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HLA-Haploidentical Peripheral Blood Stem Cell Transplantation with Post-Transplant Cyclophosphamide after Busulfan-Containing Reduced-Intensity Conditioning

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A B S T R A C T

Allogeneic hematopoietic stem cell transplantation (allo-SCT) using post-transplant cyclophosphamide (PTCy) is increasingly performed. We conducted a multicenter phase II study to evaluate the safety and efficacy of PTCy-based HLA-haploidentical peripheral blood stem cell transplantation (PTCy-haploPBSCT) after busulfan-containing reduced-intensity conditioning. Thirty-one patients were enrolled; 61% patients were not in remission and 42% patients had a history of prior allo-SCT. Neutrophil engraftment was achieved in 87% patients with a median of 19 days. The cumulative incidence of grades II to IV and III to IV acute graft-versus-host disease (GVHD) and chronic GVHD at 1 year were 23%, 3%, and 15%, respectively. No patients developed severe chronic GVHD. Day 100 nonrelapse mortality (NRM) rate was 19.4%. Overall survival, relapse, and disease-free survival rates were 45%, 45%, and 34%, respectively, at 1 year. Subgroup analysis showed that patients who had a history of prior allo-SCT had lower engraftment, higher NRM, and lower overall survival than those not receiving a prior allo-SCT. Our results suggest that PTCy-haploPBSCT after busulfan-containing reduced-intensity conditioning achieved low incidences of acute and chronic GVHD and NRM and stable donor engraftment and low NRM, particularly in patients without a history of prior allo-SCT.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (allo-SCT) is a potentially curative treatment for patients with hematological malignancies. HLA-matched related or unrelated donors are the first choice but are not always available for all patients. Alternative donors who share a single HLA haplotype (HLA-haploidentical donors) with recipients are nearly always available, but HLA-haploidentical SCT (haploSCT) is associated with high incidences of graft-versus-host disease (GVHD) and graft rejection [1-3]. Over the last few decades, several strategies have been developed to overcome HLA barriers in haploSCT.

The most popular strategy has been T cell depletion of peripheral blood stem cell (PBSC) grafts mostly by immunomagnetic CD34-positive selection [4]. However, this strategy is associated with an increased risk of severe opportunistic infections and nonrelapse mortality (NRM) early after haploSCT.

More recently, T cell–replete haploSCT has been developed by using post-transplant cyclophosphamide

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(PTCy) [5-9]. The rationale of this strategy is selective depletion of alloreactive T cells, as demonstrated in mouse studies [10-13]. Remarkably, the incidence of acute GVHD, chronic GVHD, and NRM after PTCy-based haploidentical bone marrow transplantation (PTCy-haploBMT) after reduced-intensity conditioning (RIC) appears to be equivalent to those after HLA-matched SCT [5,14]. However, relapse remains a major problem after PTCy-haploBMT with RIC.

In an attempt to decrease relapse, we added busulfan (BU) to the original nonmyeloablative platform consisting of fludarabine (Flu), cyclophosphamide (Cy), and 2 Gy total body irradiation (TBI) developed by Luznik et al. [5]. In addition, PBSC grafts were used instead of bone marrow grafts. Because of the greater content of T cells in PBSC grafts compared with bone marrow grafts, PBSC transplantation (PBSCT) may be associated with better engraftment and disease control [15,16]. Herein we report the results of a prospective, multicenter, phase II study to evaluate the safety and efficacy of PTCy-based HLA-haploidentical PBSCT (PTCy-haploPBSCT) after BU-containing RIC.

METHODS

Study Design

This prospective, multicenter, phase II study (UMIN-CTR UMIN000010316) was conducted by the Japan Study Group for Cell Therapy and Transplantation. Patients from ages 15 to 65 with hematological malignancies who had no HLA-matched related or unrelated available donors were enrolled. The institutional review board of each participating institution approved the study protocol, and written informed consent was obtained from all patients in accordance with the Declaration of Helsinki.

