a historical control group of 15 patients who received conventional bone marrow transplantation (BMT) from HLA-identical sibling donors. The two groups were matched for diagnosis, stage of disease, age, preparative regimen, graft-versus host (GVHD) prophylaxis, patients' and donors' gender and cytomegalovirus (CMV) serology. Diagnoses in both groups were: chronic myelogenous leukemia (CML) in first chronic phase (=5), acute leukemia in first complete remission (CR) (=5), non-Hodgkin's lymphoma in CR (=1) and multiple myeloma (MM) with sensitive disease (=4). All patients were given cyclosporin-A (CsA) and methotrexate (MTX) for GVHD prophylaxis. Preparative regimens varied according to diagnosis and included either busulfan/cyclophosphamide combination (BU/Cy) or total body irradiation/cyclophosphamide ± melphalan (TBI/Cy±Mel).**

Results. **The patients in the PBSC group showed a more rapid hematopoietic reconstitution with a significant difference in the median times to 1×10^9 neutrophils/L (19 days vs. 26 days; p = 0.03) and to platelet transfusion independence (18 days versus 22 days; p = 0.02). This finding was associated with a significantly shorter hospitalization (28 days versus 33 days after transplantation; p = 0.01). In the PBSC series, grade II-IV acute GVHD occurred in 3 patients (20%) and grade III-IV in 1 patient (7%). In the BMT control group, grade II-IV aGVHD was reported in 2 cases (13%; p = NS) and 1 case had grade III-IV GVHD. Chronic GVHD developed in 7 patients (47%) (limited = 6; extensive = 1) undergoing PBSC transplantation and 5 patients (33%) (limited = 4; extensive = 1) in the BMT series (p=NS). No difference was found in the incidence of grade II-IV (according to the World Health Organization) mucositis, whereas PBSC recipients did have a significantly lower incidence of additional severe (grade III-IV) organ toxicity. After a median follow-up of 300 days (range 180-630), all PBSC patients are still alive with a median Karnofsky score of 100% (range 80%-100%). Thirteen patients are in CR and 2 myeloma patient are in good partial remission (PR). Also, in the BMT group the peritransplant mortality was absent; two MM patients died due to progressive disease at day +796 and +1,023, respectively; one leukemic patient died of chronic GVHD 407 days after transplantation and one additional leukemic individual relapsed 1,140 days after BMT.**

Interpretation and Conclusions. **This retrospective comparison suggests that allogeneic PBSC transplantation performed in the early stage of the disease is safe and may be associated with a more rapid hematopoietic reconstitution than BMT, as well as lower transplant-related toxicity and earlier hospital discharge with apparently no increased risk of acute and chronic GVHD.**

Key words: PBSC transplantation, hematologic malignancy, bone

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Several groups have recently reported the successful transplantation of allogeneic recombinant human granulocyte colony-stimulating factor (rhG-CSF) mobilized peripheral blood stem cells (PBSC).[1-6] As with autografting, the most striking finding of PBSC transplantation was the faster reconstitution of hematopoiesis after the preparative regimen as compared with bone marrow (BM) derived stem cells. Moreover, these preliminary results suggest that despite the infusion of 1 log or more of T-cells, compared to conventional BM transplantation (BMT), there is no increase in the incidence or severity of acute graft-versus-host-disease (aGVHD).[7,8] More recently, formal, retrospective, case-control studies have been performed to

compare outcomes of patients with advanced hematologic malignancies given allogeneic PBSC with a control group of historical patients transplanted with BM cells.[9,10] Both reports indicate that PB-mobilized stem cells may improve the clinical results of allogeneic BMT. However, the number of PBSC transplants is still limited and the series published so far include patients who are heterogenous as regards to diagnosis, status of disease at transplant (although the vast majority were transplanted in later stages of the disease), conditioning regimens and GVHD prophylaxis.[11,12] Therefore, pivotal questions regarding both the patients and the donors of allogeneic PBSC transplantation still need to be answered by large randomized trials comparing PBSC and BM allografts.

Very few patients receiving allogeneic PBSC in the early stage of their disease have been reported so far, thus making evaluation of clinical outcomes in this setting difficult. Most importantly, it needs to be evaluated whether or not the infusion of large numbers of T-cells causes an unacceptably high rate of acute and/or chronic GVHD, which may play a negative role on transplant-related morbidity and mortality in this group of *good-risk* patients. Notably, patients with acute leukemia in complete remission (CR) and patients with chronic myelogenous leukemia (CML) in first chronic phase (CP) represent the patient population under study in the European prospective randomized trial.

