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Peripheral Blood Hematopoietic Stem Cells for Transplantation of Hematological Diseases from Related, Haploidentical Donors after Reduced-Intensity Conditioning

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In a multicenter collaboration, we carried out T cell–replete, peripheral blood stem cell (PBSC) transplantations from related, HLA-haploidentical donors with reduced-intensity conditioning (RIC) and post-transplantation cyclophosphamide (Cy) as graft-versus-host disease (GVHD) prophylaxis in 55 patients with high-risk hematologic disorders. Patients received 2 doses of Cy 50 mg/kg i.v. on days 3 and 4 after infusion of PBSC (mean, 6.4 × 10^6/kg $CD34^+$ cells; mean, 2.0 × 10^8/kg $CD3^+$ cells). The median times to neutrophil (500/μL) and platelet (>20,000/μL) recovery were 17 and 21 days respectively. All but 2 of the patients achieved full engraftment. The 1-year cumulative incidences of grade II and grade III acute GVHD were 53% and 8%, respectively. There were no cases of grade IV GVHD. The 2-year cumulative incidence of chronic GHVD was 18%. With a median follow-up of 509 days, overall survival and event-free survival at 2 years were 48% and 51%, respectively. The 2-year cumulative incidences of nonrelapse mortality and relapse were 23% and 28%, respectively. Our results suggest that PBSC can be substituted safely and effectively for bone marrow as the graft source for haploidentical transplantation after RIC.

INTRODUCTION

Allogeneic hemopoietic stem cell transplantation from HLA-matched donors is curative in a proportion of patients with hematologic malignancies, as well as in those with inherited diseases, such as hemoglobinopathies and bone marrow failure syndromes. A suitable HLA-identical sibling donor will be available for about 30% to 35% of patients. For

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patients without a matched sibling donor, the likelihood of identifying a volunteer unrelated donor that is suitably matched at HLA-A, -B, -C, and -DRB1 is population specific ranging, from about 79% for Caucasian patients of European descent to 30% to 50% for patients of other ethnic backgrounds [1]. Even if a matched, unrelated donor is identified, the likelihood of proceeding to transplantation is less than 50%, largely because of progression of disease during the search process, which renders the patient ineligible for transplantation [2]. For this substantial number of patients, alternative donors, such as haploidentical family donors, unrelated umbilical cord blood donors, or mismatched unrelated donors can bridge this gap, enabling transplantation. Over the past decade, single-center and multicenter, cooperative group trials have shown that the administration of high doses of cyclophosphamide 60 to 72 hours after the infusion of bone marrow (BM) cells from related, haploidentical donors (haplo-BM) enables engraftment with low rates of rejection, acute and chronic graft-versus-host disease (GVHD), and nonrelapse mortality (NRM) [3-5]. In these studies, disease relapse remained the major cause of treatment failure. Currently, peripheral blood stem cells (PBSC) are the preferred source of allografts because of ease of collection, higher yields of $CD34^+$ progenitor cells, faster engraftment, and, in the case of matched sibling donor transplantations, improved short and long term survival [6-8]. However, PBSC products contain approximately 5 to 10 times higher numbers of $CD3^+$ T cells than BM harvests [9], which correlates with higher rates of acute and chronic GHVD compared with BM [7,10-13]. In this report, we describe outcomes after transplantation of patients with high-risk hematologic disorders after reduced-intensity conditioning (RIC), in which haplo-PBSC was substituted for haplo-BM. Prophylaxis of GVHD included post-transplantation cyclophosphamide (Cy) to eliminate alloreactive T cell populations, a calcineurin-inhibitor, and mycophenolate mofetil (MMF). Although the rate of grade II acute GVHD was higher, engraftment kinetics, grade III acute GVHD, chronic GVHD, and survival outcomes were similar to those reported for patients receiving haplo-BM.

