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Bone Marrow Compared with Peripheral Blood Stem Cells for Haploidentical Transplantation with a Nonmyeloablative Conditioning Regimen and Post-transplantation Cyclophosphamide

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A B S T R A C T

Recently, the administration of high-dose cyclophosphamide (Cy) after T cell–replete haploidentical stem cell infusion has been reported to be feasible and effective. In the original study, bone marrow (BM) was used as the source of stem cells. Here, we retrospectively analyzed the use of BM versus peripheral blood stem cells (PBSCs) in a cohort of patients receiving haploidentical T cell–replete transplantation after a nonmyeloablative conditioning regimen with postinfusion Cy. In the PBSC versus BM groups, the incidence of acute graft-versus-host disease (GVHD) was 33% versus 25%, respectively, and the incidence of chronic GVHD was 13% versus 13%, respectively. The median time to achieve a safe and unsupported absolute neutrophil and platelet count was 20 versus 21 days and 27 versus 29 days, respectively. The incidence of engraftment was also similar in the 2 cohorts. The 1-year nonrelapse mortality rate was 12% versus 22%, respectively ($P = .96$). Finally, nonsignificant differences in survival were observed. In conclusion, the use of PBSCs instead of BM after T cell–replete haploidentical transplantation did not appear to be detrimental in terms of either GVHD or engraftment rate. PBSCs could be a valid alternative to BM after transplantation from a haploidentical donor using postinfusion Cy.

INTRODUCTION

Transplantation from alternative donors has been attempted for years in patients with hematological malignancies who lack HLA-identical donors. Whereas trials conducted without specific approaches have produced disappointing results [1], feasibility and some degree of efficacy have been achieved through extensive T cell depletion using various ex vivo selection strategies [2-5] or using anti–T cell antibodies [6,7]. In these different methods, peripheral blood stem cells (PBSCs) were the preferred stem cell source because they enable the collection of a large number of CD34-positive cells for ex vivo manipulation, allowing deep T cell depletion while preserving a rich graft. However, logistical concerns, a lack of reproducibility, and problems associated with immune reconstitution have hampered the wide dissemination of this treatment modality.

More recently, different strategies have been developed to perform haploidentical transplantation without graft manipulation [7-9]. Bone marrow (BM), associated or not with PBSCs and primed or unprimed, has been used as the stem cell source in most of these methods [8,9]. When PBSCs were used alone, graft-versus-host disease (GVHD) prophylaxis was usually strengthened by the ex vivo partial T cell depletion of the graft or by the administration of more potent postgraft immune suppression [4,7].

For years, researchers at Johns Hopkins University have investigated the use of postgraft cyclophosphamide (Cy) [10] in the context of allogeneic transplantation. Recently, investigators from Johns Hopkins University pioneered the use of high-dose Cy after T cell–replete haploidentical stem cell infusion [11]. One of the key characteristics of this program was the use of BM as the stem cell source to reduce the risk of acute (aGVHD) and/or chronic graft-versus-host disease (cGVHD). Engraftment occurred rather rapidly, with median times to a safe absolute neutrophil count (ANC) and to an acceptable untransfused platelet count of 15 and 24 days, respectively. In this program, the cumulative incidences of neutrophil and platelet engraftment exceeded 90% and 80%, respectively. Among a large number of patients who underwent transplantation, the incidences of severe aGVHD and cGVHD were remarkably low (34% and 5%, respectively) [11]. In a more recent group of T cell–replete haploidentical transplant recipients who received postinfusion Cy, a small

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number of patients were prepared with a myeloablative conditioning regimen and received a PBSC graft [12]. The median times to neutrophil and platelet engraftment were 16 days and 27 days, respectively. The incidences of aGVHD (30%) and cGVHD (35%) were acceptable.

In the present study, we retrospectively analyzed the use of 2 different stem cell sources in a cohort of patients from 2 institutions; the patients underwent haploidentical T cell–replete transplantation after a nonmyeloablative conditioning (NMAC) regimen with postinfusion Cy. The aim of this study was to compare the incidences of GVHD and engraftment between patients who underwent transplantation with PBSCs or with BM.

PATIENTS AND METHODS

From April 2009 until April 2013, 69 consecutive patients undergoing T cell–replete haploidentical transplantation at 2 institutions for hematological malignancies with poor prognoses were analyzed. Every patient provided informed consent.

