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Original Article

Salvage Haploidentical Transplantation Using Low-dose ATG for Early Disease Relapse after First Allogeneic Transplantation: A Retrospective Single-center Review

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Second allogeneic stem cell transplantation (allo-SCT) is a potentially curative therapy for patients who relapse after first allo-SCT. Human leukocyte antigen (HLA)-haploidentical related donors provide the broad opportunity to conduct second SCT at the appropriate time, but the efficacy of second SCT from haploidentical donors after relapse has not been established. We retrospectively analyzed the records of 33 patients who underwent second SCT. Twenty patients underwent haplo-SCT with low-dose antithymocyte globulin (ATG), and the other 13 patients underwent conventional- SCTs, including HLA-matched related peripheral blood, unrelated bone marrow or cord blood. Three years after the second SCT, the overall survival (OS) and progression-free survival (PFS) of all patients were 32.5% and 23.9%. Multivariate analyses indicated that non-complete response at second SCT, less than 1-year interval to relapse after first- SCT, and total score ≥3 on the hematopoietic cell transplantation-specific comorbidity index were significantly associated with a lower PFS rate. The haplo- and conventional- SCT groups showed equivalent results regarding OS, PFS, cumulative incidences of relapse, non-relapse mortality and graft-versus-host disease. The neutropenic period after transplantation was significantly shorter in haplo- SCT than conventional- SCT (10.5 days vs. 16 days, $p=0.001$). Our analysis revealed that haplo-SCT could be an alternative therapeutic option for relapsed patients after first SCT.

Key words: allogeneic stem cell transplantation, haploidentical stem cell transplantation, relapse, anti-T lymphocyte globulin

Allogeneic stem cell transplantation (allo-SCT) is recognized as a curative therapy for advanced hematologic malignancies, but the relapse of the original malignancy after allo-SCT remains one of the most common causes of treatment failure and mortality. Second allo-SCT or donor lymphocyte infusion shows potential as a definitive cure in these patients [1, 2], but only a limited proportion of relapsed patients are able to receive these intensive treatments.

One of the difficulties in conducting a second SCT is donor availability. Only about one-third of the patients have a human leukocyte antigen (HLA)-matched sibling donor, and identifying and mobilizing an unrelated adult donor could take longer than three months. Relapsed patients are often in poor condition physically,

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and there is a greater likelihood for more aggressive disease after relapse; therefore, rapid access to the appropriate donor graft is critical from the clinical viewpoint. HLA-haploidentical family donors should be considered a potential option for the graft of the second SCT, as the possibility of identifying an HLA-haploidentical donor in a family is over 90% [3]. The last decade has seen substantial improvements in haploidentical transplantations (haplo-SCTs), which has enabled the use of haploidentical donors more readily [4].

Several studies have reported similar good clinical outcomes in patients with various hematological malignancies who received transplants from matched unrelated donors compared to those who received transplants from haploidentical related donors. [5-8] However, to the best of our knowledge, no study has directly analyzed the outcomes in relapsed patients who underwent a second haplo-SCT compared to those who received second SCTs from other sources (*i.e.*, HLA-matched siblings, HLA-matched unrelated donors, umbilical cord blood. or partially HLA-mismatched unrelated donors) after their first allo-SCT at a single-institute during the same time period. We thus conducted the present retrospective analysis of patients who underwent a second allo-SCT for their relapse of hematologic malignancies after a first allo-SCT, in order to assess the safety and efficacy of haplo-SCT in these patients.

Patients and Methods

Patients. This study included all consecutive adult patients at Okayama University Hospital who underwent a second allo-SCT during the period from January 2007 to December 2016 for the treatment of a relapse of their underlying disease after the first allo-SCT. Twenty patients received an HLA-haploidentical peripheral blood (PB) transplants (the haplo-SCT group). Thirteen patients received an HLA-matched or partially mismatched transplants, three underwent cord blood transplantation (CBT), 3 underwent related peripheral blood SCT (rPBSCT), and 7 underwent unrelated bone marrow transplantation (BMT). These patients who underwent a conventional transplant were assigned to the control group.