PATIENTS AND METHODS

Eligibility Criteria

Consecutive, eligible patients from 4 centers, Guy's and St Thomas' Hospital, London; King's College Hospital, London; Westmead Hospital, Sydney; and the Fred Hutchinson Cancer Research Center, Seattle, who underwent a stem cell transplantation with PBSC from related, HLA-haploidentical donors between March 2009 and February 2013 were included (Table 1). All patients had at least 160 days of follow-up. Results were analyzed as of August 2013. All patients signed consent forms approved by their local institutional review boards. Sharing of deidentified transplantation data was approved by the institutional review boards of each of the participating centers.

Patients were $\leq$ 70 years of age with a high-risk hematologic disorder but lacked a suitably matched related or unrelated donor, defined as a donor with an 9 to 10/10 locus HLA match at HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1. An unrelated donor search was not required for a patient to be eligible for this protocol, or a search could be abandoned if the clinical situation dictated an urgent transplantation. Clinical urgency was defined as 6 to 8 weeks from referral to transplantation or a low likelihood of finding a matched, unrelated donor. In this study, the only times that an unrelated donor search were not performed were in the cases of 4 patients who had rejected allografts from 10/10 matched donors and there was urgency in performing a salvage transplantation.

Patients with acute leukemia were required to be in morphologic complete remission. Patients with primary or secondary graft failure in a prior allogeneic transplantation were also eligible. Patients were required to have adequate organ function, defined as left ventricular ejection fraction $\geq$ 35%, forced expiratory volume in the first second, functional vital capacity or carbon monoxide corrected diffusion lung capacity $>$ 50% of predicted;

Table1
Patient and Disease Characteristics

Characteristics	Value
Total sample, N	55
Seattle FHCRC	20
London Guy's and St. Thomas'	7
London Kings College Hospital	18
Westmead Hospital	10
Age	
Recipient, median (range), yr	49 (14-69)
Donor, median (range), yr	40 (15-73)
Sex, n (%)	
Male	35 (64)
Female	20 (36)
Recipient ethnicity, n (%)	
Caucasian	37 (67)
Afro-Caribbean	9 (16)
Asian	9 (16)
Donor relationship, n (%)	
Mother	8 (15)
Father	5 (9)
Brother	8 (15)
Sister	12 (22)
Son	14 (25)
Daughter	8 (15)
CMV serostatus, n (%)	
Recipient −/donor −	14 (25)
Recipient +/donor −	8 (15)
Recipient −/donor +	7 (13)
Recipient +/donor +	26 (47)
ABO compatibility, n (%)	
Compatible	27 (49)
Minor mismatch	12 (22)
Major mismatch	15 (27)
Bidirectional mismatch	1 (2)
Time diagnosis-Allogeneic HCT, median months (range)	23 (2-215)
Prior chemotherapy/radiotherapy lines median (range), n	3 (1-15)
Prior transplantation, n (%)	
Autologous	12 (22)
Months from Auto, median (range)	23 (6-106)
Allogeneic	7 (13)
Months from Allo, median (range)	6 (2-39)
Disease, n (%)	
B-NHL	7 (13)
T-NHL	5 (9)
AML	16 (29)
HL	9 (16)
ALL	2 (4)
MDS	5 (9)
SAA	4 (7)
CLL	4 (7)
CML	3 (5)
Myeloid	24 (44)
Lymphoid	27 (49)
Aplastic Anemia	4 (7)
Disease status at HCT, n (%)	
CR1	20 (36)
CR $>$ 1	11 (20)
PR	9 (16)
SD	9 (16)
RD	2 (4)
PD	4 (7)
ASBMT/CIBMTR disease risk classification, n (%)	51 (excludes AA)
Low risk	12 (24)
Intermediate risk	25 (49)
High risk	14 (25)
Cell dose/kg, mean (SD)	
$CD34^+$cells ($\times 10^6$)	6.4 (1.6)
$CD3^+$ cells ($\times 10^8$) (n = 19)	2.0 (1.4)

HLA mismatches, no (%)*	HvG	GvH
0	0	0
1	0	0
2	2 (4)	3 (7)
3	5 (12)	8 (18)