Inclusion Criteria

Patients were candidates for haploidentical allogeneic HSCT if they met the following criteria: (1) they lacked an HLA-matched related or unrelated donor (in cases of previous allogeneic HSCT, the initial donor was discarded); (2) they had a history of disease relapse after several chemotherapeutic lines or after a previous autologous or allogeneic HSCT; and (3) they were refractory to conventional salvage chemotherapy and were, thus, candidates for an auto-allo strategy consisting of high-dose melphalan (200 mg/m^2) followed by haploidentical transplantation.

Patients were ineligible if they had active uncontrolled infections, a central nervous system disease, a Karnofsky performance status <60, or severe organ dysfunction, including a left ventricular ejection fraction <40%, carbon monoxide diffusion capacity <50%, or creatinine clearance <50 mL/min. The comorbidity index of each patient was calculated using the hematopoietic cell transplantation-comorbidity index system [13].

Conditioning Regimen and GVHD Prophylaxis

The conditioning regimen consisted of Cy 14.5 mg/kg on days -5 and -6, fludarabine 30 mg/m^2 from day −6 to day −2, and low-dose total body irradiationTBI (2 Gy) on day -1. The GVHD prophylaxis consisted of Cy 50 mg/kg administered on days +3 and +4, tacrolimus (Humanitas Cancer Center), or cyclosporine A (CsA; Institut Paoli Calmettes) and mycophenolate mofetil (MMF). Tacrolimus (FK 506, at a total dose of 1 mg) was administered as a continuous infusion until discharge and was converted to an oral formulation thereafter. The doses were adjusted to obtain serum levels between 10 and 20 ng/mL. CsA was dosed at 3 mg/kg as a continuous infusion until discharge and was converted to an oral formulation thereafter. The CsA doses were modified to obtain serum levels between 100 and 200 ng/mL. MMF was administered at 15 mg/kg per oral 3 times per day until day +35 and was then stopped. FK, CsA, and MMF were started on day +5. FK and CsA were tapered by day +180. Granulocyte colony–stimulating factor (G-CSF) was started on day +5 in all patients.

Stem Cell Sources and Donors

Potential family members were typed at the HLA-A, HLA-B, and HLA-DRB1 loci at an intermediate level of resolution. Selected donors were typed at the HLA-C locus at an intermediate resolution level. The DRB1 were typed at a high-resolution level. All donor/recipient pairs exhibited a median of 4 mismatches (range, 2 to 5) on the unshared haplotype. The donors underwent BM harvest under general anesthesia, and the target dose was 4×10^8 nuclear cells/kg of recipient weight. Unmanipulated BM was used for stem cell support on day 0. In Marseille, the first 12 donors underwent BM harvest, whereas the last cohort of donors (n = 23) were mobilized by the subcutaneous administration of G-CSF (Neupogen; Filgrastim; Amgen, Neuilly-sur-Seine, France) for 5 to 6 days at 10 μg/kg/day. The target was a minimum of 4×10^6 CD34/kg.

Supportive Care

At both centers, antimicrobial prophylaxis was begun in the hospital during the conditioning regimen and consisted of acyclovir 500 mg/m^2 administered 3 times per day, levofloxacin 500 mg per day, and sulfamethoxazole + trimethoprim (160 mg + 800 mg) administered as 2 tablets per day until day -2 and then resumed after hematological reconstitution at a dosage of 1 tablet every other day. The antifungal prophylaxis programs differed between the 2 centers: in Milan, caspofungin was administered (70 mg on the first day and then 50 mg per day) until day +5, when intravenous itraconazole (200 mg/day) was introduced; in Marseille, micafungin 50 mg/day was administered. After discharge, the patients received fluconazole 400 mg/day until day +75. PCR monitoring of cytomegalovirus was performed twice each week from day +15 until day +100 and then weekly until day +180. Treatment with ganciclovir or foscarnet was started when the number of copies was more than 1000/mL for 2 consecutive weeks. No maintenance treatment was used. In 1 center (Humanitas Cancer Center), weekly PCR monitoring of Epstein-Barr virus was started on day +15, and rituximab (375 mg/m^2) was administered if more than 10^4 copies/mL were detected.