The outcomes of interest were the overall survival (OS), progression-free survival (PFS), cumulative incidence of relapse (CIR), non-relapse mortality (NRM), acute graft-versus-host disease (aGVHD), chronic GVHD (cGVHD), engraftment, and the time to platelet and neutrophil recovery. We defined the OS, CIR and NRM as the number of days from the second SCT until death from any cause, relapse, and death without relapse, respectively. The conditioning intensity was classified as myeloablative conditioning (MAC) versus reduced intensity conditioning (RIC). [9] The hematopoietic cell transplantation-specific comorbidity index (HCT-CI) was collected for all patients. [10] The day of platelet recovery was defined as the first day on which the platelet count reached or exceeded 20×10^9/L without transfusion. The day of neutrophil recovery was defined as the first of 3 consecutive days on which the absolute neutrophil count exceeded 5×10^9/L. The International Bone Marrow Transplant Registry criteria were used for the aGVHD staging, [11] and the Seattle criteria were used to determine cGVHD severity. [12] Information on baseline demographics, clinical characteristics, and transplantation and its outcomes were collected from their medical records.

The conditioning regimens for the second SCTs. The conditioning regimens for the second SCTs are detailed in Table 1. The conditioning regimens included fludarabine (Flu), busulfan (BU), melpharan (Mel) and cytarabine (CY), and the regimes for the haplo-SCT cohort were classified into the following 3 groups: Flu/BU-based (n = 14), Flu/Mel-based (n = 5), and Flu/CY-based (n = 1). In addition, low-dose rabbit antithymocyte globulin (ATG) was added to all regimens in the haplo-SCT group. For the patients with decreased renal function, the doses of Flu, Mel, and CY were adjusted according to the residual renal function levels. All hospitalized patients stayed in a dedicated inpatient unit in laminar airflow rooms with standard infection control procedures. For the haplo group, the GVHD prophylaxis consisted of tacrolimus and methylprednisolone. For the control group, combinations of cyclosporine or tacrolimus and short-term methotrexate were used.

Statistical analyses. We compared the primary study variables between the haploidentical group and the control group using Fisher's exact test (categorical variables) and the Mann–Whitney *U*-test (continuous variables). The Kaplan-Meier method was used to estimate OS and PFS. We used the log-rank test to assess group differences in OS and PFS. Cox proportional hazard regression models were fit for our evaluation of

Table 1 Second transplant preparative regimens

Regimen Type (no. of patients)	BU mg/kg	TBI cGy	Flu mg/m^2	CY mg/kg	Mel mg/m^2	VP-16 mg/kg	AraC g/m^2	ATG mg/kg
Haplo (20)								
Flu-BU-ATG (3)	6.4		180					8
Flu-BU-ATG (4)	6.4		180					2.5
Flu-BU-ATG (1)	12.8		180					2.5
AraC-Flu-BU-ATG (2)	6.4		180				8	2.5
AraC-Flu-BU-ATG (4)	12.8		180				4.4	2.5
Flu-Mel-TBI-ATG (3)		400	180		140			2.5
AraC-Flu-Mel-TBI-ATG (2)		400	180		140		8	2.5
AraC-Flu-CY-TBI-ATG (1)		400	120	120			8	2.5
Control (13)								
Flu-CY (1)			180	50				
Flu-BU (4)	6.4		180					
Flu-Mel (2)			125		140			
BU-CY (1)	16 (p.o.)			120				
BU-CY-VP-16 (1)	12.8			120		30		
Flu-TBI (1)		200	125					
Flu-BU-TBI (1)	6.4	200	180					
Flu-BU-TBI (1)	6.4	400	180					
Flu-Mel-TBI (1)		400	125		140			

Flu, fludarabine; BU, busulfan; Mel, melpharan; CY, cytarabine; TBI, total body irradiation; ATG, antithymocyte globulin; AraC, cytarabine; haplo, haploidentical; VP-16, etopiside.

the prognostic effects of demographic and clinical measures of interest on the OS and PFS. The cumulative incidence (CI) curves of relapse, transplant-related mortality (TRM), acute GVHD, and neutrophil and platelet recovery were estimated using the CI function. The competing risks for relapse and TRM included death and relapse, respectively. The competing risks for acute GVHD included death and relapse. [13] Death without count recovery was considered a competing risk for count recovery. The CI curves were compared using Gray's test. All tests of significance were two-sided, and p-values < 0.05 were considered significant. Statistical analyses were performed with EZR (Saitama Medical Centre, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria, 2.13.0) [14].