(*Continued on next page*)

Table1
(*continued*)

HLA mismatches, no (%)*	HvG	GvH
4	13 (29)	10 (22)
5	25 (56)	24 (53)
Median (range)	5 (2-5)	5 (2-5)

AML indicates acute myeloid leukemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkin's lymphoma; CLL, chronic lymphocytic leukemia; HL, Hodgkin lymphoma; ATLL, acute T leukemia lymphoma; T NHL, T non-Hodgkin's lymphoma; SAA, severe aplastic anemia; CML, chronic myeloid leukemia; CR1, first complete remission; CR > 1, subsequent complete remission; PR, partial remission; SD, stable disease; RD, refractory/progressive disease; FHCRC, Fred Hutchinson Cancer Research Center; CMV, cytomegalovirus; B-NHL, B cell non-Hodgkin lymphoma; ALL, acute lymphoblastic leukemia; ASBMT/CIBMTR, American Society for Blood and Marrow Transplantation/Center for International Blood and Marrow Transplant Research.

* n = 45.

total bilirubin ≤ 2.5 mg/dL, and aspartate and alanine aminotransferases and alkaline phosphatase < 5 times the upper limit of normal, and serum creatinine within the normal range for age or creatinine clearance or calculated glomerular filtration rate > 40 mL/min/1.73 m^2. A Karnofsky performance score of ≥ 60 was required. Patients who had undergone a prior autologous transplantation were eligible, provided 3 months had elapsed since the procedure.

Characteristics at Hematopoietic Cell Transplantation

Details regarding patient and donor characteristics are outlined in Table 1. Of the 5 patients with myelodysplastic syndrome, prior chemotherapy was not administered in 2 patients with an International Prognostic Scoring System score of low risk, 1 of whom had rejected 2 prior allografts from the same HLA-matched, unrelated donor, and the other patient had rejected a double allograft from 2 unrelated umbilical cord blood donors. Two of 3 International Prognostic Scoring System high-risk patients were treated with 4 cycles of 5-azacytidine (75 mg/m^2 for 7 days every 28 days). One of these 2 patients had cytogenetic progression and was treated subsequently with induction chemotherapy (daunorubicin 50 mg/m^2 for 3 days and cytarabine 100 mg/m^2 twice daily for 10 days) (DA 3 + 10) before proceeding to haplo PBSC. The third high-risk patient had previously received 10 cycles of lenalidomide and on disease progression to refractory anemia with excess blasts stage 2 was treated with 2 cycles of induction chemotherapy (first with DA 3 + 10and then DA 3 + 8[8 days of cytarabine at the same dose as above]).

All 4 patients with severe aplastic anemia (SAA) had failed prior immunosuppressive therapy and 2 of the 4 patients had subsequently rejected stem cell transplantations from HLA-matched, unrelated donors. None of the patients received further chemotherapy within 90 days of haplo-PBSC transplantation. One patient with SAA/paroxysmal nocturnal hemoglobinuria was treated with eculizumab before and during the transplantation. MMF was stopped at day +35; however, tacrolimus was maintained at levels of 10 to 15 ng/mL for 9 to 12 months after transplantation and then tapered over a 3-month period.

HLA Matching and Donor Selection

Haplo-PBSC donors were required to be first-degree relatives of the patient, defined as biologic parents, siblings, children, or half-siblings. Donor and recipients were typed at HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1 at the allelic level for the 45 patients enrolled at the London and Seattle transplantation centers. At the Westmead center, 3 of 10 donor-recipient pairs were typed at HLA-A, HLA-B, and HLA-DRB1 and 7 of 10 donor-recipient pairs were typed at HLA-A, HLA-B, HLA-C, and HLA-DRB1, as per the preference of the local Red Cross laboratory. Donor-recipient pairs were considered HLA haploidentical if they were genotypically identical for 1 allele at each of the typed loci. Donors were required to be ≥ 16 years of age and were screened as per the American Association of Blood banks and Foundation for Accreditation of Cellular Therapy/Joint Accreditation Committee ISCT EBMT guidelines. Donors were excluded if the recipient's serum contained antidonor HLA antibodies. If more than 1 possible donor were identified, donor selection hierarchy was as follows: (1) donor-recipient matching for cytomegalovirus (CMV) serology, and (2) donor-recipient red blood cell compatibility. A previous report showed that increasing donor-recipient HLA-mismatch did not have a detrimental effect after nonmyeloablative HLA-haploidentical BM transplantation with post-transplantation Cy [14]. Therefore, higher degrees of HLA matching were not prioritized in donor selection.