In this study, we analyze in detail the clinical outcome of 15 patients with hematologic malignancies who were transplanted with allogeneic PBSC early in the stage of their disease. The controlled marrow transplants were represented by 15 patients receiving allogeneic BMT and matched to the study population for the variables most affecting stem cell transplantation. Our results demonstrate that the use of allogeneic PBSC may be associated with a faster engraftment of donor cells coupled with a significant decrease in peritransplant morbidity and hospitalization. Moreover, we did not find an increased incidence of GVHD as compared to BMT.

Patients and Methods

Patients

Between June 1995 and September 1996, 15 consecutive patients with an early stage hematologic malignancy received an allogeneic PBSC transplantation from a HLA-identical sibling donor. Patients were eligible for transplant with PBSC if they were between the age of 18 to 55 and had a disease for which stem cell transplantation was indicated. Patients who presented pregnancy or lactation (female recipients), human immunodeficiency virus (HIV) positivity, any major organ system dysfunction or inability to give written informed consent were excluded from the study. This study included individuals with acute leukemia in first CR, CML in first CP, non-Hodgkin's lymphoma (NHL) in remission and patients with multiple myeloma (MM) with sensitive disease. The clinical characteristics of the study patients are shown in Table 1. In the same period of time, 7 additional patients received allogeneic PBSC transplantation in a more advanced stage of their disease. These patients, however, were excluded from analysis.

Table 1. Patient and donor characteristics.

	PBSC	*BM*
No. of patients	15	15
Dates of transplant	6/95-9/96	11/90-3/96
Median age in years (range)		
Patients	37 (22-51)	37 (20-46)
Donors	29 (21-49)	36.5 (17-52)
Gender*		
Patient female	5 (33%)	5 (33%)
Donor female	8 (53%)	8 (53%)
Donor multiparous	6 (40%)	7 (47%)
CMV serology		
Patient and donor negative	2 (13%)	1 (12%)°
Diagnosis		
AML	4 (27%)	4 (27%)
ALL	1 (7%)	1 (7%)
NHL	1 (7%)	1 (7%)
CML	5 (33%)	5 (33%)
MM	4 (27%)	4 (27%)
Phase of disease		
CR	6 (40%)	6 (40%)
CML in CP	5 (33%)	5 (33%)
MM in PR	4 (27%)	4 (27%)
Median follow-up in days (range)	300 (180-630)	979 (331-2131)

*Abbreviations: PBSC, peripheral blood stem cells; BM, bone marrow; CMV, cytomegalovirus; AML, acute myeloblastic leukemia; ALL, acute lymphoblastic leukemia; NHL, non-Hodgkin's lymphoma; CML, chronic myelogenous leukemia; MM, multiple myeloma; CR, complete remission; CP, chronic phase; PR, partial remission. *No. of patients (% of patients). °Only data from 8 patient-donor pairs were available.*

Donors and mobilization procedures

The main donor characteristics are shown in Table 1. PBSC donors received glycosilated rhG-CSF (Lenograstim; Rhone-Poulenc Rorer, Milan, Italy) administered subcutaneously at 10 μg/kg/day for 5-6 days. All donors were related and had full HLA match with the recipient. Inclusion and exclusion criteria have already been reported.[13] Leukapheresis were performed on days 5-7 as previously described[13] to collect a minimum number of 4×10^6 $CD34^+$ cell/recipient body weight. Apheresis products were then cryopreserved in liquid nitrogen until transplantation.[14] Flow cytometry analysis of $CD34^+$ cells as well as culture assays in methylcellulose for the assessment of hematopoietic progenitors were

performed as reported in previous papers.[13,14] Analysis of lymphoid subpopulations was performed by direct immunofluorescence on whole blood using a panel of fluorescein (FITC)- or phycoerythrin (PE)-conjugated monoclonal antibodies. All samples were analysed for the expression of the following antigens: CD3, CD4, CD8, CD19, CD56. All reagents were purchased from Becton Dickinson (BD, San Josè, CA, USA).

None of the donors required central line placement to undergo apheresis. BM cells were collected according to standard procedures.[15] In BMT patients, marrow cells were reinfused on day 0 after the completion of the preparative regimen.

