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

Hirohisa Nakamae1, Hiroshi Okamura1,2, Asao Hirose1, Hideo Koh1, Yasuhiro Nakashima1, Mika Nakamae1,2, Mitsutaka Nishimoto1, Yosuke Makuuchi1, Masatomo Kuno1, Naonori Harada1, Teruhito Takakuwa1, and Masayuki Hino1

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 × 10^6/kg CD34+ cells and >1.85 × 10^9/kg but ≤3.70 × 10^9/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 transplantation3–6. Therefore, it may not be an overstatement that PTCy-haplo is becoming the preferred platform for treatment of hematological malignancies.
PTCy was optimal based on these more recent findings, it days 3 and 4 prevented fatal GVHD; notably, 25 mg/kg/day demonstrated that PTCy doses of 10 to 50 mg/kg/day on identical murine hematopoietic cell transplantation model standard. The primary endpoint was the proportion (jRCTs051180144). The primary endpoint was the proportion 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. 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. Based on these more recent findings, it may be time to conduct a prospective clinical trial and revisit the dose of PTCy to identify an optimal dose that improves the outcome with minimum adverse effects.

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 ≥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, serum creatinine <2.0 mg/dl, left ventricular ejection fraction ≥50%, and ratio of vital capacity to the predicted vital capacity ≥40%, the ratio of forced expiratory volume in 1 s to forced vital capacity ≥50%, or oxygen saturation ≥90% without oxygen treatment); and (6) patients who have given their consent to participate in the study.

The conditioning regimen consisted of fludarabine (15 mg/m², twice a day on days −8 and −7, and 30 mg/m² once a day on days −6 and −3), cytarabine (2.0 g/m² twice a day on days −8 and −7), melphalan (100 mg/m² 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 described19,20.

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 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 nonlinear21. 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 index22. 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 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 × 10^6/kg CD3^+ cells. Neutrophil engraftment was achieved in all patients in a median of 17 (range, 12–33) days. Platelet engraftment was achieved
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.

**Table 1.** Patient, Donor, and Graft Characteristics.

| Characteristic                                      | n (% or range) | n (% or range) |
|-----------------------------------------------------|----------------|----------------|
| Patient gender (M/F)                                | 20/13          |                |
| Median age (range) years                             | 47 (19–66)     | 5 (15%)        |
| Diagnosis                                           |                |                |
| AML                                                 | 11 (33%)       | 15 (45%)       |
| ALL                                                 | 8 (24%)        | 13 (39%)       |
| MDS                                                 | 9 (27%)        |                |
| CML                                                 | 2 (6%)         | 7 (21%)        |
| ML                                                  | 3 (9%)         | 6 (18%)        |
| Nonremission disease                                | 13 (39%)       | 9 (27%)/10 (30%)|
| Refined DRI                                         | 4/8            |                |
| Low/intermediate                                     | 5/8            | 18 (55%)/17 (52%)|
| High/very high                                      | 6/8            | 3 (9%)/4 (12%) |
| HCT-CI                                              | 7/8            | 2 (6%)/2 (6%)  |
| 0                                                   | 8/8            | 1 (3%)/0 (0%)  |
| 1–2                                                 | 12 (36%)       |                |
| ≥3                                                  | 13 (39%)       |                |
| History of prior transplantation                     | 17 (52%)       |                |
| Donor-recipient CMV status                           | 8 (24%)        | 6.10 (2.45–17.6)|
| Positive/positive                                   | 23 (70%)       |                |
| Positive/negative                                    | 5 (15%)        |                |
| Negative/positive                                   | 3 (9%)         |                |
| Negative/negative                                   | 2 (6%)         |                |

**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/or 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,
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%).
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 \( >4.54 \times 10^6/\text{kg} \), and the optimal CD3\(^+\) cell count/kg thresholds were \( 1.85 \times 10^8/\text{kg} \) and \( 3.70 \times 10^8/\text{kg} \), respectively.

Infused grafts containing \( >4.54 \times 10^6/\text{kg} \) 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 \( \leq 1.85 \times 10^8/\text{kg} \) 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 \( \leq 1.85 \times 10^8/\text{kg} \) CD3\(^+\) cells died of primary disease.

Infused grafts containing \( >3.70 \times 10^8/\text{kg} \) 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 \( >1.85 \times 10^8/\text{kg} \) but \( \leq 3.70 \times 10^8/\text{kg} \) 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).
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 $3.70 \times 10^8$/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
allogeneic hematopoietic stem cell transplantation with PBSCs for acute myeloid leukemia. 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. 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\textsuperscript{30}. 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\textsuperscript{31} 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\textsuperscript{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\%\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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.

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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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**ORCID iD**

Hirohisa Nakamae https://orcid.org/0000-0003-4203-990X

**Supplemental material**

Supplemental material for this article is available online.

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