The primary endpoint was the incidence of NRM at 100 days. The expected NRM was estimated to be 30% and the threshold NRM, determined according to previous studies [17], was set to be 60%. Secondary endpoints included overall survival (OS), disease-free survival (DFS), the incidence of engraftment, acute GVHD, chronic GVHD, relapse, and incidence of noninfectious fever early after PTCy-haploPBSCT. The required sample size was 19 eligible patients for an 80% power to detect a 30% difference in the NRM at 100 days from the threshold with a 1-sided Type I error of .05. The planned sample size was 21 with the expectation that 10% would be ineligible.

Eligibility Criteria

Eligibility criteria were as follows: ages 15 to 65 years, diagnosis of hematological malignancies, a good performance status (Eastern Cooperative Oncology Group performance status of 0 to 2), and good organ function (bilirubin < 2.0 mg/dL, aspartate/alanine aminotransferases < 3 times upper limit of normal, creatinine clearance > 30 mL/min, cardiac ejection fraction $> 50\%$, and peripheral capillary oxygen saturation (SpO_2) at room air $> 95\%$). Patients who had antibodies targeting mismatched donor HLAs (donor-specific antibodies) were excluded.

Conditioning Regimens and GVHD Prophylaxis

The conditioning regimen consisted of Flu (30 mg/m^2/day on days -6 to -2), Cy (14.5 mg/kg/day on days -6 and -5), BU (3.2 mg/kg/day on days -3 and -2), and TBI (2 Gy on day -1). GVHD prophylaxis consisted of Cy (50 mg/kg/day on days 3 and 4), tacrolimus (days 5 to 180), and mycophenolate mofetil (days 5 to 60).

Definitions

Disease risk of the patients was determined according to the refined Disease Risk Index (DRI) [18]. Neutrophil engraftment was defined as an absolute neutrophil count of $.5 \times 10^9$/L for 3 consecutive days. Platelet engraftment was defined as a platelet count $> 20 \times 10^9$/L without transfusion for the 7 preceding days. Time to engraftment was defined as the time required to reach the first day of engraftment. Acute and chronic GVHD were defined and graded according to standard criteria [19,20]. Time to relapse/progressive disease was calculated from PBSCT to the day of the documented event. OS was calculated from the day of PBSCT, with patients alive at the time of last follow-up censored. NRM was defined as death due to any cause of death without relapse. Noninfectious fever was defined as fever ($>38^\circ$C) without clinical or microbiological documentation of infection.

Statistical Analysis

The primary endpoint was the incidence of NRM at 100 days. Cumulative incidence curves were used in a competing-risks setting to calculate the probabilities of neutrophil recovery, acute and chronic GVHD, relapse, and NRM. For neutrophil recovery, competing events were death or relapse before engraftment [21]. For GVHD, death without GVHD and relapse were competing events. For relapse, death without relapse was the competing event, and for NRM, relapse was the competing event. Unadjusted OS and event-free survival were estimated by Kaplan-Meier curves. Differences in NRM and OS between subgroups were evaluated using Gray's test and a log-rank test, respectively [22]. $P < .05$ is considered as significant. All statistical analyses were performed with EZR [23].

Table 1
Patient Characteristics

Characteristic	Value
Median age, yr (range)	48 (21-65)
Sex, n (%)	
Male	22 (71.0)
Female	9 (29.0)
Diagnosis, n (%)	
Acute myeloid leukemia	17 (54.8)
Acute lymphoblastic leukemia/ lymphoblastic lymphoma	8 (25.8)
Myelodysplastic syndrome/ myeloproliferative neoplasms	4 (12.9)
Diffuse large B cell lymphoma	1 (3.2)
Follicular lymphoma	1 (3.2)
Disease status, n (%)	
CR1	7 (22.6)
CR2 or subsequent CR	5 (16.1)
Not in remission	19 (61.3)
Refined DRI, n (%)	
Very high	11 (35.5)
High	11 (35.5)
Intermediate	8 (25.8)
Low	1 (3.2)
HCT-CI, n (%)	
0	19 (61.3)
1-2	9 (29.0)
3-4	3 (9.7)
History of prior allo-SCT, n (%)	
No	18 (58.1)
Yes	13 (41.9)
Median duration from diagnosis to haploPBSCT, mo (range)	12 (3-72)

CR1 indicates first complete remission; CR2, second complete remission; CR, complete remission; HCT-CI, hematopoietic cell transplantation–specific comorbidity index.