Table 1
Patient Characteristics

Characteristic	BM	PBSC	*P* Value
No. of patients	46	23	
Age, median (range), yr	44 (19-68)	54 (25-65)	.06
Disease			.10
HL	23 (50%)	6 (27%)	
NHL	15 (33%)	9 (35%)	
CLL	1 (2%)	3 (12%)	
AML/MDS	2 (4%)	2 (19%)	
MM	2 (4%)	2 (8%)	
ALL	2 (4%)	0	
Previous HDC	34 of 46 (74%)	8 of 23 (35%)	.002
Previous ALLO	1 of 46 (2%)	4 of 23 (17%)	.022
Tandem auto-allo	15 of 46 (33%)	2 of 23 (9%)	.03
Disease status at haplo			.6
CR	28 (61%)	13 (57%)	
PR	11 (24%)	7 (30%)	
SD/PD	7 (15%)	3 (13%)	
HCT-CI, median (range)	1 (0-5)	3 (0-6)	.008
HLA mismatches, median (range)	4 (3-6)	4 (4-6)	.10
Conditioning regimen			
FCyTBI	46	23	NA
Sex D/R			.90
F/F	11 (24%)	4 (17%)	
F/M	16 (35%)	5 (22%)	
M/F	12 (26%)	6 (26%)	
M/M	21 (46%)	8 (35%)	
CMV D/R			
+/+	27 (59%)	11 (48%)	.60
+/−	6 (13%)	3 (13%)	
−/+	8 (17%)	4 (17%)	
−/−	5 (11%)	5 (22%)	
ABO compatibility*			
Compatible	31 (67%)	14 (61%)	.80
Minor	9 (20%)	6 (26%)	
Major	6 (13%)	3 (13%)	
CD34+ cells infused, median (range), × 10^6/kg	3 (.8-7)	5.1 (3.2-14)	<.0001
CD3+ cells content, median (range), × 10^6/kg	34 (17-92)	273 (119-530)	<.0001
Post-transplantation IS			
CsA	12 (26%)	23 (100%)	<.0001
FK	34 (74%)	0	
Follow-up, median (range), d	721 (365-728)	332 (135-498)	<.0001

BM indicates bone marrow; PBSC, peripheral blood stem cells; HL, Hodgkin lymphoma; NHL, non-Hodgkin lymphoma; MM, multiple myeloma; CLL, chronic lymphocytic leukemia; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukemia; HDC, high-dose chemotherapy; FCyTBI, fludarabine, cyclophosphamide, and low-dose total body irradiation; D/R, donor/recipient; IS, immunesuppression; CsA, cyclosporine A; FK, tacrolimus; HCT-CI, hematopoietic cell transplantation-comorbidity index.
Data presented are n (%) unless otherwise indicated.
* One patient in the BM group with bidirectional ABO incompatibility was included in the major incompatibility group.

Engraftment and GVHD Evaluation

Neutrophil engraftment was defined as the first of 3 consecutive days with an ANC of .5 × 10^9/L after transplantation. Platelet engraftment was

Table 2
Engraftment Results for all the Patients and for BM- or PBSC-supported Transplantation

Engraftment Results	BM	PBSC	*P* Value
ANC >.5 × 10^9/L all pts	20 (14-32)		
ANC >.5 × 10^9/L	21 (14-32)	20 (14-27)	.18
PLT >20,000/L all pts	29 (14-52)		
PLT >20,000/L	29 (16-46)	27 (14-52)	.13

ANC indicates absolute neutrophil count; PLT, platelet count; BM, bone marrow; PBSC, peripheral blood stem cells; Pts, patients.

defined as a platelet count of 20,000/mL with no transfusions during the preceding 7 days. Acute GVHD was graded according to the Keystone criteria [14], and chronic GVHD was retrospectively graded according to the National Institutes of Health criteria [15].

HLA Antibody Screening with Microarray Bead–based Assay

IgG anti-HLA reactivity in the sera was tested with a bead-based screening assay. Briefly, we used the LABScreen Mixed kit (One Lambda Inc., Canoga Park, CA) which simultaneously detects class I and class II antibodies with microbeads coated with purified class I and class II HLA antigens. Results above a cut-off value of 3.0 were considered positive. The Single Antigen kit (One Lambda) was also used to identify HLA specificities. The tests were carried out according to the manufacturer instructions and the analysis was performed with One Lambda software (HLA Fusion Version 3.0). Fluorescence intensity, measured on a Luminex analyzer, indicates the relative amount of antibody bound to the test sample. All sera with a mean fluorescence intensity value > 1000 were considered positive.