Conditioning Regimen and Immunosuppressive Therapies

Recipients were conditioned with fludarabine 30 mg/m^2/day i.v. daily from days -6 to -2 (total dose of 150 mg/m^2), Cy 14.5 mg/kg i.v. on days -6 and -5, and 2 Gy total body irradiation in a single dose on day -1 as previously described [4,5]. The dose of fludarabine was adjusted for creatinine clearance as clinically indicated. For patients with an actual body weight of > 125% ideal body weight (IBW), Cy was dosed based on adjusted IBW. Adjusted IBW was computed as the sum of the IBW and 25% of the difference between the actual and IBW. Sodium 2-sulfanylethanesulfonate (MESNA) and i.v. hydration were administered for uro-protection. GVHD prophylaxis consisted of Cy 50 mg/kg by i.v. infusion over 1 to 2 hours on days +3 (between 60 to 72 hours after PBSC infusion) and +4 after transplantation. Patients received tacrolimus and MMF beginning day +5 after infusion of haplo-PBSC. MMF was given at a dose of 15 mg/kg every 8 hours with the maximum daily dose not exceeding 3 grams. MMF prophylaxis was discontinued on day +35 or continued at the discretion of the treating center, if active GVHD was present. Tacrolimus was administered to achieve a target trough level of 5 to 10 ng/mL with the goal of discontinuing at day +180 after transplantation. Filgrastim was initiated at day +5 at a dose of 5 μg/kg/day and continued until the neutrophil count was ≥ 1000/μL for 3 consecutive days.

Collection of Hemopoietic Stem Cells and Supportive Care

Filgrastim at a dose of 10 μg/kg (London and Sydney) or 16 μg/kg (Seattle) actual body weight was administered subcutaneously once daily or equally divided twice daily from day -5 to day -1, followed by collection of PBSC by apheresis on day -1. The target dose of PBSC for infusion was 5 to 6 × 10^6 $CD34^+$ cells/kg. If the target dose was met after the first apheresis procedure, PBSC were stored overnight at 4°C before infusion on day 0. If the target dose was not met, a second apheresis was performed the next day. PBSC in excess of the target dose were cryopreserved.

Antimicrobial prophylaxis was administered as per the institutional protocols. All patients received prophylaxis for *Pneumocystis jirovici* pneumonia, herpes simplex/zoster and *Candida albicans*. Prophylaxis for mold was as per institutional protocols (secondary prophylaxis in Seattle and Sydney and primary prophylaxis in London). Neutropenic patients received prophylaxis with quinolones. Blood products were irradiated to 25 Gy before infusion. Transfusions for blood and platelets followed institutional protocols. All patients, including CMV-negative patients, received leuko-depleted blood products ("CMV-safe"). CMV DNA viral load was monitored at least weekly by PCR of serum until day 100. Pre-emptive therapy with either ganciclovir (5 mg/kg/i.v. twice daily) or foscarnet (90 mg/kg twice daily) was initiated as per the institutional guidelines. Patients in London were treated as in-patients from conditioning to engraftment of neutrophils, whereas conditioning and transplantation in Seattle and Sydney were outpatient procedures. Patients in Seattle and Sydney were admitted as indicated for regimen-related toxicity or infection.