Table 2
Donor and Graft Characteristics

Characteristic	Value
HLA match	
(GVH direction)	
4/8	18 (58.1%)
5/8	12 (38.7%)
6/8	1 (3.2%)
(HVG direction)	
4/8	20 (64.5%)
5/8	9 (29.0%)
6/8	2 (6.5%)
Donor relationship	
Parent	7 (22.6%)
Sibling	9 (29.0%)
Child	14 (45.1%)
Other	1 (3.2%)
Cytomegalovirus serostatus	
D+R+	24 (77.4%)
D−R+	5 (16.1%)
D+R−	1 (3.2%)
D−R−	0 (0%)
NA	1 (3.2%)
Cell dose, median (range)	
CD34 ($\times 10^6$/kg)	4.0 (1.4-10.5)

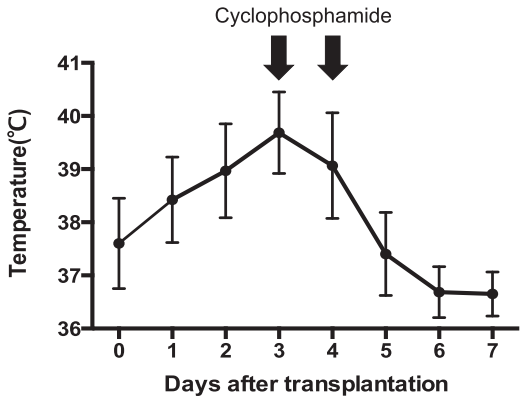

Figure 1. Time course of body temperature early after haploPBSCT. Data represent mean ± standard deviation of the maximum temperature of each day.

RESULTS

Patient Characteristics

Patient characteristics are summarized in Table 1. Thirty-one patients with a median age of 48 (range, 21 to 65) were enrolled in this study between May 2013 and April 2014. Pateitn diagnoses included acute myeloid leukemia (n = 17), acute lymphoblastic leukemia/lymphoblastic lymphoma (n = 8), myelodysplastic syndrome/myeloproliferative neoplasms (n = 4), and lymphoma (n = 2). According to the refined DRI [18], patients were classified as low risk (n = 1), intermediate risk (n = 8), high risk (n = 11), and very high risk (n = 11). Nineteen patients (61%) were not in remission, and 13 patients (42%) had a history of prior allo-SCT once (n = 10) or twice (n = 3). For these patients, haploPBSCT was undertaken for relapse. Transplantation-related donor and recipient information is shown in Table 2. The median number of $CD34^+$ cells was 4.0×10^6/kg (range, 1.4 to 10.5).

Noninfectious Fever Early after HaploPBSCT

All patients developed high fever without evidence of documented infection with a peak at day 3 and quickly resolved after administration of PTCy (Figure 1). No patients received corticosteroids before administration of PTCy.

Engraftment

Neutrophil engraftment was achieved in 27 patients (87%) at a median of 19 days (range, 15 to 27) (Figure 2A). The median time to platelet engraftment was 35 days (range, 13 to 224) (Figure 2B). Peripheral blood or bone marrow samples were collected on days 30 post-transplant, and donor chimerism of whole nucleated cells and $CD3^+$ cells were determined. Complete donor chimerism of both whole cells and $CD3^+$ cells defined by >95% donor chimerism was achieved in all patients. Four patients (13%) were not engrafted. Two patients died before neutrophil recovery because of sepsis on day 17 or sinusoidal obstructive disease on day 34. One patient relapsed after PBSCT, and 1 patient achieved engraftment after rescue transplantation.

Acute and Chronic GVHD

The cumulative incidence of grades II to IV and III to IV acute GVHD at 100 days was 23% (95% confidence interval [CI], 10% to 39%) and 3% (95% CI, 0% to 14%), respectively (Figure 3A). Cumulative incidence of chronic GVHD was 15% (95% CI, 4% to 32%) at 1 year (Figure 3B). One patient developed mild chronic GVHD, and 2 patients developed moderate chronic GVHD. No patients developed severe chronic GVHD.