Statistical Analysis

Categorical variables were expressed as proportions and continuous variables were expressed as the medians with the respective range. Comparisons between groups were performed with the chi-squared and Mann-Whitney tests for categorical and continuous variables, respectively. The cumulative incidences of aGVHD and cGVHD were estimated considering disease progression or death as competing events [16]. Nonrelapse mortality (NRM) was defined as death with no evidence of progression or relapse; death after disease progression was treated as a competing event in the NRM calculation. The Kaplan-Meier method was used for the engraftment, overall survival (OS), and progression-free survival (PFS) analyses [17]. PFS was defined as the probability of survival without disease recurrence or progression. All outcomes were calculated from the date of transplantation. Only patients with successful ANC engraftment were evaluated for aGVHD, and cGVHD was evaluated only in patients with a minimum follow-up of 100 days. A Cox regression [18] was performed to identify any associations between GVHD (acute grade II to IV or chronic) and the following variables: patient age (continuous), donor/patient gender (female/male versus other), donor/patient cytomegalovirus serology, ABO matching, donor type (mother versus other), stem cell source, GVHD prophylaxis (FK versus CsA), and CD34 + infusion (continuous). *P* values ≤.05 were considered significant. SPSS v16.0 and R v2.12.0 software programs were used for the analysis.

RESULTS

The patient characteristics are displayed in Table 1. As expected, higher numbers of CD34 + cells were infused in the PBSC group. Because of the different types of GVHD prophylaxis used in each center, the patients infused with BM received either CsA or FK, whereas the patients treated with PBSCs (originating from Marseille only) received only CsA. In addition, the PBSC group had a higher hematopoietic cell transplantation-comorbidity index score.

Engraftment

For the population as a whole, the median time for the ANC to increase to more than .5 × 10^9/L was 20 days (range,14 to 32), and the median time to an acceptable transfusion-independent platelet count was 29 days (range, 14 to 52). There were no significant differences between the 2 cohorts in terms of the median time to ANC and platelet reconstitution (Table 2). The cumulative incidences of ANC and platelet engraftment (Figure 1) were 87% and 95% after BM and PBSC infusion, respectively ($P = .18$). The median time to obtain full donor chimerism was 60 days (range, 15 to 108), which was similar between the BM and PBSC groups.

GVHD and infectious complications

The incidence of grade II to IV aGVHD was 25% and 33% after BM and PBSC infusions, respectively ($P = .43$), and the incidence of cGVHD was 13% after both BM and PBSC infusions ($P = .21$) (Figures 2 and 3; Table 3). There were no significant associations between any of the variables and acute or chronic GVHD (data not shown). The univariate hazard ratios according to stem cell source (PBSC versus BM) were 1.61 (95% confidence interval [CI]: .50 to 5.22, $P = .43$) and .95 (95% CI: .17 to 5.22, $P = .96$) for aGVHD grade II to IV and cGVHD, respectively.

The cumulative incidence of grades III to IV acute GVHD was 14% in PBSC and 3% in BM group, with a *P* value of .10.

No major differences between the 2 cohorts were observed in term of infectious complications (Table 4).

Survival and NRM

Twenty-one patients eventually died: 11 because of progressive disease and 10 because of transplantation-related complications. In the latter group, the cause of death was infection in 7 patients, organ failure in 2 patients, and aGVHD in 1 patient. After a median follow-up of 18 months (range, 4 to 52 months), the 2-year OS and PFS probability estimates were 68% and 62%, respectively. The OS and PFS values were

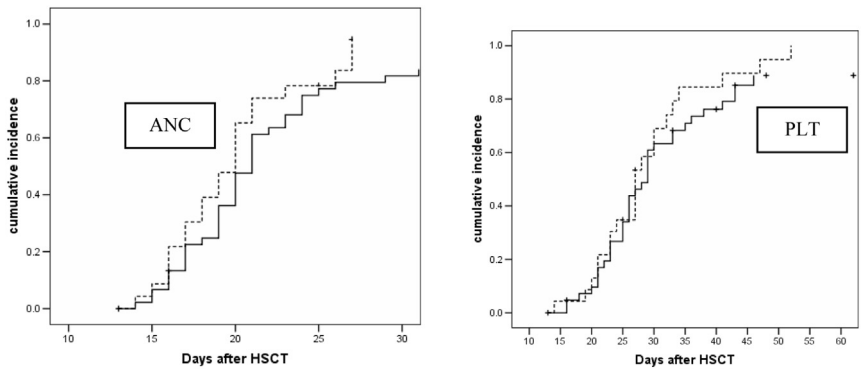

Figure 1. Cumulative incidence of engraftment by stem cell source. (Left) Shows incidence of absolute neutrophil count and (Right) shows platelet count. BM indicates bone marrow (continuous line); PBSC, peripheral blood stem cells (dashed line).