GVHD Grading and Treatment

Acute GVHD was graded according to the consensus criteria [15]. Chronic GVHD was assessed as per the National Institutes of Health criteria [16]. Each institution treated GVHD according to their local protocols. Typically, progressive or grade III or IV acute GVHD was treated initially with 1 to 2 mg/kg of methylprednisolone parenterally with optimization of the dose of tacrolimus or the addition of MMF to tacrolimus.

Analyses of Donor Chimerism

Peripheral blood donor chimerism was tested at least on day +28 after transplantation and then as per institutional protocols. Chimerism was studied by PCR analysis of short or variable nucleotide tandem repeats unique to the donor and recipient. Chimerism was performed on at least peripheral blood, $CD3^+$ cells, and $CD33^+$ or $CD15^+$ cells. Patients were considered fully donor chimeric if their unfractionated, $CD3^+$ and $CD33^+$/$CD15^+$ fractions were ≥ 95% donor.

Statistical Methods

The outcomes are reported as of August 2013. The main outcomes of interest were engraftment of neutrophils and platelets, incidence and severity of both acute and chronic GVHD, NRM, overall survival (OS), and event-free survival (EFS). Neutrophil engraftment was defined as the time from infusion of donor stem cells and the first of 3 consecutive days with an absolute neutrophil count of ≥ 500/μL. Platelet engraftment was similarly defined as the interval between donor stem cell infusion and the first day of a platelet count > 20,000/μL without a platelet transfusion in the preceding 7 days. Donor engraftment was defined as a donor chimerism ≥ 95%. Graft failure was defined as ≤ 5% donor cells after transplantation not due to progressive disease. NRM was defined as death in the absence of detectable

disease relapse or progression. Probabilities of OS and EFS were estimated using the Kaplan Meier method [17] on an intent-to-treat basis. Probabilities of acute GVHD, chronic GVHD, relapse, and NRM were summarized using cumulative incidence estimates [18]. Death without engraftment was considered a competing risk for engraftment; death without relapse was a competing risk for relapse; relapse was a competing risk for NRM; graft failure, relapse or death, without GVHD, were considered competing risks for GVHD.

RESULTS

Patient and Graft Characteristics

By American Society for Blood and Marrow Transplantation/Center for International Blood and Marrow Transplant Research disease risk criteria, 24% of patients were characterized as low risk, 49% as intermediate risk, and 25% as high risk (Table 1). Nineteen patients had received prior stem cell transplantations: 12 (22%) had relapsed after a previous autograft, whereas 2 (4%) had relapsed after previous allografts (matched sibling × 1, n = 1; double umbilical cord blood × 1, n = 1), and 5 (9%) had failed to engraft after prior allografts (matched sibling × 2, n = 1; matched unrelated donor × 1, n = 1; matched unrelated donor × 2, n = 2; double umbilical cord blood × 1, n = 1). Patients with hematologic malignancies were divided approximately equally between myeloid (44%) and lymphoid (49%) disorders. Two-thirds of patients were Caucasian, whereas 32% were from an ethnic minority population (Afro-Caribbean, n = 9; Asian, n = 9).

Donor characteristics are also listed in Table 1. Donors were children of recipients in 22 (40%) cases, siblings in 20 (37%) cases, and parents in 13 (24%) cases. In the 45 cases where HLA typing was performed at 5 loci, there were a median of 5 mismatches (range, 2 to 5) in the host-versus-graft direction and a median of 5 mismatches (range, 2 to 5) in the graft-versus-host direction.

Engraftment

The median time to neutrophil recovery was 17 days (range, 12 to 29 days). The median time to platelet recovery was 21 days (range, 11 to 48 days); 6 patients did not reach a platelet nadir < 20,000/μL. Primary graft failure occurred in 2 patients (4%): a patient with MDS/acute myeloid leukemia who underwent a subsequent salvage transplantation from an HLA-mismatched, unrelated donor but ultimately died of relapsed disease and a patient with chronic myeloid leukemia in second chronic phase who had autologous neutrophil recovery at day +23 and who is alive on tyrosine kinase inhibitor therapy at day +622. Full donor chimerism was detected in unfractionated peripheral blood or $CD3^+$ and $CD33^+$ or $CD15^+$ fractions of peripheral blood in the remaining 53 patients (96%) by day 28 and was sustained.