The protocol was approved by the ethical committee of the Bologna University Hospital and both patients and donors gave written informed consent.

Historical control group

The control group included 15 patients who had received BMT. They were chosen from our database among 240 individuals, starting from the patients most recently transplanted with BM from HLA-identical sibling donors in our institution. Each patient who entered in this study was matched to 1 historical control patient according to the factors most influencing the outcome of stem cell transplantation: diagnosis, disease stage at transplant, patient age±5 years, GVHD prophylaxis, conditioning regimen, patients' and donors' gender, CMV serology.

Conditioning regimens and GVHD prophylaxis

Cytotoxic conditioning therapy was administered according to our institutional protocols (Table 2). Patients with myeloproliferative disorders (= 9, plus 9 controls) received busulfan (Bu; 16 mg/kg) and cyclophosphamide (Cy; 200 mg/kg) (BU/Cy) (13); patients with lymphoproliferative disease (= 2, plus 2 BMT patients) were treated with Cy (120 mg/kg) and total body irradiation (TBI; 10 Gy given in single dose with lungs shielding at 8 Gy) (TBI/Cy), whereas MM patients received either TBI (10 Gy), Cy (120 mg/kg) and melphalan (120 mg/kg) (PBSC=3; BMT=1) or BU/Cy (PBSC=1; BMT=3). Acute GVHD was graded according to Przepiorka *et al.*[16] and chronic GVHD was classified according to the criteria proposed by Shulman *et al*.[17] GVHD prophylaxis included cyclosporin A (CsA; 3 mg/Kg intravenously from day –1 and then orally) to maintain blood levels between 200 and 300 µmol/L, and methotrexate (MTX; 15 mg/m^2 on day +1 and 10 mg/m^2 on days +3, +6, +11). Folinic acid was not used to rescue MTX cytotoxicity. Detailed description of supportive care measures is reported elsewhere.[15] G-CSF was not routinely used to accelerate neutrophil engraftment. CMV infection prophylaxis (short course of Gancyclovir 6 mg/kg/day) was only performed in patients who were positive, in two subsequent determinations for CMV antigens in PB. In case of documented infection, the treatment consisted of 10 mg/kg/day of Gancyclovir and hyperimmune anti-CMV immunoglobulin (Cytotect).

Table 2. Treatment characteristics.

	PBSC	*BM*
No. of patients	15	15
Conditioning regimen		
BU/Cy	10 (67%)	12 (80%)
Cy/TBI	2 (13%)	2 (13%)
Cy/TBI/Mel	3 (20%)	1 (7%)
GVHD prophylaxis		
CsA/MTX	15 (100%)	15 (100%)
Post-transplant cytokines	2 (13%)	2 (13%)

Abbreviations: PBSC, peripheral blood stem cells; BM, bone marrow; BU/Cy, busulfan and cyclophosphamide; TBI, total body irradiation; Mel, melphalan; GVHD, graft-versus host disease; CsA, cyclosporin-A; MTX, methotrexate.

Engraftment

Blood counts were performed daily until hospital discharge and then at least once a week for the first 3 months. Granulocyte engraftment was defined as the first of 3 consecutive days with an absolute neutrophil count (ANC) > 0.5×10^9/L. Similarly, platelet (Plts) engraftment was defined as the first of 3 consecutive days with an unsupported Plts count > 20×10^9/L. Additional parameters which were considered important to document hematologic reconstitution and analyzed in this study are: day to 1×10^9 neutrophil/L and day to 50×10^9 Plts/L.

The origin of engrafted cells in peripheral blood and BM was determined in selected cases by conventional cytogenetics in mismatched sex donor-recipient pairs and by analysis of variable number tandem repeat (VNTR) polymorphism in samples from sex-matched pairs.

Toxicity grading

Early regimen-related toxicity (RRT) was assessed by the clinical investigators according to the *World Health Organization* (WHO) toxicity grading.

Documented infection was defined in febrile patients, as the occurence of a single blood culture that was positive for any microorganism. Individuals with invasive infections required histologic documentation or culture.

Statistical considerations

Data are presented as median values and ranges. The results were compared using the Wilcoxon rank-sum test and two-sided P values were considered significant at less than 0.05. The ζ^2 test was used to compare the frequency of GVHD and

adverse events in the study groups. The probabilities of neutrophil and platelet recovery of the patients in the two groups were compared by using the Kaplan and Meier method.