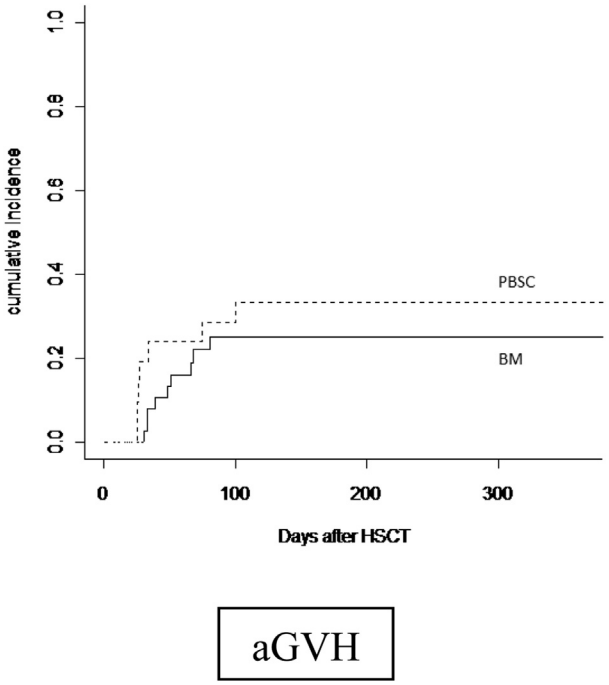

Figure 2. Cumulative incidence of acute GVHD (aGVHD) by stem cell source. BM indicated bone marrow and PBSC, peripheral blood stem cells.

not significantly different between the 2 cohorts of patients (Figures 4 and 5). The relapse incidence was similar between the 2 cohorts (Figure 6). However, patients in complete remission had a significant lower relapse incidence (14% versus 33%, $P = .04$) and superior PFS (68% versus 49%, $P = .05$) compared with those not in complete remission. The 2-year overall NRM was 18% (BM: 22%; PBSC: 12%; $P = .96$).

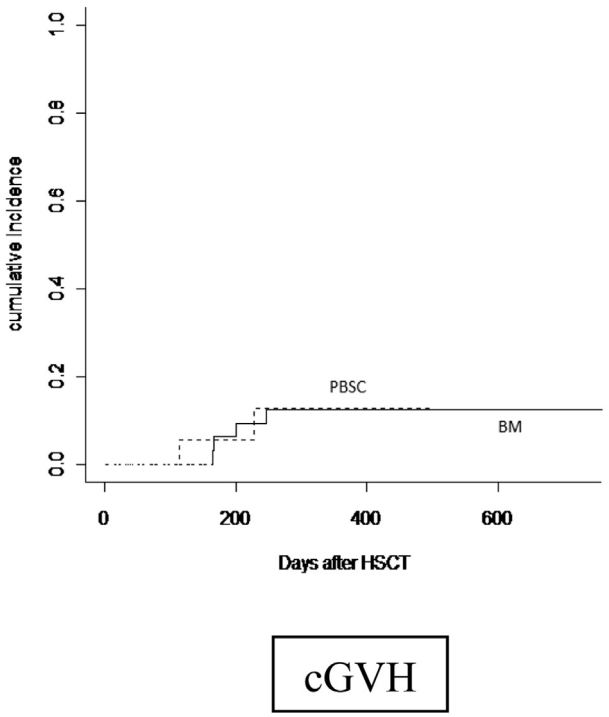

Figure 3. Cumulative incidence of chronic GVHD (cGVHD) by stem cell source. BM indicated bone marrow and PBSC, peripheral blood stem cells.

Table 3
Acute and Chronic Graft-versus-Host Disease Incidence and Nonrelapse Rate

Outcome	BM	PBSC	*P* Value
aGVHD 2-4 all pts	28%		
aGVHD 2-4	25%	33%	.43
cGVHD all pts	13%		
cGVHD	13%	13%	.21
NRM all pts	18%		
NRM	22%	12%	.96

BM indicates bone marrow; PBSC, peripheral blood stem cells; Pts, patients; aGVDH, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; NRM, nonrelapse mortality.

OS and NRM were not statistically different between the 2 cohorts of patients.

DISCUSSION

Haploidentical transplantation with the NMAC regimen, supported by BM stem cells and postinfusion Cy, has been reported to be well tolerated, with encouragingly low incidences of GVHD and host versus graft and sustained engraftment in the majority of patients [11,19].