Infections

No invasive mold infections or Epstein Barr virus reactivations were observed in any patients on study. CMV reactivation occurred in 27 of 34 patients (79%) who were at high risk for reactivation (donor seropositive/seronegative, recipient seropositive) at a median of 33 days after transplantation. Patients received pre-emptive therapy and no case of CMV disease occurred. There were no cases of primary CMV infection.

Acute and Chronic GVHD

The cumulative incidences of grades II and grade III acute GVHD at 1 year were 53% (95% confidence interval [CI], 40% to 66%) and 8% (95% CI, .4% to 15%), respectively

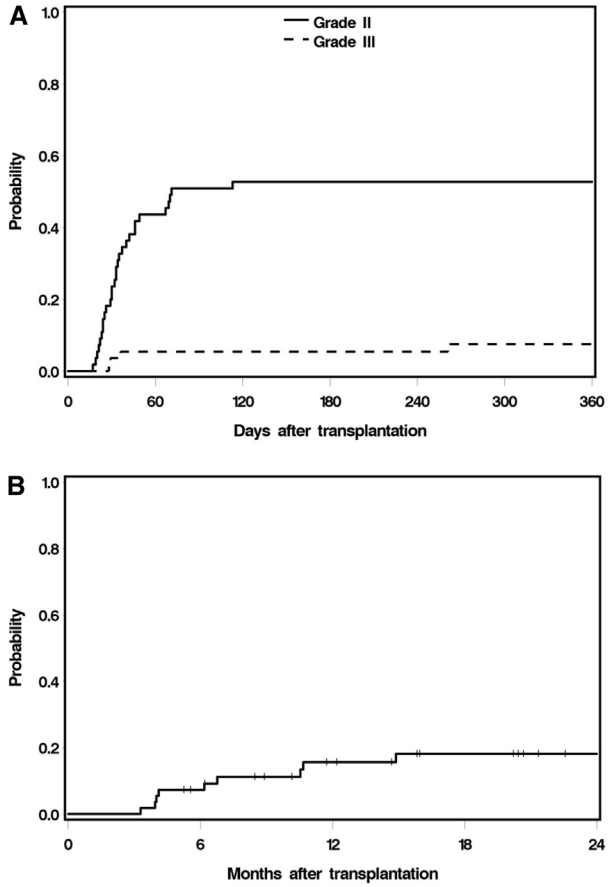

Figure 1. Cumulative Incidence of (A) Grade II and Grade III acute graft-versus-host disease and (B) NIH chronic graft-versus-host disease.

(Figure 1A). No grade IV acute GVHD was observed. The median time to onset of acute GVHD was 33 days. Two patients developed late acute GVHD of the skin and gut at 113 and 261 days, respectively. Twenty-nine patients developed grade II acute GVHD, most frequently involving skin and lower gut in 15 patients. Seven of the 29 patients developed mild stage I GVHD of the upper gastrointestinal tract confirmed by endoscopic biopsy [19] that resolved completely after a short course of prednisone and beclomethasone [20]. Six of these patients were diagnosed in the Seattle cohort. Four patients developed grade III acute GVHD; skin and gut were involved in 1 patient; skin, gut and liver in 2 patients; and gut and liver in 1 patient. Twenty-three of 33 patients developing acute GVHD were treated with systemic steroids. All patients responded to initial therapy; 5 patients responded to .5 mg/kg of steroids, 9 patients responded to 1 mg/kg of steroids, and 9 patients responded to 2 mg/kg of steroids, including the 4 patients with grade III GVHD.