Results

Donors

rhG-CSF administration was generally well tolerated. Moderate bone pain occurred in > 90% of the donors and was relieved by acetominophene. G-CSF dose was halved at day 4 in 20% of the donors due to a WBC > 50×10^9/L. However, no donors required the discontinuation of the drug. Leukapheresis procedures were performed without complications.

Study populations and collection of hematopoietic stem cells

As shown in Table 1 and 2, the PBSC and BMT groups were well matched according to diagnosis, age, stage of disease, CMV serology, conditioning regimen and GVHD prophylaxis. Due to prior spinal cord irradiation, 2 MM patients in the BM group received Bu rather than TBI. At the time of writing, the median follow-up is 300 days (range 180-630) for PBSC patients and 979 days (range 331-2,131) for individuals in the control group ($p < 0.02$). Patients receiving PBSC were infused with 12.5×10^8 nucleated cells/kg, 7.4×10^6 $CD34^+$ cells/kg, 36×10^4 CFU-GM/kg and 267×10^6 $CD3^+$ cells/kg (Table 3). Three patients were transplanted with 3 apheresis products, 9 patients received 2 aphereses and 3 individual received only 1 PBSC collection. The patients in the historical BM group were infused with a median of 3.4×10^8 nucleated cells and 13.7×10^4 CFU-GM/kg of recipient body weight.

Engraftment

Engraftment results, supportive care data and hospital stay are reported in Table 4.

Although folinic acid was not used to rescue MTX toxicity, 87% and 73% of PBSC and BM patients respectively, received the four scheduled doses of the drug (p=NS). Only two patients in each group were treated with cytokines (G-CSF=2, PBSC; GM-CSF=1, BMT; erythropoietin=1, BMT) to accelerate engraftment.

The patients in the PBSC group showed a more rapid hematopoietic reconstitution compared to BMT. A statistically significant difference was found regarding the median time to 1×10^9 neutrophil/L and 20×10^9 platelet/L (Figure 1). However, we did not find any statistical difference in the incidence of documented infections, the use of intravenous antibiotics (data not shown) or the transfusion requirement (Table 4). Conversely, PBSC patients were discharged 5 days earlier than BM individuals (p= 0.01).

One leukemic patient in the PBSC group did not show a full platelet reconstitution, perhaps due to the recovery and reinfusion of only 10% of cryopreserved cells (1.1×10^4 CFU-GM/kg and 2.2×10^6 $CD34^+$ cells/kg). Subsequent post-transplant VNTR assays showed that more than 80% of cells were of donor origin. At day +367 after transplantation he is still alive and in CR.

Table 3. Cellular composition of 15 apheresis collections.

Median (range)	*Collected*	*Reinfused*
Nucleated cells ($\times10^8$/kg)	12.7 (7-29.5)	12.5 (2.6-24.8)
$CD34^+$ cells ($\times10^6$/kg)	10.4 (6-24.7)	7.4 (2.2-12.9)
CFU-GM ($\times10^4$/kg)	109.6 (2.4-271.7)	36 (1.1-286.1)
$CD3^+$ cells ($\times10^6$/kg)	345 (199.7-944)	267 (83.6-637.8)
$CD4^+$ cells ($\times10^6$/kg)	239 (122.6-559)	132 (57.5-253)
$CD8^+$ cells ($\times10^6$/kg)	150 (47.6-324)	89.7 (47-153)
$CD19^+$ cells ($\times10^6$/kg)	77.6 (31.7-129)	30.4 (8.8-70)
$CD56^+CD3^-$ cells ($\times10^6$/kg)	13.8 (8.8-20.7)	9.2 (2.5-12.9)

Normal donors underwent a median of 2 leukaphereses (range: 1-3).

Table 4. Engraftment data and supportive care.