NRM and Relapse

Rates of NRM were 19% (95% CI, 8% to 35%) at 100 days and 23% (95% CI, 10% to 39%) at 1 year (Figure 4A). Cumulative incidence of relapse was 19% (95% CI, 8% to 35%) at 100 days and 45% (95% CI, 25% to 64) at 1 year (Figure 4A).

OS, DFS, and Cause of Death

After a median follow-up of 287 days (range, 226 to 517), 15 patients (48%) were alive. Rates of OS were 74% (95% CI, 55% to 86%) at 100 days and 45% (95% CI, 26% to 62%) at 1 year. Rates of DFS were 62% (95% CI, 42% to 76%) at 100 days and 34% (95% CI, 16% to 53%) at 1 year (Figure 4B). Nine patients (29%) died of relapse and 7 patients (23%) died of NRM (Table 3).

Engraftment, NRM, and OS According to the History of Prior Allo-SCT

Subgroup analysis showed that patients who had a history of prior allo-SCT (n = 13) had lower engraftment (69% versus 100%, $P = .04$; Figure 5A), higher NRM (39% versus 11%, $P = .07$; Figure 5B), and lower OS (29% versus 56%, $P = .05$; Figure 5C) than those without a history of prior allo-SCT (n = 18). In 8 patients who had no prior allo-SCT and were transplanted in remission, 1 patient (13%) died of NRM, and 2 patients (25%) relapsed. In 10 patients who had no prior history of prior SCT and were not transplanted in remission, 1 patient (10%) died of NRM and 7 patients (70%)

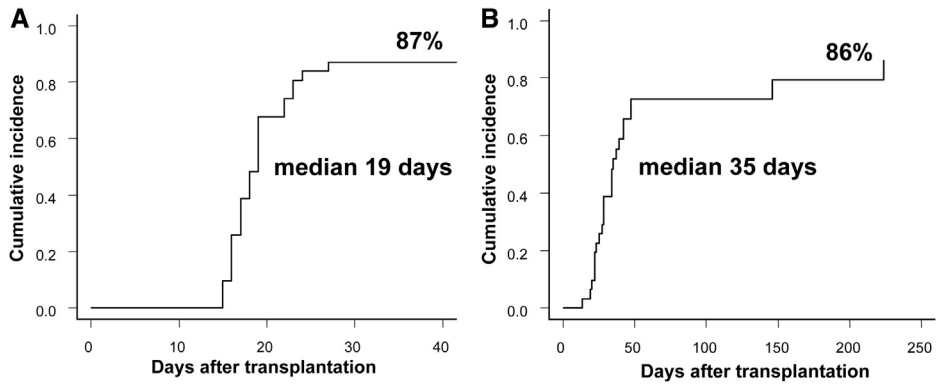

Figure 2. Engraftment. Cumulative incidences are shown for (A) neutrophil engraftment and (B) platelet engraftment.

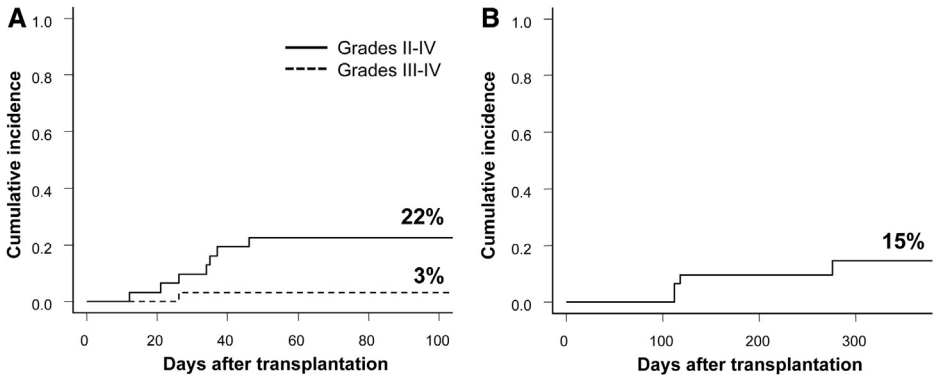

Figure 3. GVHD. Cumulative incidences are shown for (A) grades II to IV (*solid line*) and grades III to IV (*dashed line*) acute GVHD and (B) chronic GVHD.

relapsed. In 4 patients who had a history of prior allo-SCT and were transplanted in remission, 2 patients (50%) died of NRM and 2 patients (50%) relapsed. In 9 patients who had a history of prior allo-SCT and were not transplanted in remission, 3 patients (33%) died of NRM and 4 patients (57%) relapsed.