The cumulative incidence of chronic GHVD was 16% (95% CI, 6% to 26%) at 1 year and 18% (95% CI, 7% to 29%) at 2 years (Figure 1B). Nine cases of chronic GVHD were observed. Four cases were mild in severity, involving eye, gut, or liver. Three cases were moderate, with 1 involving the skin, 1 involving the lungs, and 1 involving serosal surfaces. Two cases were severe, with 1 involving the lungs and 1 involving the eye, skin, and liver. One patient experienced immune myopathy and neuropathy at day +40, consistent with the diagnosis of

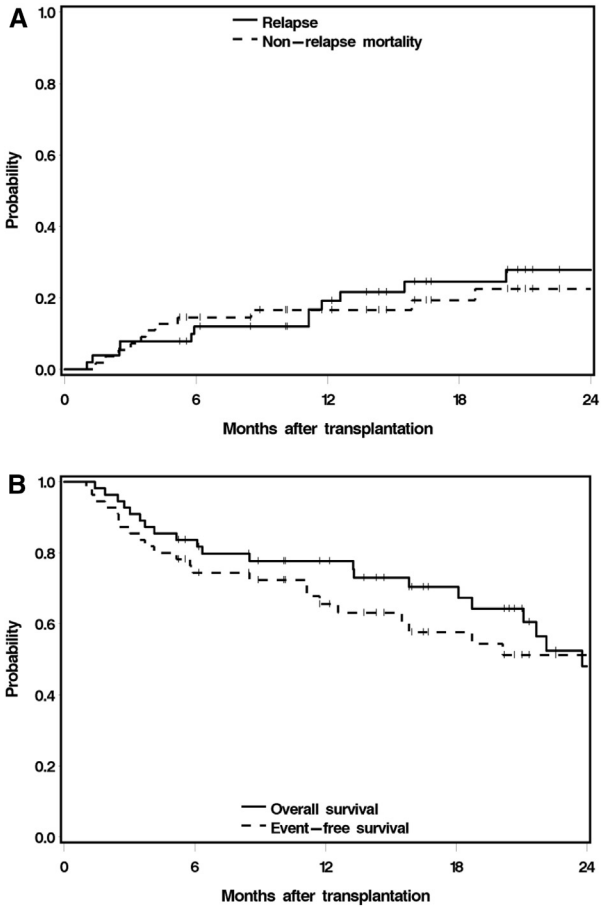

Figure 2. (A) Cumulative incidence of relapse and nonrelapse mortality. (B) Overall and event-free survival at 2 years after transplantation.

Guillain-Barre syndrome that resolved after treatment with steroids and i.v. immunoglobulin.

NRM and Relapse

The incidence of NRM was 17% (95% CI, 7% to 27%) at 1 year and 23% (95% CI, 10% to 35%) at 2 years after transplantation (Figure 2A). There were 12 deaths from nonrelapse causes. One patient died from complications associated with GVHD and 1 patient died from diffuse alveolar hemorrhage. There were 10 deaths from infections, including 4 viral infections (parainfluenza, n = 2; adenovirus, n = 2), 4 bacterial infections (*P. aerugenosa*, n = 2; combined *E. coli* and *K. pneumonia*, n = 1; and *R. planticola*, n = 1), 1 yeast infection (*S. cerevisae*), and 1 likely infection of unclear etiology. As shown in Figure 2A, the cumulative incidence of relapse (excluding the 4 patients with SAA) at 2 years after transplantation was 28% (95% CI, 14% to 42%). Relapse occurred in 12 patients (acute T leukemia lymphoma, n = 3; acute myeloid leukemia, n = 3; HIV-associated non-Hodgkin lymphoma, n = 1; anaplastic large cell lymphoma, n = 1; chronic lymphoblastic leukemia, n = 1; Hodgkin lymphoma, n = 2; chronic myeloid leukemia-second chronic phase, n = 1).

