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A Prospective Study of an HLA-Haploidentical Peripheral Blood Stem Cell Transplantation Regimen Based on Modification of the Dose of Posttransplant Cyclophosphamide for Poor Prognosis or Refractory Hematological Malignancies

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Abstract

The optimal dose of posttransplant cyclophosphamide (PTCy) for use in patients undergoing HLA-haploidentical hematopoietic cell transplantation with posttransplant cyclophosphamide (PTCy-haplo) has not been sufficiently examined. This study evaluates the safety and efficacy of HLA-haploidentical hematopoietic cell transplantation with a reduced dose of PTCy for patients with a poor prognosis or those with refractory hematological malignancies. We conducted a prospective clinical study of PTCy-haplo with peripheral blood stem cells (PBSCs) using a modified PTCy dosage regimen consisting of 50 mg/kg on day 3 posttransplantation and a reduced dose of 25 mg/kg on day 4. The cumulative incidences of grades II to III and IV acute graft-versus-host disease (GVHD) at day 100 posttransplantation were 30% and 0%, respectively. The cumulative incidence of moderate-to-severe chronic GVHD after transplantation was 7.0%. The cumulative incidence of nonrelapse mortality at 1 year posttransplantation was 6.1%. Overall survival (OS) at 1 year was 66%. In addition, the restricted cubic-spline Cox regression analysis showed nonlinear relationship between the number of infused $CD34^+$ cells and $CD3^+$ cells, and OS. A graft composition of $>4.54 \times 10^6$/kg $CD34^+$ cells and $>1.85 \times 10^8$/kg but $\leq 3.70 \times 10^8$/kg $CD3^+$ cells was significantly associated with better survival, irrespective of the disease status (hazard ratio, 0.13; 95% confidence interval, 0.04–0.41; $P < 0.001$). These results suggest that PTCy-haplo with PBSCs using a de-escalated dose of 50 mg/kg on day 3 and 25 mg/kg on day 4 posttransplantation is a feasible option.

Keywords

peripheral blood stem cells (PBSCs), HLA-haploidentical hematopoietic cell transplantation, a reduced dose of posttransplant cyclophosphamide (PTCy), graft cellular composition

Introduction

Allogeneic hematopoietic cell transplantation with posttransplant cyclophosphamide from an HLA-haploidentical related donor is used worldwide, particularly for patients lacking an HLA-matched donor[1,2]. Several recent meta-analyses demonstrate that the risk of chronic graft-versus-host disease (GVHD) after HLA-haploidentical hematopoietic cell transplantation with posttransplant cyclophosphamide (PTCy-haplo) was significantly lower than that after HLA-matched transplantation[3–6]. Therefore, it may not be an overstatement that PTCy-haplo is becoming the preferred platform for

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HLA-haploidentical hematopoietic cell transplantation due to its ease and feasibility. However, the risk of relapse after PTCy-haplo is higher than after transplantation from an HLA-matched unrelated donor (MUD)[6]. It was also shown, in a murine model, that PTCy profoundly decreased graft-versus-leukemia (GVL) effects and that it could lead to rapid relapse[7]. Furthermore, the alloreactivity of natural killer (NK) cells plays a crucial role in the GVL effects after PTCy-haplo[8–11]; however, the posttransplant recovery of NK cells may be suppressed by PTCy, suggesting that PTCy may reduce the GVL effects mediated by NK cells in the PTCy-haplo setting[11].

Currently, PTCy-haplo is performed by administering 50 mg/kg of cyclophosphamide on days 3 and 4 posttransplantation; however, the optimal dose of PTCy has not been examined clinically. A dose of 50 mg/kg was chosen because it had been used as a high-dose cyclophosphamide therapy for aplastic anemia and is very nearly the maximum tolerable dose in humans[12]. With respect to the timing of PTCy, a previous study in an allogeneic skin transplantation model shows that the administration of high-dose cyclophosphamide on days 2 and 3 prevents graft rejection. To minimize toxicity and maximize the interval from conditioning to PTCy, day 3 was chosen[12]. In addition, a two-center comparison of clinical trials showed that the administration of a double dose (50 mg/kg on days 3 and 4) was associated with a reduced risk of extensive chronic GVHD in comparison with a single dose of 50 mg/kg on day 3[13]. Based on these results, a double dose of 50 mg/kg PTCy has become the standard.

However, a recent experimental study in an MHC-haploidentical murine hematopoietic cell transplantation model demonstrated that PTCy doses of 10 to 50 mg/kg/day on days 3 and 4 prevented fatal GVHD; notably, 25 mg/kg/day PTCy was optimal[14]. Based on these more recent findings, it may be time to conduct a prospective clinical trial and revisit and optimize the procedure for PTCy-haplo to identify an optimal dose that improves the outcome with minimum adverse effects. Furthermore, the optimal dose of PTCy may differ depending on whether PTCy-haplo is performed with peripheral blood stem cells (PBSCs) or bone marrow (BM).

We conducted a prospective clinical study of PTCy-haplo using PBSCs and a modified PTCy dose of 50 mg/kg on day 3, followed by a reduced dose of 25 mg/kg on day 4 posttransplantation for patients with a poor prognosis or those with refractory hematological malignancies.

Patients and Methods

Study Design

This prospective, single-center phase II trial was registered at the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR; identification number UMIN000026028) and the Japan Registry of Clinical Trials (jRCTs051180144). The primary endpoint was the proportion of patients who survived and showed engraftment at day 100 posttransplantation. Secondary endpoints included treatment-related toxicity, the probability of overall survival (OS) and relapse/progression at 1 year posttransplantation, and non-relapse mortality (NRM) at day 100 and 1 year posttransplantation; the rates of engraftment and graft failure; the incidence and severity of acute GVHD and chronic GVHD; infectious complications; immune reconstitution; and the impact of the composition of stem cell and leukocyte subsets in donor grafts on the prognosis. As additional endpoints, we calculated OS at 3 years and relapse/progression-free survival (RFS) after PTCy-haplo using data that had already been collected by the end of follow-up.