Results

Patient characteristics. The characteristics of the 33 consecutive patients who underwent a second SCT are summarized in Table 2. The median age of the patients in the haplo group was 49.5 years (range 19-64 years), and that of the control group 54 years (range 19-69 years) ($p = 0.58$). There were no significant differences in sex ($p = 0.148$), HCT-CI score ($p = 0.431$), or hematopoietic progenitor cells (HPCs) for the first SCT ($p = 0.31$). Preparative regimens for the first SCT did not differ significantly; 12 of 20 (60%) patients in the haplo group had undergone a TBI-based preparative regimen compared to 5 (38.5%) patients in the control group ($p = 0.097$). The median time from the first SCT to the relapse diagnosis in the haplo group (232.5 days; range, 57-1,004 days) was significantly shorter than that in the control group (667 days; range, 84-2,235 days) ($p = 0.009$). The median time between the first and second SCTs in the haplo group (388 days, range 85-1,144 days) was significantly shorter than that in the control group (833 days, range 252-3,123 days) ($p = 0.027$). The median time between the relapse and second SCT ($p = 0.428$) and the period of the second SCT ($p = 0.27$) did not differ significantly between the haplo and control groups. The diagnoses for the second SCT included acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), acute lymphoblastic leukemia (ALL), malignant lymphoma (ML) and adult T-cell leukemia (ATL), with no significant difference between the haplo and control groups ($p = 0.839$). The indication for second SCT was the same as that for the first SCT in the

Table 2 Patient characteristics

Characteristic	Haplo (n = 20)	Control (n = 13)	*P*
Years of age at second SCT, median (range)	49.5 (19–64)	54 (19–69)	0.58
Male, n (%)	14 (70.0)	5 (38.5)	0.148
HCT-CI total scores, median (range)	1 (0–6)	1 (0–3)	0.431
Disease status at first SCT, n (%)			
CR	14 (70.0)	9 (69.2)	1
No CR	6 (30.0)	4 (30.8)	
HPC for first SCT, n (%)			
rBMT	1 (5.0)	1(7.7)	0.31
rPBSCT	9 (45.0)	5 (38.5)	
UR-BMT	6 (30.0)	2 (15.4)	
CBT	2 (10.0)	5 (38.5)	
Haploidentical	2 (10.0)	0 (0.0)	
Preparative regimen: first SCT, n (%)			
BU-based	7 (35.0)	3 (23.1)	0.097
TBI-based	12 (60.0)	5 (38.5)	
Others	1 (5.0)	4 (30.8)	
Unknown	0 (0.0)	1 (7.7)	
Interval no. of days to relapse after first SCT, median (range)	232.5 (57–1,004)	667 (84–2,235)	0.009*
Time between relapse and 2nd SCT, median (range)	142.5 (24–723)	165 (85–888)	0.428
No. of days between first and second SCT, median (range)	388 (85–1,144)	833 (252–3,123)	0.027*
Period of 2nd transplant			
2007 to 2011	5 (25.0)	6 (46.2)	0.27
2012 to 2016	15 (75.0)	7 (53.8)	
Diagnosis for second SCT, n (%)			
AML	11 (55.0)	6 (46.2)	0.839
MDS	2 (10.0)	1 (7.7)	
ALL	3 (15.0)	3 (23.1)	
ML	4 (20.0)	2 (15.4)	
ATL	0 (0.0)	1 (7.7)	
Same diagnosis for first and second SCT, n (%)	20 (100.0)	11 (84.6)	0.148
Disease status at second SCT, n (%)			
CR	9 (45.0)	7 (53.8)	0.728
No CR	11 (55.0)	6 (46.2)	
DLI before second SCT, n (%)	6 (30.0)	1 (7.7)	0.202
Conditioning intensity for second SCT, n (%)			
MAC	5 (25.0)	3 (23.1)	1.000
RIC	15 (75.0)	10 (76.9)	

SCT, stem cell transplantation; MAC, myeloablative conditioning; RIC, reduced intensity; HCT-CI, hematopoietic cell transplantation-specific comorbidity index; PBSCT, peripheral blood SCT; BMT, bone marrow transplantation; r, related; UR, unrelated; CBT, cord blood transplantation; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukemia; ML, malignant lymphoma; ATL, adult T-cell leukemia. *$P < 0.05$

majority of cases. The disease status at the first and second SCTs ($p = 1.000$ and 0.728, respectively), donor leukocyte infusion (DLI) before the second SCT ($p = 0.202$), and the conditioning intensity for the second SCT ($p = 1.000$) did not differ significantly between the haplo and control groups.