	PBSC	*BM*	*p*
Day ANC $\geq 0.5\times10^9$/L	17 (14-29)	19 (15-31)	NS
Day ANC $\geq 1\times10^9$/L	19 (15-32)	26 (17-38)	0.03°
Day PLT $\geq 20\times10^9$/L	18 (10-25)	22 (17-75)	0.02°
Day PLT $\geq 50\times10^9$/L	24 (14-376*)	28 (16-116)	NS
Transfusion requirement			
RBC units	6 (0-14)	6 (0-14)	NS
PLT (single donor aphereses)	4 (2-18)	5 (3-11)	NS
Hospital discharge	28 (25-45)	33 (27-53)	0.01°

*The results are expressed as median (range) number of days. Abbreviations: PBSC, peripheral blood stem cells; BM, bone marrow; ANC, absolute neutrophil count; PLT, platelet; RBC, red blood cells; NS, not significant. °Statistically significant. *One PBSC patient did not achieve a full PLT reconstitution after 376 days from transplantation. For further details see text.*

GVHD

In the PBSC group, grade II-IV aGVHD occurred in 3 patients (20%) and grade III-IV in 1 patient (7%) (Table 5). In the control group, grade II-IV aGVHD was observed in 2 patients (13%; p= NS) and grade III-IV in 1 patient (7%). Notably, the median day of onset of aGVHD in patients receiving allogeneic PBSC was +28 (range 21-41) as compared to day +16 (range 15-24) in the BMT group (p=0.04).

Clinical chronic GVHD (cGVHD) developed in 7

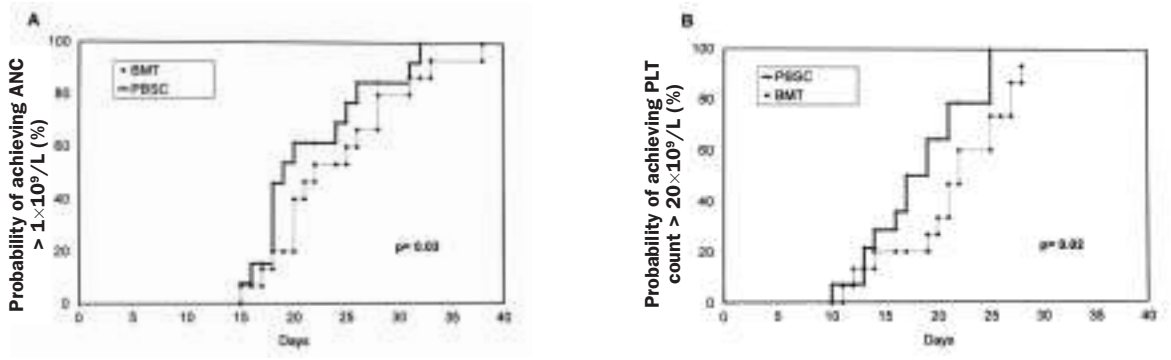

Figure 1. Kaplan-Meier plot of probability of recovery of neutrophils to 1×10⁹/L (A) and recovery to an unsupported platelet count of 20×10⁹/L (B) in transplanted patients.

patients transplanted with PBSC and in 5 BMT patients (p=NS). One patient in each group developed extensive GVHD while the remaining patients showed clinical signs of limited GVHD. No difference was found in the median time of onset of cGVHD.

In the same period of time, 7 additional patients received allogeneic PBSC in a more advanced stage of their disease (4 with resistant or relapsed leukemia; 3 with CML in accelerated or blastic phase). In this series, hematopoietic reconstitution was superimposable to that of *good risk* patients (data not shown). Three patients developed grade III-IV aGVHD. Two individuals died of aGVHD and 1 due to disease progression. With a median follow-up of 115 days, 4 patients are still alive and in CR.

Peritransplant morbidity and survival

Grade II-IV stomatitis occurred in 13 patients (87%) in both the PBSC and the BMT group. Additional individual organ toxicity was recorded in BMT patients as follows: grade III-IV hepatotoxicity in 4 individuals (27%), grade III-IV bladder toxicity in 2 patients (13%), grade III-IV gastrointestinal toxicity in 4 patients (27%) (Table 6).

In comparison to BMT recipients, only 1 patient in the PBSC group showed signs of severe organ toxicity (grade III-IV hepatotoxicity). Overall, PBSC recipients had a significantly lower incidence of cumulative grade III-IV RRT (p=0.03) (Table 6). Six patients transplanted with circulating stem cells received a short course of Gancyclovir for CMV infection prophylaxis. Subsequently, none of them developed CMV disease. No patients in the BM group received CMV prophylaxis or treatment.

After a minimum follow-up of 180 days (median 300 days) all PBSC patients are still alive with a median Karnofsky score of 100% (range 80-100). Thirteen patients are in CR, while 2 patients with MM show > 90% tumor burden reduction. Similarly, in the BMT group transplant-related mortality within 180 days from transplant was absent. Two myeloma patients died due to progressive disease at day +796 and +1,023, respectively. One leukemic patient died of cGVHD and concomitant infections after 407 days after the transplantation, while one

Table 5. GVHD.