This study was approved by the Osaka City University Hospital Certified Review Board and was conducted in accordance with the Clinical Trials Act and the tenets set down in the Declaration of Helsinki, and with the Ethical Guidelines for Medical and Health Research Involving Human Subjects of Japan. All participants provided their written informed consent.

Eligibility Criteria

The inclusion criteria were as follows: (1) patients with a poor prognosis or refractory hematological disorder; (2) patients with no HLA serological identical related donor and HLA-haploidentical donor; (3) age $\geq$15 and $<$70 years; (4) Eastern Cooperative Oncology Group (ECOG) performance status 0–1; (5) major organ function is preserved (total bilirubin level $<$2.0 mg/dl; aspartate aminotransferase/alanine aminotransferase $<3 \times$ upper limit of normal; serum creatinine $<$2.0 mg/dl; left ventricular ejection fraction $\geq$50%; and ratio of vital capacity to the predicted vital capacity $\geq$40%, the ratio of forced expiratory volume in 1 s to forced vital capacity $\geq$50%, or oxygen saturation $\geq$90% without oxygen treatment); and (6) patients who have given their consent to participate in the study.

Conditioning Regimens and GVHD Prophylaxis

The conditioning regimen consisted of fludarabine (15 mg/m^2, twice a day on days –8 and –7, and 30 mg/m^2 once a day on days –6 and –3), cytarabine (2.0 g/m^2 twice a day on days –8 and –7)[15,16], and melphalan (100 mg/m^2 per day on day –2). Cyclophosphamide (50 mg/kg) was given on day 3 and then again (25 mg/kg) on day 4 postgraft infusion. PBSCs were used as the donor source. Acute GVHD prophylaxis consisted of continuous intravenous tacrolimus and oral mycophenolate mofetil (MMF; 1,000 mg 3 times a day) from day 5. Granulocyte colony-stimulating factor (G-CSF) was initiated on day 5. If GVHD did not occur by day 40, MMF was discontinued and the administration of tacrolimus commenced, with tapering between days 60 and 100; it was planned to be discontinued by day 180 (or later at the discretion of the physician).

Definition

The date of neutrophil engraftment was defined as the first day of three consecutive days of evaluation on which the neutrophil count exceeded 0.5×10^9/l. The date of platelet engraftment was defined as the first day of three consecutive days of evaluation on which the platelet count exceeded 20×10^9/l, without the need for platelet transfusion during the preceding 7 days. Acute and chronic GVHD were diagnosed and graded according to the standard criteria[17,18].

OS was defined as the interval from transplantation to death from any cause. RFS was defined as the interval from transplantation to relapse/progression or death from any cause. The refined disease risk index (rDRI) and hematopoietic cell transplantation–specific comorbidity index (HCT-CI) were stratified as described[19,20].

Statistical Analysis

Based on the previous pilot study, the expected and threshold success rates for survival with engraftment at day 100 post-transplantation in this study were estimated to be 72% and 50%, respectively. Using the Minimax method, the required number of patients was set at 30. Simon's two-stage design yielded a one-sided alpha error of 0.05 and power of 0.8. The expectation was that 10% of patients would discontinue treatment or drop out of the study; therefore, the target number of patients was set at 33.

The incidences of neutrophil and platelet engraftment, NRM, relapse/progression, and GVHD following PTCy-haplo were calculated using cumulative incidence estimates. Death without engraftment was treated as a competing event with respect to neutrophil and platelet engraftment. NRM and relapse/progression were treated as mutually competing events. The occurrence of relapse/progression or death without GVHD was treated as competing risks for GVHD.

The probability of OS was estimated using the Kaplan–Meier method. The log-rank test was used to compare groups. A Cox proportional hazards model was used for the univariate and multivariable analyses of factors associated with OS and RFS. The proportionality of hazard assumption was evaluated using scaled Schoenfeld residuals. The Fine–Gray subdistribution hazard model was used to analyze factors associated with relapse/progression. Patient age, disease status, rDRI, HCT-CI, a history of prior transplantation, donor relationship, female-to-male transplantation, and cytomegalovirus (CMV) serology were included in the univariate analysis. Variables showing statistical significance for OS, relapse/progression, or RFS in the univariate analysis were entered in the multivariate analysis.

Restricted cubic-spline tests allowed us to investigate whether the association was nonlinear[21]. We estimated the trend in the risk of the $CD34^+$ and $CD3^+$ cells dose for OS by a restricted cubic-spline Cox regression analysis with three "knots" (cell dose percentiles 10, 50, and 90). The results of the analysis are presented as smoothed plots with 95% confidence intervals (CIs) for the overall risk of OS. The restricted cubic-spline Cox regression analysis revealed a nonlinear association between the infused $CD34^+$ and $CD3^+$ cell dose and OS. Therefore, we evaluated the infused dose of $CD34^+$ and $CD3^+$ cells as tertiles and plotted the time-dependent receiver-operating characteristic (ROC) curves and calculated the best cutoff dosages using the Youden index[22]. The number of $CD34^+$ cells were reciprocally transformed before the ROC curve analysis. As relationship between the numbers of $CD3^+$ cells and OS showed a nonlinear relationship, we performed quadratic transformation of the number of $CD3^+$ cells using the following equation before the time-dependent ROC analysis of the $CD3^+$ cell values: $(x - x \text{ mean value})^2$ (where x: numbers of $CD3^+$cell).

We used R version 4.1.0 and R package rms version 6.2.0 for the restricted cubic spline to assess the nonlinear relationship. We used EZR version 1.54 (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria),[22] and R package survival ROC version 1.0.3 to plot the time-dependent ROC curves and calculate the best cutoff values using the Youden index. Other analyses were also conducted using EZR version 1.54. Factors that were identified as statistically significant in a univariate analysis were entered into the multivariate analysis. All statistical analyses were two-sided and P values of ≤ 0.05 were considered statistically significant.