Relapse, DFS, and OS According to the Refined DRI

Nine patients (29%) were classified as low/intermediate risk and 22 patients (71%) as high/very high risk according to the refined DRI [18]. The incidences of relapse at 1 year were 0% in patients with low/intermediate risk and 61% in patients with high/very high risk. The incidences of OS and DFS at 1 year were 67% and 67%, respectively, in patients with low/intermediate risk and 37% and 22%, respectively, in patients with high/very high risk.

DISCUSSION

Luznik et al. [5,12] pioneered a novel GVHD prophylaxis using PTCy in the haploidentical setting based on animal studies on tolerance induction by PTCy. Their initial protocols used a nonmyeloablative regimen and bone marrow as the source of hematopoietic stem cells. This is because a combination of HLA-mismatch, myeloablative conditioning, and PBSCT represents the highest risk for acute GVHD [24,25]. Although PTCy-haploBMT after a nonmyeloablative regimen ensures good GVHD controls, relapse remains a problem. We developed a strategy in which BU was added to the Johns Hopkins' nonmyeloablative regimen and PBSCs were used as the stem cell source in the setting of PTCy-haploSCT to augment disease control and prospectively demonstrated that this strategy was feasible and safe.

Several groups have developed PTCy-haploSCT after BU-based myeloablative conditioning. Solomon et al. [26] reported the results of PTCy-haploPBSCT after myeloablative conditioning consisting of Flu (125 to 180 mg/m^2), Cy (29 mg/kg), and BU (440 to 520 mg/m^2). The incidences of relapse, DFS, and OS at 1 year were 40%, 50%, and 69%, respectively. Raiola et al. [27] reported the result of PTCy-haploBMT after myeloablative conditioning consisting of Flu (150 mg/m^2), BU (3.2 to 9.6 mg/kg), and thiotepa (10 mg/kg) or Flu (120 mg/m^2) and TBI (9.9 to 12 Gy). The incidences of relapse, DFS, and OS at 18 months were 22%, 51%, and 62%, respectively. Our study used RIC regimen containing BU (6.4 mg/kg) and included larger numbers of patients not in remission and those with a history of prior allo-SCT than other studies; therefore, it is not surprising that a 1-year relapse rate of 45% was higher than that of other studies. Nonetheless, 1-year DFS and OS rates of 34% and 45%, respectively, appear to be lower than in other studies, suggesting that the use of a RIC regimen in the setting of PTCy-haploPBSCT is associated with increased relapse but reduced treatment-related mortality. The myeloablative dose used in the BU-based regimen, however, is being increasingly studied in the setting of PTCy-haploSCT [26,27].

GVHD prophylaxis using PTCy has been tested by several groups in the setting of haploPBSCT [26,28-30]. In these studies engraftment was achieved in 94% to 100%, and the incidences of grades II to IV acute GVHD were 33% to 53%. In general, it seems so far that PBSCT is associated with an

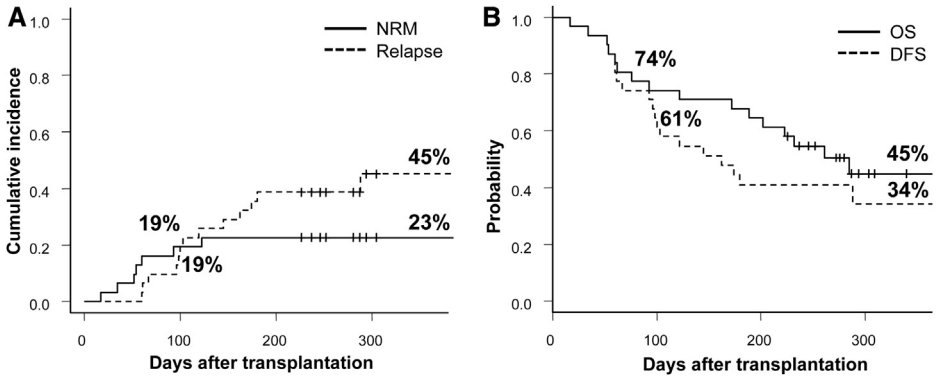

Figure 4. NRM, relapse, OS, and DFS. Cumulative incidences are shown for (A) NRM (*solid line*) and relapse (*dashed line*). Probabilities are shown for (B) OS (*solid line*) and DFS (*dashed line*).