Overall survival and progression-free survival. The median follow-up time was 1,276 days (range 505-3,747 days) among the survivors. At the 3-year follow-up, the OS and PFS rates of all patients who underwent a second SCT were 32.5% (95% CI 17.3-48.7%) and 23.9% (95% CI 10.2-40.6%), respectively (Fig. 1A and B). There were no significant differences in OS at 3 years between the haplo and control groups; 25.0% (95% CI 9.1-44.9%) and 44.9% (95% CI 17.7-69.0%), respectively($p = 0.366$) (Fig. 2A, Table 3). The PFS at 3 years was 25.0% (95% CI, 9.1-44.9%) for the haplo group and 23.9% (95% CI, 4.6-51.6%) for the

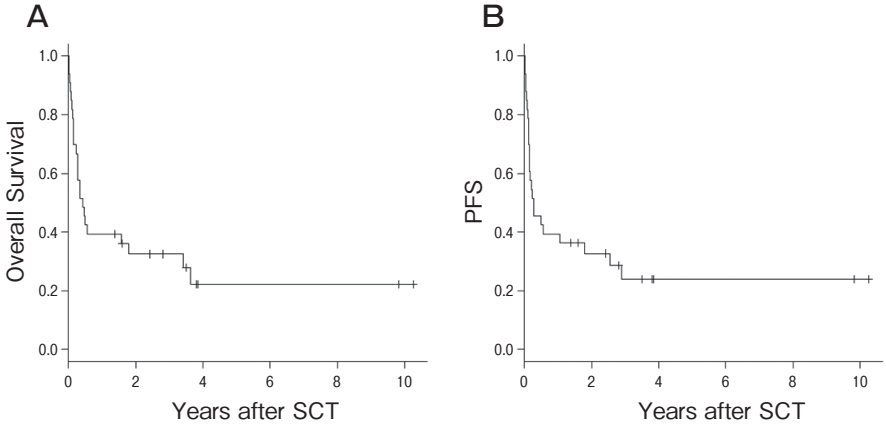

Fig. 1 **A**, OS after second SCT; **B**, PFS after second SCT.

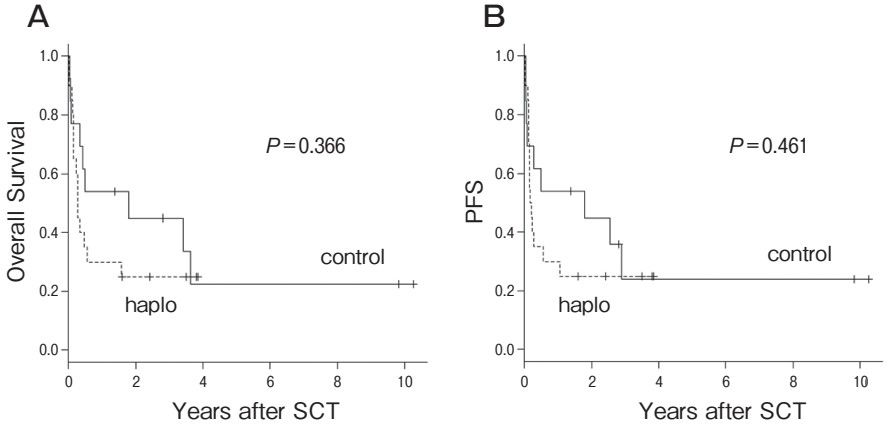

Fig. 2 **A**, OS after second SCT (haplo vs. control); **B**, PFS after second SCT (haplo vs. control).