One of the cornerstones of the Baltimore approach was the use of BM as a stem cell source. The reason for using BM instead of PBSCs, even after NMAC, is based on mouse models, in which different levels of mixed chimerism were obtained by infusing a marrow stem cell dose of fewer than 10×10^6 cells, corresponding to 5×10^8 mononuclear cells/kg in humans [20]. Furthermore, the lower number and different functional characteristics of the CD3+ cells contained in BM caused the use of BM to lower the incidence of acute and chronic GVHD [21,22]. In human clinical studies involving NMAC or reduced-intensity conditioning regimens, BM has been replaced by PBSCs as a stem cell source because of the higher engraftment rates due to the larger number of CD34+ stem cells and because of a putatively higher antitumoral effect linked to a larger number of T cells. However, acute and, in particular, chronic GVHD occurred more frequently after PBSC infusions, particularly when NMAC regimens [23] without in vivo T cell depletion were used [24,25].

In a prospective phase II study of haploidentical PBSC infusions using a myeloablative conditioning regimen and postinfusion Cy, Salomon et al. showed that the cumulative incidences of grade II to IV aGVHD and cGVHD were 30% and 35% (5% severe), respectively, and that the 1-year NRM was 10%. In that study, 2 peculiar clinical features were observed: late BK-linked hemorrhagic cystitis (75% of patients) and cytokine-related fever (90%) after stem cell infusion [12].

Table 4
Incidences of Infectious Complications Observed in All Patients

Infectious Complications	BM n = 46	PBSCs n = 23	*P* Value
FUO	22%	13%	.50
Bacterial sepsis	46%	30%	.30
Gram −	28%	26%	
Gram +	18%	4%	
Pneumonia	39%	22%	.10
Bacterial	4%	13%	
Viral	9%	0	
Fungal	6%	0	
Not documented	20%	9%	
CMV viremia	37%	48%	.40
BK-virus HC	11%	0	.10

FUO indicates fever of unknown origin; BM, bone marrow; PBSC, peripheral blood stem cells; CMV, cytomegalovirus; HC, hemorrhagic cystitis.

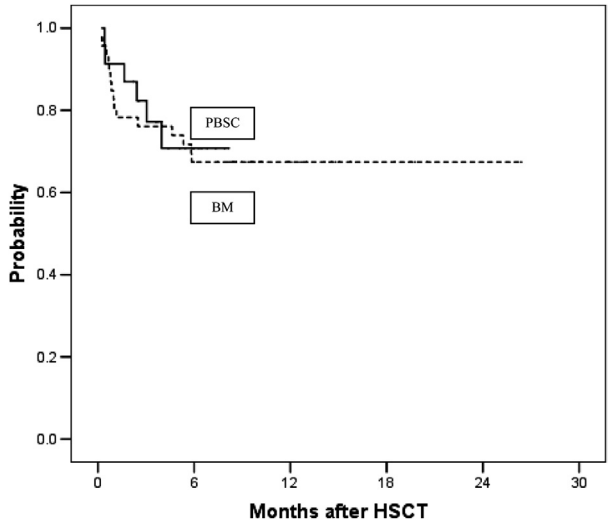

Figure 4. Overall survival (OS) for patients received bone marrow (BM) or peripheral blood stem cells (PBSC).

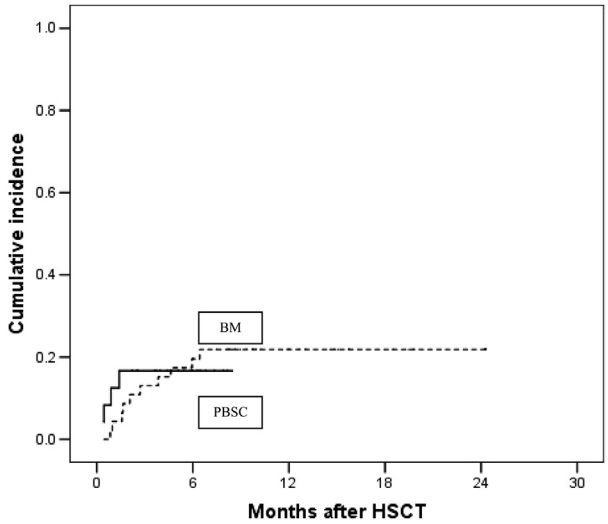

Figure 6. Relapse incidence for patients received bone marrow (BM) or peripheral blood stem cells (PBSC).

In the present retrospective study, we analyzed the outcomes of patients after haploidentical transplantation using NMAC and postinfusion Cy supported by BM or PBSCs. The analysis of the first clinical endpoint, the incidences of acute and chronic GVHD, did not reveal any differences based on the stem cell graft source. We did not observe any significant differences in the rates of acute and chronic GVHD between the patients receiving PBSCs and those receiving BM.