Results

Patients, Donors, and Graft Characteristics

A total of 34 patients were registered from February 2017 to November 2019. One patient could not receive PTCy due to cardiac failure, which was probably related to toxicity induced by the conditioning regimen. Therefore, this patient was excluded from the analysis of outcomes. The remaining 33 patients, the donors, and the graft characteristics are summarized in Table 1.

The median follow-up period of the surviving patients was 1,045 days. The disease status of 13 (39%) of the 33 patients at the time of transplantation was classified as "non-remission." Eight (24%) had a history of prior allogeneic hematopoietic stem cell transplantation.

Engraftment and Donor-Recipient T-Cell Chimerism and Immune Reconstitution

G-CSF–mobilized, unmanipulated PBSCs were used as a stem cell source for all patients. Infused grafts contained a median of 6.10×10^6/kg $CD34^+$ and 2.44×10^8/kg $CD3^+$ cells. Neutrophil engraftment was achieved in all patients in a median of 17 (range, 12–33) days. Platelet engraftment was achieved

Table 1. Patient, Donor, and Graft Characteristics.

Characteristic	*n* (% or range)		*n* (% or range)
Patient gender (M/F)	20/13	Donor–recipient relationship	
Median age (range) years	47 (19–66)	Parent–child	5 (15%)
Diagnosis		Sibling–sibling	15 (45%)
AML	11 (33%)	Child–parent	13 (39%)
ALL	8 (24%)	Donor–recipient sex mismatch	
MDS	9 (27%)	Match	20 (61%)
CML	2 (6%)	Female to male	7 (21%)
ML	3 (9%)	Male to female	6 (18%)
Nonremission disease	13 (39%)	HLA disparity (antigen, GVH/HVG direction)	
Refined DRI		4/8	9 (27%)/10 (30%)
Low/intermediate	16 (48%)	5/8	18 (55%)/17 (52%)
High/very high	17 (52%)	6/8	3 (9%)/4 (12%)
HCT-CI		7/8	2 (6%)/2 (6%)
0	12 (36%)	8/8	1 (3%)/0 (0%)
1–2	13 (39%)	Infused cell numbers	
≥3	8 (24%)	$CD34^+$ cells ($\times 10^6$/kg)	6.10 (2.45–17.6)
History of prior transplantation	8 (24%)	$CD3^+$ cells ($\times 10^8$/kg)	2.44 (1.15–5.67)
Donor-recipient CMV status			
Positive/positive	23 (70%)		
Positive/negative	5 (15%)		
Negative/positive	3 (9%)		
Negative/negative	2 (6%)		

ALL: acute lymphoblastic leukemia; AML: acute myelogenous leukemia; CML: chronic myeloid leukemia; CMV: cytomegalovirus; DRI: disease risk index; GVH: graft-versus-host; HCT-CI: hematopoietic cell transplantation–specific comorbidity index; HVG: host-versus-graft; MDS: myelodysplastic syndrome; ML: malignant lymphoma.

in 88% of patients in a median 35 (range, 19–95) days. In 91% of patients, complete donor T-cell chimerism was achieved from day 30 ± 7 after transplantation. Overall, 91% of patients survived with graft engraftment at day 100 posttransplantation, which was the primary endpoint of the study. Secondary graft failure was observed in three patients (9%).

The median $CD45^+CD3^+$ T cell, $CD45^+CD3^+CD8^+$ T cell, $CD45^+CD3^+CD4^+$ T cell, $CD45^+CD3^-$ $CD56^+$ NK cell, and $CD45^+CD3^-$ $CD19^+$ B cell counts in peripheral blood at 1 year posttransplantation were 1,294/μl (range, 398–2.783/μl), 849/μl (range, 149–2,086/μl), 354/μl (range, 166–672/μl), 101/μl (range, 40–400/μl), and 459/μl (range, 4–1,303/μl), respectively.

Treatment-Related Toxicity

The major nonhematological treatment-related toxicities that occurred in the first 100 days posttransplantation are listed in Supplemental Table 1. All of the patients experienced febrile neutropenia. Two (6%) of the 33 patients experienced grade ≥3 cardiac failure in the first 28 days posttransplantation. Among the two cases, one patient had grade 4 cardiac failure that was possibly related to PTCy. In the first 28 days posttransplantation, two (6%) patients experienced grade 3 arrhythmia for which a relationship with PTCy could not be denied.

Infectious Complications

We observed cytomegalovirus (CMV) infection after transplantation in 70% patients; however, no CMV disease was observed during the follow-up period. The infectious complications during the first 180 days posttransplantation are summarized in Supplemental Table 2. Three (9%) patients had invasive pulmonary aspergillosis. BK polyomavirus and adenovirus hemorrhagic cystitis occurred in eight (24%) patients and two (6%) patients, respectively. Three of eight patients with BK virus (BKV) hemorrhagic cystitis resolved with only fluid replacement with/without diuretics. Four received Choreito[23] and three received levofloxacin. One patient received continuous urinary bladder drainage and hematoma sequestration due to severe hematoma in the urinary bladder. Another patient received nephrostomy due to the concurrent occurrence of adenovirus-induced cystitis, ureteritis, and nephritis. Eventually, BKV hemorrhagic cystitis resolved in all eight patients. In the other patient, adenovirus overlapping with BKV hemorrhagic cystitis also improved with Choreito.