Table 3
Causes of Death

Causes of Death	First SCT	Second/Third SCT	Total
Relapse	5	4	9
NRM			
Sinusoidal obstruction syndrome	0	2	2
Acute respiratory distress syndrome	0	1	1
Infection	1	1	2
Multiple organ failure	1	1	2

improved engraftment rate but a higher incidence of acute GVHD when compared with PTCy-haploBMT [5]. In our study all patients without a history of prior SCT achieved engraftment. The incidence of GVHD was lower than that from other studies of PTCy-haploPBSCT (grades II to IV acute GVHD, 23% versus 33% to 53%) [26,28-30]. This may be due to a longer duration of mycophenolate mofetil administration of 60 days in our study versus 35 days in the others and/or genetic homogeneity in the Japanese population. The incidence of chronic GVHD was remarkably low (15%), similar to previous reports of PTCy-haploBMT [5]. Furthermore, the absence of severe chronic GVHD was noteworthy, although a median follow-up of 287 days was not sufficient to assess the incidence of chronic GVHD.

Noninfectious fever developed in all patients with a peak at day 3 but was quickly relieved by PTCy administration. Noninfectious fever is the major manifestation of haploimmunostorm syndrome seen in HLA-mismatched cellular therapy and is associated with vigorous proliferation of alloreactive T cells and subsequent inflammatory milieu in the absence of post-transplant immunosuppressants until day 5 despite HLA haplotype mismatch [31]. This syndrome is likely to be more frequent after PTCy-haploPBSCT than PTCy-haploBMT [26,32] and as frequent after PTCy-based HLA-matched BMT [32], suggesting that haploPBSCT is a risk for haploimmunostorm syndrome. Interestingly, Colvin et al. [33] suggested that haploimmunostorm syndrome conferred an antitumor effect [33]. It is intriguing to determine the impact of haploimmune syndrome on disease control in the setting of PTCy-haploPBSCT. Administration of immunosuppressants before PTCy administration may theoretically diminish the effect of PTCy [34]. However, this hypothesis has never been tested clinically, and in fact tacrolimus was given before PTCy in several studies [27].

The cumulative incidence of relapse was 45% at 1 year in our study. Despite the fact that our cohort included 61% of patients not in remission and 42% of patients having a history of prior allo-SCT, the relapse rate was equivalent to that from previous studies of PTCy-haploBMT, in which most patients were in remission without a history of prior allo-SCT [5]. In our study only 2 of 8 patients relapsed, who received PTCy-haploPBSCT in remission without prior history of allo-SCT. Recently, McCurdy et al. [35] reported the risk-stratified outcomes of PTCy-haploBMT after nonmyeloablative conditioning using a refined DRI. Cumulative incidences of 1-year relapse were 17%, 38%, and 58% in low, intermediate, and high/very high risk patients, respectively. In our study, although a 61% relapse rate of high-risk patients was equivalent, 0% relapse rate of low/intermediate risk patients was lower compared with McCurdy's study. A combination of RIC and PBSCT in the setting of PTCy-haploSCT might be promising to reduce relapse in patients with low/intermediate risk, but efficacy of this strategy needs to be investigated in a larger study involving a larger number of a more