	PBSC	*BM*	*p*
No. of evaluable patients	15	15	
aGVHD			
*Grade 0**	7 (47%)	11 (73%)	NS
Grade I	5 (33%)	2 (13%)	NS
Grade II	2 (13%)	1 (7%)	NS
Grade III-IV	1 (7%)	1 (7%)	NS
Median day of onset	28 (21-41)	16 (15-24)	0.04°
cGVHD			
Extensive	1 (7%)	1 (7%)	NS
Limited	6 (40%)	4 (27%)	NS
Median day of onset	175 (90-310)	225 (112-660)	NS

*Abbreviations: PBSC, peripheral blood stem cells; BM, bone marrow; aGVHD, acute graft-versus host disease; cGVHD, chronic GVHD; NS, not significant. *No. of patients (% of patients).°Statistically significant.*

Table 6. Regimen-related toxicity.

	PBSC	*BM*
Grade II-IV stomatitis	13 (87%)*	13 (87%)
Grade III-IV hepatotoxicity	1 (7%)	4 (27%)
Grade III-IV bladder toxicity	–	2 (14%)
Grade III-IV gastrointestinal toxicity	–	4 (27%)

**Number of patients (% of patients).*

additional leukemic individual relapsed at 1140 days from BMT and subsequently achieved a second CR after salvage treatment.

Discussion

According to recently published data, the administration of G-CSF to normal donors allows the mobilization of an adequate number of PBSC for allogeneic transplantation.[1-8] Circulating stem cells may reduce the time to engraftment after myeloablative therapy and thus decrease early morbidity and mortality of the transplant procedure.[2] In addition, an earlier immune reconstitution has been reported following infusion of allogeneic PBSC.[7,18] Their collection may also prove to be a more tolerable procedure for donors with respect to BM harvest. However, transplantation of mobilized stem cells is associated with the infusion of high numbers of T-cells and NK-cells; as a consequence, the incidence and severity of acute and chronic GVHD and graft-versus-leukemia (GVL) effect must be considered.

In this paper we report in detail the clinical data of 15 patients enrolled in a pilot study of allogeneic transplantation of mobilized PBSC who were transplanted in the early stage of their disease and have a minimum follow-up of more than 4 months. The interest in this patient population arises from the fact that the vast majority of PBSC patients reported so far[9,12,19] have been transplanted in the advanced stage of the disease. In addition, thanks to the consistency of the policy on the use of supportive care measures (including the use of post-transplant growth factors GVHD prophylaxis and conditioning regimens in our center), we have been able to perform a formal retrospective case-control study with a historic control group of 15 patients receiving conventional BMT and matched to the study population for all the major variables affecting stem cell transplantation. In the present study, leukapheresis products were cryopreserved upon collection, since storage permits a more precise evaluation of the hematopoietic progenitor cell content and because of the advantage of independently scheduling stem cell collection and the transplant procedure.

Regarding engraftment, our findings are consistent with previous reports of prompt and durable reconstitution of hematopoiesis after PBSC transplantation. Moreover, patients receiving circulating stem cells showed a more rapid recovery of BM function as compared to BMT. Notably, 40% of PBSC patients received gancyclovir for CMV prophylaxis, compared to none of the patients in the BM group. The administration of the full course of MTX treatment in 87% of patients (with no rescue of folinic acid) and the lack of post-transplant growth factors may account for the slight delay in the rate of engraftment observed in this study compared to earlier reports.[2,9] The acceleration of hematopoietic reconstitution did not result in a lower transfusion requirement and lesser antibiotic therapy. However, it should be taken into account that the need for transfusions is modest (see Table 4) in most good risk patients engrafted with BM; therefore the relatively small sample size of this study may not have the stastistical power to detect minimal differences.