Acute and Chronic GVHD

The cumulative incidences of grades II to III and III acute GVHD at day 100 posttransplantation were 30% (95% confidence interval [CI], 16%–46%) and 15% (95% CI,

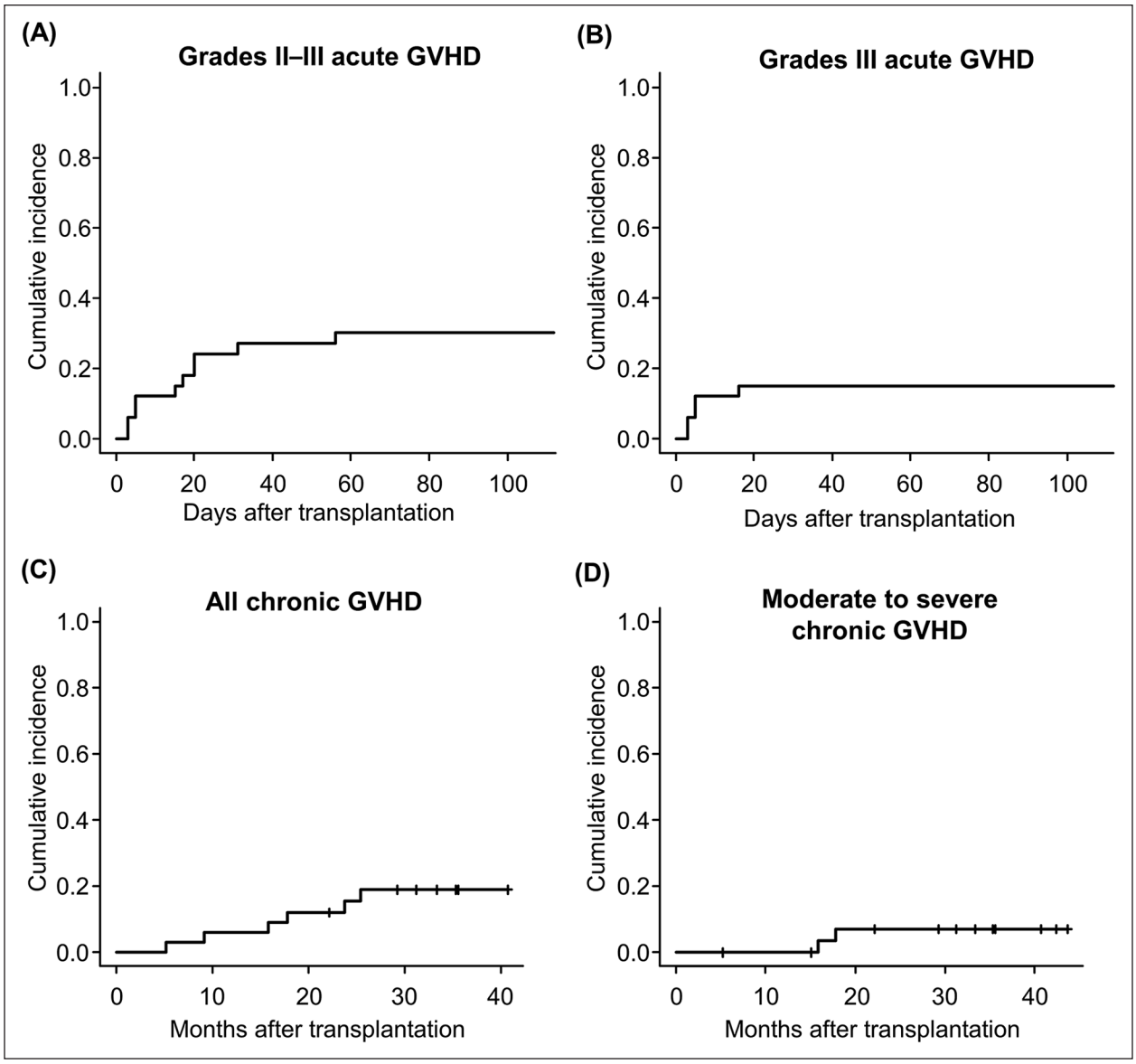

Figure 1. Cumulative incidences of acute GVHD and chronic GVHD. Cumulative incidences of (A) grades II to III and (B) grade III acute GVHD. There was no grade IV acute GVHD after transplantation. Cumulative incidences of (C) all and (D) moderate-to-severe chronic GVHD. GVHD: graft-versus-host disease.

5.4%–30%), respectively (Fig. 1). There was no grade IV acute GVHD after transplantation. The cumulative incidences of all grade and moderate-to-severe chronic GVHD were 19% (95% CI, 7.3%–35%) and 7.0% (95% CI, 1.1%–21%), respectively.

NRM, Relapse/Progression, and Survival

During the follow-up period, 17 patients died. Among them, 15 patients died of primary disease and two patients died of idiopathic pneumonia syndrome and acute GVHD overlapping with infection.

The cumulative incidences of NRM at day 100 and 1 year posttransplantation were 6.1% (95% CI, 1.0%–18%) and 6.1% (95% CI, 1.0%–18%), respectively. The probability of OS at 1 year and 3 years posttransplantation was 66% (95% CI, 47%–80%) and 45% (95% CI, 27%–62%), respectively (Fig. 2). For patients in remission at the time of transplantation, the probability of OS at 1 year posttransplantation was 79% (95% CI, 53%–92%) (Fig. 2). For patients not in remission, the probability of OS at 1 year posttransplantation was 46% (95% CI, 19%–70%). The cumulative incidence of relapse/progression at 1 year posttransplantation was 49% (95% CI, 30%–64%).