The second clinical endpoint analyzed was the engraftment rate. Unpredictably, PBSCs and BM were associated with similar engraftment rates to achieve both stable ANC and platelet counts, although the number of CD34-positive cells was significantly higher in the PBSC cohort. This finding differed from the usual results of randomized studies comparing PBSCs with BM after myeloablative conditioning for HLA-identical transplantation. In a meta-analysis of 9 such studies, the median number of days to safe ANC and platelet levels was significantly shorter in the PBSC group.

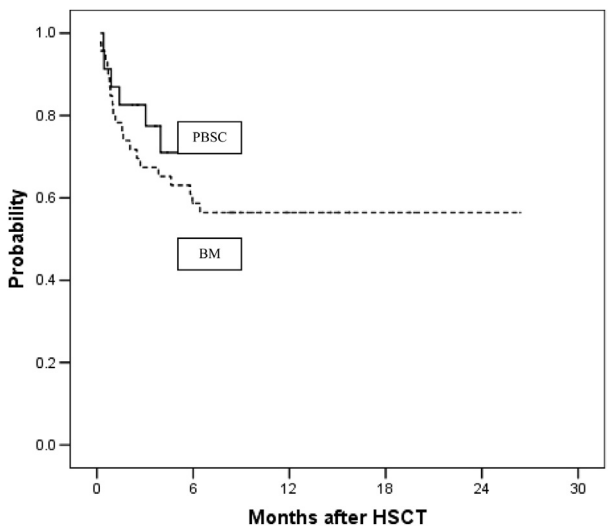

Figure 5. Progression-free survival (PFS) for patients received bone marrow (BM) or peripheral blood stem cells (PBSC).

Overall, the time to engraftment in our cohort of patients was longer than that reported by the Baltimore group. In fact, the latter patients showed a shorter median time to a safe ANC (15 days versus 20 days in the BM group and 21 days in the PBSC group), whereas the time to platelet reconstitution was similar (29 days versus 28 days) between the groups. When only patients receiving BM were considered, the numbers of mononuclear cells infused were also similar in the 2 studies. The most likely reason for this difference is the time until G-CSF was begun, which was delayed in our patients until day +5, as opposed to day +1 in the Baltimore cohort [26]. Finally, the rates of infectious complications were similar in the 2 groups. Overall, we confirmed that the risk of opportunistic infections was not enhanced after haploidentical transplantation.

This study has several limitations due to its retrospective nature. The number of patients was quite low in the PBSC cohort, immunosuppression other than postinfusion Cy was not homogeneous, and selection bias cannot be excluded.

Overall, this is the first study to suggest that, in the setting of haploidentical transplantation with postinfusion Cy and NMAC regimens, the use of PBSCs did not appear to be detrimental to the patients in terms of both GVHD and engraftment. This finding could be useful in cases with difficulties accessing an operating room or with a great disparity in weight between the recipient and donor.

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REFERENCES

1. Anasetti C, Amos D, Beatty PG, et al. Effect of HLA compatibility on engraftment of bone marrow transplants in patients with leukemia or lymphoma. *N Engl J Med*. 1989;320:197-204.
2. Aversa F, Terenzi A, Tabilio A, et al. Full haplotype-mismatched hematopoietic stem-cell transplantation: a phase II study in patients with acute leukemia at high risk of relapse. *J Clin Oncol*. 2005;23:3447-3454.
3. Federmann B, Bornhauser M, Meisner C, et al. Haploidentical allogeneic hematopoietic cell transplantation in adults using CD3/CD19 depletion and reduced intensity conditioning: a phase II study. *Haematologica*. 2012;97:1523-1531.