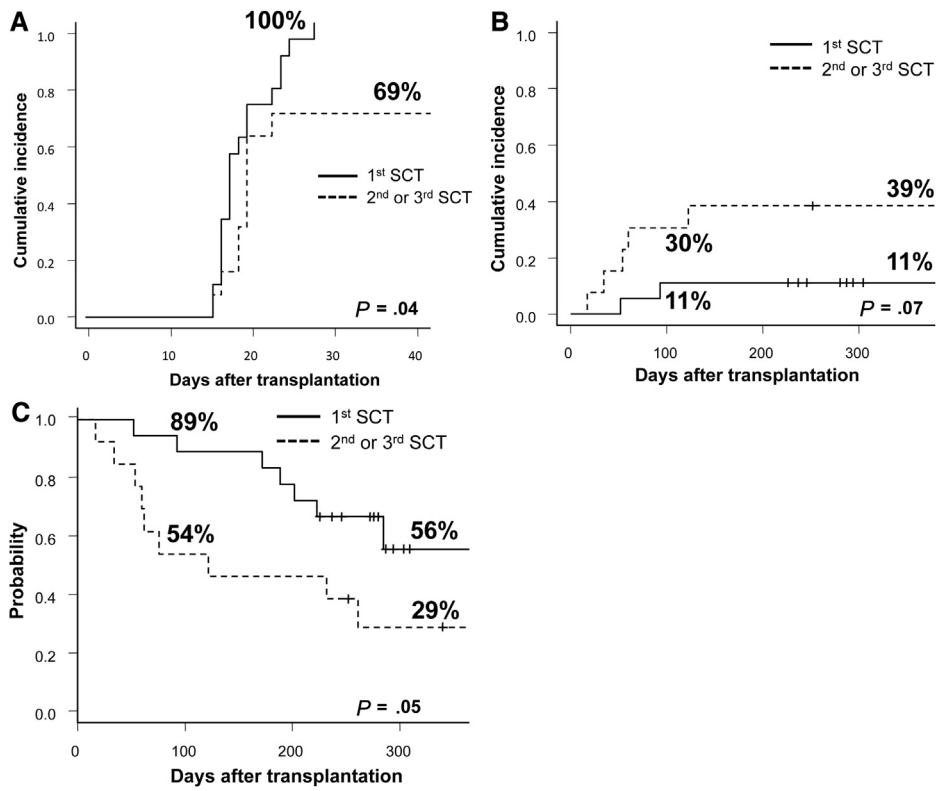

Figure 5. Engraftment, NRM, and OS according to the history of prior allo-SCT. (A) Cumulative incidence of neutrophil engraftment. (B) Cumulative incidence of NRM. (C) Probability of OS.

homogeneous population such as acute myeloid leukemia in remission. However, for patients with advanced disease, a myeloablative conditioning regimen may provide better disease control, given high rates of relapse in these patients.

A unique aspect of this study was the inclusion of a large proportion of patients with a history of prior allo-SCT. Because most previous studies of PTCy-haploSCT did not include patients with a history of prior allo-SCT [5,26,27], little data regarding outcomes of PTCy-haploSCT used as a second or third allo-SCT are available in literature. Remarkably, 31% of such patients failed to achieve engraftment, and NRM reached 33%. On the other hand, all patients without a history of prior allo-SCT achieved sustained engraftment and NRM was only 11%, which was comparable with reported incidences of 15% NRM in PTCy-haploBMT [5]. High incidence of graft failure in patients with a history of prior allo-SCT suggests that recipient T cells derived from prior SCT donors are associated with rejection. Notably, 3 of 4 patients who experienced graft rejection had a history of prior SCT from HLA-mismatched donors and had developed GVHD after a prior SCT, suggesting that host-reactive memory T cells derived from the previous donor were associated with rejection of PBSCs from a haploidentical family donor who shared mismatched HLA antigens with recipients. This is still speculative but may be supported by a recent study demonstrating that antigen-specific memory T cells survive PTCy [36]. Thus, outcomes of PTCy-haploPBSCT in patients who had a history of prior allo-SCT are not satisfactory, as in other transplant modalities. However, the numbers of patients are small, and the results of our subgroup analysis should be confirmed in larger studies.

In conclusion, our results suggest that PTCy-haploPBSCT after BU-containing RIC achieves stable donor engraftment and low incidences of acute and chronic GVHD. NRM and the incidence of GVHD were remarkably low; particularly, there was no extensive chronic GVHD. Given the promising results of GVHD and NRM, we are now conducting a phase II study of PTCy-haploPBSCT using myeloablative conditioning in patients without prior allo-SCT.

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