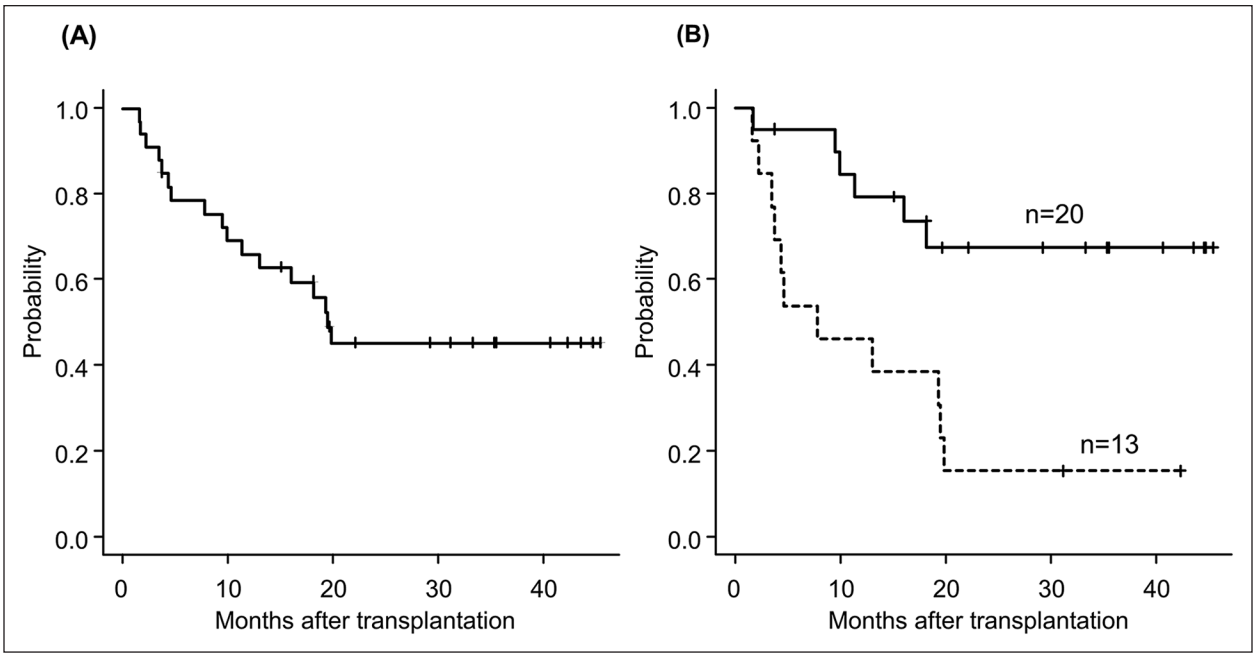

Figure 2. Kaplan–Meier estimates of overall survival (OS) after HLA-haploidentical transplantation with posttransplant cyclophosphamide. (A) OS and (B) OS stratified by remission status. The solid line indicates OS for patients in remission at transplantation. The dash line indicates OS for patients in a state of nonremission at transplantation.

The univariate analysis using a Cox regression proportional model revealed that a disease status of nonremission was significantly associated with worse OS (hazard ratio [HR], 4.2; 95% CI, 1.5–11; $P = 0.0049$). Patient age, high/very high rDRI, HCT-CI, a history of prior transplantation, donor relationship, female-to-male transplantation, and CMV serology were not associated with worse survival. A univariate Fine–Gray subdistribution hazard model showed that a disease status of nonremission and high/very high rDRI were significant risk factors for relapse/progression (HR, 3.8; 95% CI, 1.5–9.9; $P = 0.0062$ and HR, 2.9; 95% CI, 1.2–7.5; $P = 0.024$). Patient age, HCT-CI, a history of prior transplantation, donor relationship, female-to-male transplantation, and CMV serology were not associated with the risk of relapse/progression.

The Impact of the Cellular Composition of the Infused Graft on OS and Relapse/Progression

The restricted cubic-spline Cox regression analysis revealed a significant nonlinear relationship between the number of infused $CD34^+$ cells and $CD3^+$ cells, and OS ($P = 0.020$ and $P = 0.0022$, respectively). In addition, the number of infused $CD3^+$ cells had a statistically significant effect on the HR for OS ($P = 0.0014$). The number of infused $CD34^+$ cells was suggested to affect the HR for OS ($P = 0.062$) (Fig. 3). There was no significant correlation between the number of $CD34^+$ cells and the number of $CD3^+$ cells ($r = -0.0044$, $P = 0.98$). We also evaluated the HR of the infused dose of $CD34^+$ and $CD3^+$ cells for OS as tertiles (Supplemental Table 3). In the ROC analysis, the numbers of infused $CD34^+$ cells and $CD3^+$ cells had a favorable predictive ability for 1 year OS (area under the curve: 0.79 and 0.90, respectively) (Supplemental Figure). We found that the optimal $CD34^+$ cell count/kg threshold was $>4.54 \times 10^6$/kg, and the optimal $CD3^+$ cell count/kg thresholds were 1.85×10^8/kg and 3.70×10^8/kg, respectively.

Infused grafts containing $>4.54 \times 10^6$/kg $CD34^+$ cells were significantly associated with favorable OS and a low risk of relapse/progression (HR, 0.37; 95% CI, 0.14–0.98, $P = 0.046$; and HR, 0.31; 95% CI, 0.12–0.81, $P = 0.016$, respectively) (Supplemental Table 4). In contrast, infused grafts containing $\leq 1.85 \times 10^8$/kg $CD3^+$ cells were significantly associated with poorer OS and a higher risk of relapse/progression (HR, 3.19; 95% CI, 1.16–8.79, $P = 0.025$; HR, 5.44; 95% CI, 1.44–20.4, $P = 0.012$, respectively). Six of seven patients who received $\leq 1.85 \times 10^8$/kg $CD3^+$ cells died of primary disease.

Infused grafts containing $>3.70 \times 10^8$/kg $CD3^+$ cells were also significantly associated with inferior OS (HR, 5.91; 95% CI, 2.00–17.5, $P = 0.0013$) but not with an increased risk of relapse/progression (HR, 1.33; 95% CI, 0.50–3.57, $P = 0.57$). In contrast, infused grafts containing $>1.85 \times 10^8$/kg but $\leq 3.70 \times 10^8$/kg $CD3^+$ cells were significantly associated with better OS and a low risk of relapse/progression (HR, 0.12; 95% CI, 0.04–0.35, $P < 0.001$; and HR, 0.27; 95% CI, 0.11–0.71, $P = 0.0076$, respectively) (Supplemental Table 4).