Conversely, the use of PBSC seems to significantly shorten hospital stay. This finding may be due to the combination of a more rapid hematopoietic recovery and the marked reduction of severe extra-hematological toxicity associated, in this study, with the infusion of PBSC. For this reason, the MD Anderson Hospital team has recently reviewed the clinical outcomes of 74 adults with advanced hematologic malignancy to determine the short-term benefit of allogeneic PBSC over BMT.[20] The authors found that PBSC recipients had a lower RRT, fewer early deaths, earlier hospital discharge and an improved day 180 survival. These results may be partly attributed to the anti-inflammatory effects of G-CSF which decreases the secretion of tumor necrosis factor (TNF)-α[21] by accessory cells which are largely represented in PBSC collections. In addition, G-CSF is able to increase the production of antagonists, such as soluble TNF-α receptor and IL-1 receptor,[22] of two cytokines which have been involved in the pathogenesis of organ toxicity in the transplant setting.[23,24] In fact, plasma levels of TNF-α have been correlated to the development and severity of liver veno-occlusive disease (VOD), diffuse non-infectious pneumonia and aGVHD.[23]

More importantly, our pair-matched analysis did not indicate a higher incidence of moderate-severe aGVHD in PBSC recipients compared to patients receiving BM cells. Notably, the probability of developing aGVHD in the BM control group as well as in PBSC patients (as low as 13-20% for grade II-IV and 7% for grade III-IV) was lower than that reported when the standard combination of CsA/MTX was used for prophylaxis[25] in BMT and comparable to that recorded in young patients with early leukemia receiving BM cells and CsA/MTX and prednisone.[26] Therefore, based on our preliminary results and on data from previous studies, the fear of an unacceptably high rate of severe and perhaps uncontrollable aGVHD after allogeneic PBSC transplantation can be dismissed with a certain degree of confidence even when good risk patients are considered for this procedure. Although this was not a prospective randomized study, it compared two groups of patients that were well matched for all the major risk factors and treated with the same protocols in the same institution. Interestingly enough, aGVHD occurred later in PBSC recipients rather than in BMT patients. There is no obvious explanation for this finding which

needs confirmation in a larger series, although one may hypothesize that G-CSF itself and/or cryopreservation may affect T-cell proliferation and function. Regarding this, however, early reports suggesting the deleterious effect of long-term storage on lymphocytes have been largely unconclusive. Moreover, although the follow-up is significantly shorter in the PBSC group and few patients are still under CsA treatment, there did not appear to be a higher incidence of either extensive or limited chronic GVHD (7% and 27%, respectively) compared to BMT. This is in agreement with the studies from Bensinger *et al*.[9] and Russel *et al.,*[10] who made a similar case-control analysis, although direct comparisons of the three studies in terms of overall incidence of acute and chronic GVHD cannot be made due to the much higher percentage of high-risk individuals present in the Canadian and Seattle studies. On the contrary, Majolino *et al.*,[27] found a higher incidence of extensive cGVHD in PBSC recipients.

Possible explanations for the lack of excessive GVHD in PBSC recipients have been discussed in detail elsewhere;[12] they include the presence of a suppressor cell activity in PBSC collections, the differentiation, by treatment with G-CSF, of $CD4^+$ cells toward T helper type-2 (Th2) cells responsible of the secretion of antiinflammatory cytokines and the decrease of TNF activity.

Overall, after a median follow-up of 10 months, all PBSC patients are still alive and in excellent physical conditions and 13 out of 15 patients are in CR. Although we cannot demonstrate a survival advantage for a PBSC transplantation performed in early stage of the neoplastic disease, this strategy appears to be safe, and is perhaps associated with a significant difference in the speed of engraftment and short-term morbidity. However, our analysis has a number of intrinsic limitations; therefore only prospective, randomized trials comparing conventional BMT and PBSC transplantation, which are currently in progress in Europe and the USA with the inclusion of good risk patients, should answer the questions related to engraftment, incidence and severity of GVHD, relapse rate, and long-term survival.

Contributions and Acknowledgments

A. Fortuna, S. Mangianti, M.R. Motta and S. Rizzi developed and carried out cryopreservation and thawing procedures, CFU-C assay and flow cytometry assay. C. Tassi was responsible for PBSC collection. G. Leopardi, A. Rosti, A. Bonini, D. Rondelli, C. Remiddi and A. Curti were all involved in the clinical assessment of both the patients and the donors in the inpatient and outpatient departments. G. Bandini and M. Cavo drafted the paper and helped the principal investigator (RML) for data analysis and interpretation. R.M. Lemoli was the principal investigator: he designed the study, was responsible for ethical approval of the program, funding and direct supervision. R. Conte and S. Tura revised critically the paper and gave the final approval for publication.

The order of authorship has been made according to the substantial contribution given to the study.

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Disclosures

Conflict of interest: none.

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