OS and EFS

The median follow-up of surviving patients was 509 (range, 160 to 1203) days. OS and EFS at 1 year after transplantation were 78% (95% CI, 64% to 87%) and 66% (95% CI, 51% to 77%), respectively (Figure 2B). At 2 years after transplantation, OS and EFS were similar at 48% (95% CI, 30% to 64%) and 51% (95% CI, 35% to 65%), respectively. The OS and EFS curves appear to cross at 2 years due to small sample variability (n = 11). There were no significant differences in EFS or OS between patients with myeloid malignancies compared with patients with lymphoid malignancies (data not shown).

DISCUSSION

We have shown that substituting PBSC for BM as the graft source in the Hopkins protocol for haploidentical transplantation after RIC is feasible and does not adversely affect outcomes. Our results suggest that BM or PBSC could be used interchangeably as allograft sources for haploidentical transplantation using this regimen.

The fixed dose of $CD34^+$ cells in the PBSC allograft (5 to 6×10^6/kg) was chosen for 2 reasons. Firstly, it approximated the median $CD34^+$ cell dose in BM allografts reported previously for this particular haploidentical protocol [4]. Secondly, it standardized the T cell dose, since an increased number of T cells in PBSC products compared with BM products has been associated with increased rates of acute and chronic GVHD in the setting of HLA-matched related [7,10,11] or unrelated donors [12,13]. In this study, the mean number of $CD3^+$ T cells in PBSC allografts (2.0×10^8 $CD3^+$ cells/kg) was about 5-fold higher than that reported previously for BM allografts [4]. The most striking effect of increased T cell dose was an almost 2-fold increase in the rate of grade II GVHD (53% with haplo-PBSC compared with 28% to 32% with haplo-BM [4,5]), which responded completely to steroid therapy in all treated cases.

The incidences of grade III acute GVHD and chronic GVHD were low and similar to those reported previously for haplo-BM [3,4]. Similar low rates of acute and chronic GVHD were also observed in 2 small studies of haplo-PBSC transplantation after myeloablative conditioning. The first involved a 2-step approach to haploidentical transplantation, in which patients were conditioned with high-dose total body irradiation followed by donor lymphocyte infusion of 2×10^8 $CD3^+$ cells/kg (the same dose as in this study) and then high-dose Cy before infusion of $CD34^+$-selected cells from a filgrastim-mobilized PBSC donor [21]. In the second study, patients were conditioned with busulfan, fludarabine, and Cy followed by a PBSC allograft targeted at 5×10^6 CD34 cells/kg and post-transplantation Cy, tacrolimus, and MMF as in this study [22].

When compared with the haplo-BM protocol of RIC, rates of nonengraftment (4% for haplo-PBSC, 2% to 16% for haplo-BM [4,5,14]) and of neutrophil and platelet recovery (median 17 and 21 days, respectively, for haplo-PBSC; 16 and 24 days, respectively, for haplo-BM [4,5]) were similar. Sustained engraftment was achieved in the 5 patients where haplo-PBSC was used as salvage for graft failure after prior allogeneic transplantation from matched sibling, matched unrelated, or double umbilical cord blood donors. Sustained engraftment also was achieved in 5 patients with myelodysplastic syndrome and in 4 patients with SAA, which has not been reported previously for haplo-BM using this regimen.

With a median follow-up for surviving patients of 17 months, the 2-year cumulative incidence of NRM was 23% for haplo-PBSC compared with 16% for haplo-BM [4]; the 2-year cumulative incidence of relapse was 28% for haplo-PBSC compared with 58% for haplo-BM [4]; the 2-year probability of EFS was 51% for haplo-PBSC compared with 26% for haplo-BM [4] and the 2-year probability of OS was

also 48% for haplo-PBSC compared with 36% for haplo-BM [4]. For a comparable number of patients who underwent transplantation in 4 separate centers using haplo-PBSC compared with the multicenter study conducted by the Blood and Marrow Transplant Clinical Trials Network using haplo-BM [5], it appeared that transplantation outcomes of patients with high-risk hematologic disorders after transplantation with haplo-PBSC were comparable to those after transplantation with haplo-BM.

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