4. Ciceri F, Bonini C, Lupo Stanghellini MT, et al. Infusion of suicide-gene-engineered donor lymphocytes after family haploidentical haemopoietic stem-cell transplantation for leukaemia (the TK007 trial): a non-randomised phase I–II study. *Lancet Oncol*. 2009;10:489-500.
5. Dodero A, Carniti C, Raganato A, et al. Haploidentical stem cell transplantation after a reduced-intensity conditioning regimen infusions for the treatment of advanced hematologic malignancies: posttransplantation CD8-depleted donor lymphocyte contribute to improve T-cell recovery. *Blood*. 2009;113:4771-4779.
6. Rizzieri DA, Koh LP, Long GD, et al. Partially matched, nonmyeloablative allogeneic transplantation: clinical outcomes and immune reconstitution. *J Clin Oncol*. 2007;25:690-697.
7. Lee K-H, Lee J-H, Lee J-H, et al. Hematopoietic cell transplantation from an hla-mismatched familial donor is feasible without ex vivo T cell depletion after reduced-intensity conditioning with busulfan, fludarabine, and antithymocyte globulin. *Biol Blood Marrow Transplant*. 2009;15:61-72.
8. Huang XJ, Zhu HH, Chang YJ, et al. The superiority of haploidentical related stem cell transplantation over chemotherapy alone as post-remission treatment for patients with intermediate- or high-risk acute myeloid leukemia in first complete remission. *Blood*. 2012;119:5584-5590.
9. Di Bartolomeo P, Santarone S, De Angelis G, et al. Haploidentical, unmanipulated, G-CSF-primed bone marrow transplantation for patients with high-risk hematologic malignancies. *Blood*. 2013;121:849-857.
10. Santos GW, Owens AH Jr. A comparison of the effects of selected cytotoxic agents on allogeneic skin graft survival in rats. *Bull Johns Hopkins Hosp*. 1965;116:327-340.
11. Luznik L, O'Donnell PV, Symons HJ, et al. HLA-haploidentical bone marrow transplantation for hematologic malignancies using nonmyeloablative conditioning and high-dose, post-transplantation cyclophosphamide. *Biol Blood Marrow Transplant*. 2008;14:641-650.
12. Solomon SR, Sizemore CA, Sanacore M, et al. Haploidentical transplantation using T cell replete peripheral blood stem cells and myeloablative conditioning in patients with high-risk hematologic malignancies who lack conventional donors is well tolerated and produces excellent relapse-free survival: results of a prospective phase II trial. *Biol Blood Marrow Transplant*. 2012;18:1859-1866.
13. Sorror ML, Maris MB, Storb R, et al. Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. *Blood*. 2005;106:2912-2919.
14. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant*. 1995;15:825-828.
15. Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. *Biol Blood Marrow Transplant*. 2005;11:945-956.
16. Gooley TA, Leisenring W, Crowley J, Storer BE. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. *Stat Med*. 1999;18:695-706.
17. Kaplan EL, Meier P. Non-parametric estimation from incomplete observations. *J Am Stat Assoc*. 1958;53:457-481.
18. Cox D. Regression models and life tables. *J R Stat Soc (B)*. 1972;34:187-220.
19. Luznik L, Jones RJ, Fuchs EJ. High-dose cyclophosphamide for graft-versus-host disease prevention. *Curr Opin Hematol*. 2010;17:493-499.
20. Luznik L, Jalla S, Engstrom LW, et al. Posttransplantation cyclophosphamide facilitates engraftment of major histocompatibility complex–identical allogeneic marrow in mice conditioned with low-dose total body irradiation. *Biol Blood Marrow Transplant*. 2002;8:131-138.
21. Ottinger HD, Beelen DW, Scheulen B, et al. Improved immune reconstitution after allotransplantation of peripheral blood stem cells instead of bone marrow. *Blood*. 1996;88:2775-2779.
22. Korbling M, Anderlini P. Peripheral blood stem cell versus bone marrow allotransplantation: does the source of hematopoietic stem cells matter? *Blood*. 2001;98:2900-2908.
23. Storb R, Gyurkocza B, Storer BE, et al. Graft-versus-host disease and graft-versus-tumor effects after allogeneic hematopoietic cell transplantation. *J Clin Oncol*. 2013;31:1530-1538.
24. Peggs KS, Sureda A, Qian W, et al. Reduced-intensity conditioning for allogeneic haematopoietic stem cell transplantation in relapsed and refractory Hodgkin lymphoma: impact of alemtuzumab and donor lymphocyte infusions on long-term outcomes. *Br J Haematol*. 2007;139:70-80.
25. Crocchiolo R, Esterni B, Castagna L, et al. Two days of antithymocyte globulin are associated with a reduced incidence of acute and chronic graft-versus-host disease in reduced-intensity conditioning transplantation for hematologic diseases. *Cancer*. 2013;119:986-992.
26. Burroughs LM, O'Donnell PV, Sandmaier BM, et al. Comparison of outcomes of HLA-matched related, unrelated, or HLA-haploidentical related hematopoietic cell transplantation following nonmyeloablative conditioning for relapsed or refractory Hodgkin lymphoma. *Biol Blood Marrow Transplant*. 2008;14:1279-1287.