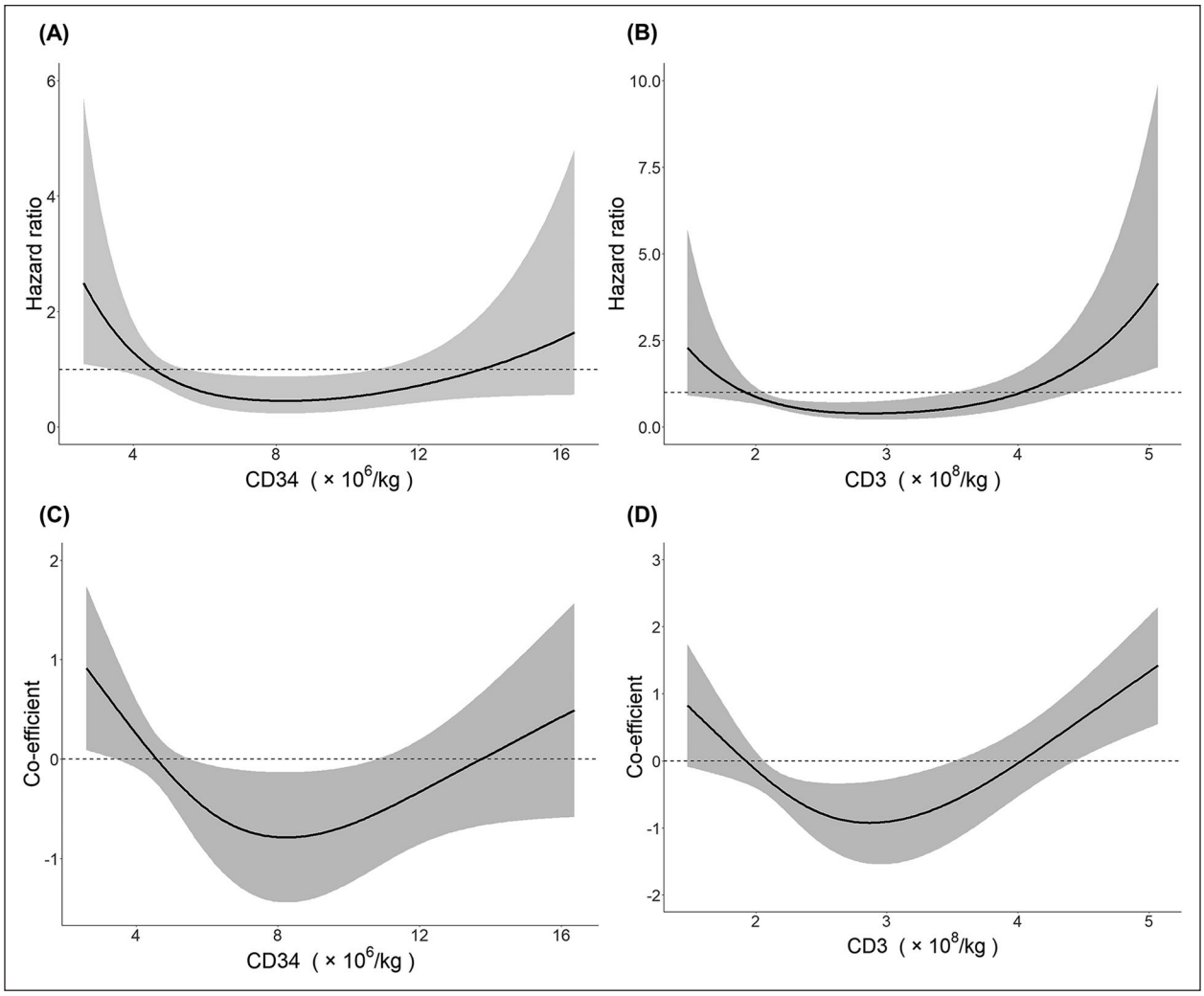

Figure 3. Smoothed plot of the hazard ratios (HRs) of the number of infused $CD34^+$and $CD3^+$ cells for overall survival (OS). The HRs of the number of infused $CD34^+$ (A) and $CD3^+$ cells (B) for OS were estimated by a restricted cubic-spline Cox regression analysis. Log (e)-transformed HRs of the number of (C) $CD34^+$ or (D) $CD3^+$ cells for OS were also plotted. The solid line and the gay area indicate hazard ratios and 95% confidence intervals, respectively.

As a consequence, the 16 patients (48%) who received a graft containing $>4.54 \times 10^6$/kg $CD34^+$ cells and $>1.85 \times 10^8$/kg but $\leq 3.70 \times 10^8$/kg $CD3^+$ cells showed far better OS and lower relapse/progression than those who received other grafts ($P < 0.001$ and $P = 0.02$, respectively) (Fig. 4). A multivariate analysis and a univariate analysis revealed that grafts containing $>4.54 \times 10^6$/kg $CD34^+$ cells and $>1.85 \times 10^8$/kg but $\leq 3.70 \times 10^8$/kg $CD3^+$ cells were significantly associated with better OS and with a lower risk of relapse/progression, irrespective of the disease status (Table 2 and Supplemental Table 4).

Discussion

The results of the present study demonstrate that PTCy-haplo with PBSCs using a modified regimen (75 mg/kg, given in two doses: 50 mg/kg on day 3 and 25 mg/kg on day 4) after transplantation is a valid option, particularly due to the low incidences of chronic GVHD and NRM. However, the strategy of reduced-dose PTCy in the present study did not obviously contribute to a reduction of relapse/progression, whereas the infused graft cellular composition had a significant effect on survival and relapse/progression, irrespective of the disease status. Indeed, OS of patients with a disease status of nonremission who received a graft containing $>4.54 \times 10^6$/kg $CD34^+$ cells and between 1.85 and 3.70×10^8/kg $CD3^+$ cells was much greater than that of patients who received other grafts 100% versus 31% at 1 year and 73% versus 19% at 2 years. This result suggests that there may be optimal components of the infused graft in PTCy-haplo. A recent report also demonstrates that the $CD34^+$ cell dose affects clinical outcomes after T-cell-replete haploidentical

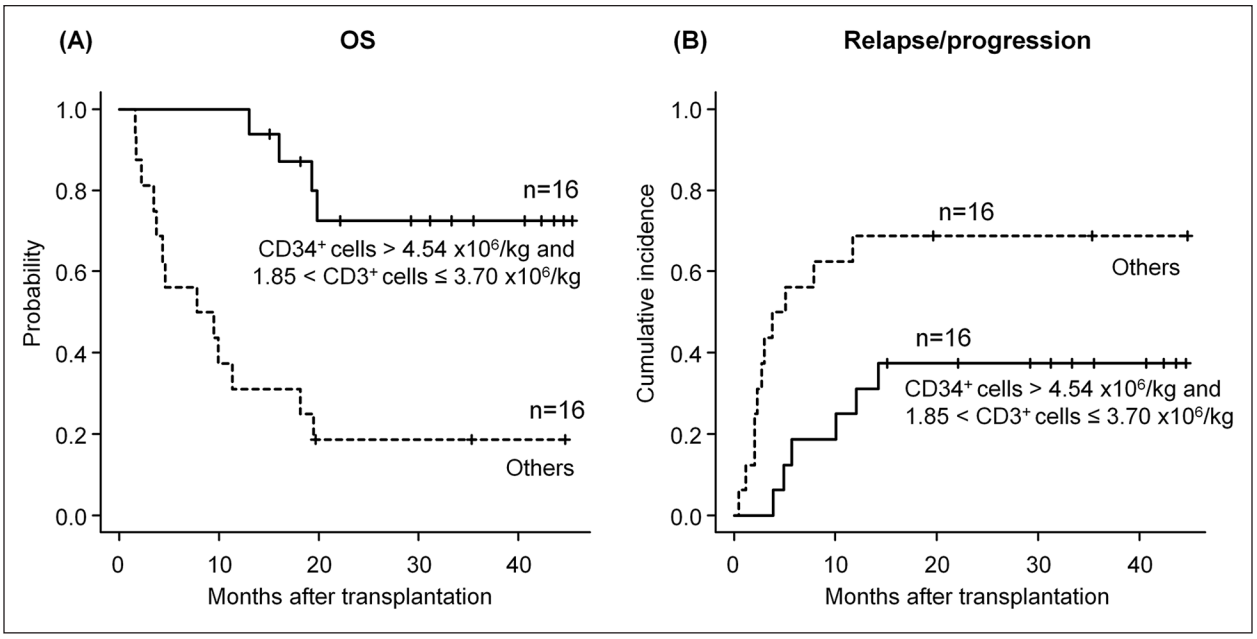

Figure 4. Comparison of OS and relapse/progression after HLA-haploidentical transplantation with posttransplant cyclophosphamide, stratified by infused graft composition. Comparison of (A) OS and (B) relapse/progression of patients who received a graft of $>4.54 \times 10^6$/kg $CD34^+$ cells and 1.85×10^6/kg $<CD3^+$ cells $\leq 3.70 \times 10^6$/kg in comparison with patients who received other grafts. In one case, information about the infused dose of $CD3^+$ cells was missing. OS: overall survival.

Table 2. Multivariable Analyses of OS and Relapse/Progression.

	OS		Relapse/progression		RFS	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Model 1						
$CD34^+$ cell dose: >4.54 ($\times 10^6$/kg) and $CD3^+$ cell dose: 1.85–3.70 ($\times 10^8$/kg)	0.13 (0.04–0.41)	<0.001	0.31 (0.10–0.94)	0.038	0.12 (0.04–0.40)	<0.001
Nonremission disease	4.89 (1.73–13.8)	0.0027	4.62 (1.48–14.4)	0.0082	8.97 (2.77–29.1)	<0.001
Model 2						
$CD34^+$ cell dose: >4.54 ($\times 10^6$/kg) and $CD3^+$ cell dose: 1.85–3.70 ($\times 10^8$/kg)	0.14 (0.05–0.46)	0.0010	0.34 (0.13–0.88)	0.027	0.22 (0.08–0.58)	0.0025
rDRI (high/very high vs low/intermediate)	2.28 (0.83–6.28)	0.11	2.71 (0.98–7.52)	0.055	2.72 (1.05–7.04)	0.039

The infused $CD34^+$ and $CD3^+$ cell dose, as a continuous variable, showed a nonlinear association with the clinical outcomes. The optimal cutoff value for the number of infused $CD34^+$ and $CD3^+$ cells was identified in a time-dependent receiver-operating characteristic analysis. 1.85–3.70 indicates >1.85 but ≤ 3.70. The analysis of nonrelapse mortality was not performed due to the small number of events. CI: confidence interval; HR: hazard ratio; OS: overall survival; rDRI: refined disease risk index; RFS: relapse/progression-free survival.

allogeneic hematopoietic stem cell transplantation with PBSCs for acute myeloid leukemia[24]. However, we should consider that the optimal cellular composition of the infused graft may depend on dose of PTCy and whether a BM or a PBSC graft is used.

Our results regarding the effects according to the number of infused $CD34^+$ and $CD3^+$ cells were not consistent with some previous reports[25–28]. The contradictory results might, in part, be associated with a heterogeneous group of patients with different disease statuses and transplantation characteristics. However, one critical difference was the analytical method. Most previous studies used linear models. Indeed, in our cohort, a linear analysis failed to detect a significant prognostic relationship with the numbers of infused $CD34^+$ or $CD3^+$ cells. However, using a nonlinear model, we found that the number of infused cells had a significant impact on OS.

In the present study, the incidence of chronic GVHD was low despite the reduced dose of PTCy. Our previous study showed that even a reduced dose of 25 mg/kg of PTCy on days 3 and 4 (total, 50 mg/kg) increased the proportion of regulatory T cells, which contributes to the inhibition of

chronic GVHD[29]. However, in this previous study, the incidence of grades II to IV acute GVHD was high, despite the low incidence of NRM. Therefore, based on the present study, we consider that a further decrease in the dosage of PTCy may lead to an unfavorable increase in the incidence of acute GVHD in PTCy-haplo with PBSCs. Nevertheless, if BM is used as the stem cell source, it is possible that we can decrease the dose of PTCy to a double dose of 25 mg/kg. Reports on reduced-dose PTCy are summarized in Supplemental Table 5. A prospective comparison study is required to define the optimal dose of PTCy for each source or disease.

Bashey et al[30] reported, in patients with leukemia, the performance of BM transplantation is associated with a higher risk of relapse in comparison with transplantation using mobilized PBSCs. Furthermore, Russo et al performed a detailed analysis of the recovery of subsets of NK cells after PTCy-haplo and found that PTCy removed most of the mature NK cells that proliferated early after transplantation. A high killer-cell immunoglobulin-like receptors population within the remaining mature NK cells at 30 days after PTCy-haplo was significantly associated with a low incidence of relapse[11]. These results suggest that a high dose of PTCy might attenuate the anti-leukemic effects of mature NK cells. We hypothesized that a reduced dose of PTCy and use of PBSCs would act synergistically to contribute to the favorable outcomes in the present study. However, a reduced dose of PTCy did not result in a low incidence of relapse/progression. Considering that 39% of patients were in a state of nonremission at the time of transplantation in our study, we should compare outcomes between dose-modified PTCy-haplo and standard-dose PTCy-haplo in a cohort of patients with a similar disease risk.

In a prospective multicenter study using standard-dose PTCy with RIC in Japan (Haplo14 RIC), the cumulative incidence rates of grades II to IV and III to IV acute GVHD on day 100 were 14% and 5%, respectively, whereas that of moderate-to-severe chronic GVHD at 2 years was 20%[31]. In our study, the cumulative incidence rates of grades II to IV and III to IV acute GVHD on day 100 were 30% and 15%, respectively, whereas that of moderate-to-severe chronic GVHD at 2 years was 7%. Although the results of the two studies cannot be simply compared, it is likely that the reduction in the PTCy dose increased the incidence of acute GVHD but not moderate-to-severe chronic GVHD.

In addition, another Japanese multicenter study of Haplo16 and 17 showed that PTCy-haplo with a reduced intensity conditioning regimen using PTCy at a reduced dose of 40 mg/kg/day on days 3 and 4 was safe and feasible due to the low incidence of grades II to IV acute GVHD, III to IV acute GVHD, and NRM[32]; however, the incidence of moderate-to-severe chronic GVHD was a little higher than that observed in our study. We do not know the exact reason for this, but we speculate that the pace of tapering and discontinuation of systemic immunosuppressants and/or a difference in the characteristics of the cohorts might play a role.

In contrast, the incidence of grade III GVHD in our setting was higher than that in the Haplo16 and 17 study[32], although it did not translate into a high incidence of NRM. This may imply that a higher PTCy dose on day 4 than on day 3 is more effective against acute GVHD. In fact, a study in a murine MHC-haploidentical hematopoietic cell transplantation model suggests that PTCy is maximally effective when administrated on day 4[12].

The incidence of grade $\geq$2 cardiac failure in our study was 9% (3/33). Recently, a study investigated cardiac toxicity in 585 patients who received the standard-dose PTCy regimen in MD Anderson Cancer Center[33]. In that report, the incidence rates of cardiotoxicity and/or cardiac failure after standard-dose PTCy were 6.5% and 2.4%, respectively. The incidence of grade $\geq$ 2 cardiac failure in our study (9%) was not reduced, at least when compared with standard-dose PTCy. However, the disease status at transplantation of the three patients who presented cardiac failure was nonremission in all cases, which might have influenced the incidence.

In this study, BKV cystitis occurred in eight of 33 (24%) patients. The reported incidence of BKV cystitis after standard-dose PTCy-haplo ranged from 11% to 75%, depending on the type of conditioning[34–37]. Based on previous reports, myeloablative conditioning and/or busulfan may be associated with a high incidence of BKV cystitis. We reduced intensity conditioning without busulfan in this study. Our conditioning and/or reduced dose of PTCy may have contributed to the decreased incidence of BKV cystitis.

The present study was associated with some limitations, including the small sample size, various underlying diseases, and the fact that it was a single-center study; however, the effects of the infused cell composition were still clear, despite the small cohort size, which suggests that the effect size of the infused cell composition on survival was not small.

In conclusion, PTCy-haplo with PBSCs using a de-escalated dose of PTCy (50 mg/kg on day 3 and 25 mg/kg on day 4 after transplantation) is a feasible option. The results of the present study also support the undertaking of a further clinical study to explore clinical impact of the composition of infused cells when BM or PBSCs are used as a stem cell source in a larger cohort.

Acknowledgments

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Author Contributions

HN, AH, MN (Nakamae), and HM participated in research design. HN, HO, and MN (Nakamae) wrote the paper. HN, HO, HK, YN, MN (Nishimoto), YM, MK, NH, TT, and MH performed the research. HN, HO AH, and MN performed the data analysis.

Ethical Approval

This study was approved by the Osaka City University Hospital Certified Review Board (UMIN-CTR; identification number UMIN000026028 and jRCTs051180144).

Statement of Human and Animal Rights

All procedures in this study were conducted in accordance with the Clinical Trials Act and the tenets set down in the Declaration of Helsinki, and with the Ethical Guidelines for Medical and Health Research Involving Human Subjects of Japan and the Osaka City University Hospital Certified Review Board approved protocols (UMIN-CTR; identification number UMIN000026028 and jRCTs051180144).

Statement of Informed Consent

All participants provided their written informed consent in accordance with the Declaration of Helsinki.

Declaration of Conflicting Interest

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Supplemental material

Supplemental material for this article is available online.

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