Table 3 Outcomes of second transplant

Characteristic	Haplo (n = 20)	Control (n = 13)	*P*
Donor engraftment, n (%)	18 (90.0)	10 (76.9)	0.36
No. of days until PMN >500/μL, median (range); n=18, haplo; n=10, control	10.5 (9–23)	16 (12–21)	0.001
No. of days until platelet count >20,000/μL, median (range); n = 12, haplo; n = 10, control	16 (10–82)	20.5 (14–70)	0.06
Chronic GVHD			
None	9 (75.0)	6 (60.0)	0.348
Limited	0 (0.0)	2 (20.0)	
Extensive	3 (25.0)	2 (20.0)	
Follow-up survivors, median days (range)	1,276 (584–1,403)	2,307.5 (505–3,747)	0.624
OS at 3 years (%), (95% CI)	25.0 (9.1–44.9)	44.9 (17.7–69.0)	0.366
PFS at 3 yeasr (%), (95% CI)	25.0 (9.1–44.9)	23.9 (4.6–51.6)	0.461
CI relapse at 3 years (%), (95% CI)	25.0 (8.5–45.9)	28.6 (4.8–59.7)	0.794
CI of NRM at 3 years (%), (95% CI)	50.0 (26.0–70.0)	47.4 (17.7–72.5)	0.755
CI of aGVHD II-IV at 120 days (%), (95% CI)	30.0 (11.4–51.3)	46.2 (17.0–71.4)	0.304
CI of aGVHD III-IV at 120 days (%), (95% CI)	20.0 (5.7–40.5)	15.4 (2.1–40.2)	0.742

NRM, non-relapse mortality; aGVHD, acute graft-vs.-host disease; CI, cumulative incidences; OS, overall survival; PFS, progression-free survival.

control group ($p=0.461$) (Fig. 2B, Table 3).

The results of univariate and multivariate time-to-event Cox regression analyses of OS and PFS are shown in Table 4A and B. The effects of donor type (haplo vs. conventional), conditioning regimen (MAC vs. RIC), and older patient age were not significant as univariate risk factors for OS or PFS. The multivariate analyses showed that non-complete response (CR) status at the second SCT (hazard ratio (HR) 2.41, 95% CI 1.01-5.74; $p=0.047$), a <1-year interval to relapse after the first SCT (HR 3.23, 95% CI 1.15-9.06; $p=0.026$), and an HCT-CI score ≥3 (HR 5.85, 95% CI 1.99-17.17; $p=0.0013$) were significantly associated with poor PFS.

Relapse and NRM. The CIR at 3 years were 25.0% (95% CI 8.5-45.9%) and 28.6% (95% CI 4.8-59.7%) for the haplo and control groups, respectively ($p=0.794$; Fig. 3A, Table 3). The cumulative incidence of NRM at 3 years were 50.0% (95% CI, 26.0-70.0%) for the haplo group and 47.4% (95% CI, 17.7-72.5%) for the control group ($p=0.755$; Fig. 3B, Table 3). Neither

Table 4A Univariate and multivariable risk factors for OS

Factors	Univariate HR (95% CI)	*P*	Multivariate HR (95% CI)	*P*
Non-CR status at second SCT	2.83 (1.19–6.72)	0.019	2.39 (0.98–5.80)	0.055
Interval to relapse after first SCT < 1 year	2.16 (0.89–5.23)	0.088	2.59 (0.98–6.87)	0.055
HCT-CI total score > 3	3.12 (1.25–7.82)	0.015	4.64 (1.69–12.72)	0.0029
Transplant (haplo vs. conventional)	1.46 (0.64–3.38)	0.37		
Conditioning regimen (MAC vs. RIC)	1.46 (0.54–3.92)	0.46		
Patient age > 51 years	1.08 (0.48–2.4)	0.86		

Table 4B Univariate and multivariable risk factors for PFS

Factors	Univariate HR (95% CI)	*P*	Multivariate HR (95% CI)	*P*
Non-CR status at second SCT	2.86 (1.2–6.82)	0.018	2.41 (1.01–5.74)	0.047
Interval to relapse after first SCT < 1 year	2.12 (0.87–5.13)	0.097	3.23 (1.15–9.06)	0.026
HCT-CI total score > 3	3.49 (1.41–8.63)	0.0068	5.85 (1.99–17.17)	0.0013
Transplant (haplo vs. conventional)	1.37 (0.59–3.16)	0.46		
Conditioning regimen (MAC vs. RIC)	1.28 (0.55–3.01)	0.57		
Patient age > 51 years	1.10 (0.49–2.45)	0.83		

SCT, stem cell transplantation; HCT-CI, hematopoietic cell transplantation-specific comorbidity index; MAC, myeloablative conditioning; RIC, reduced intensity.

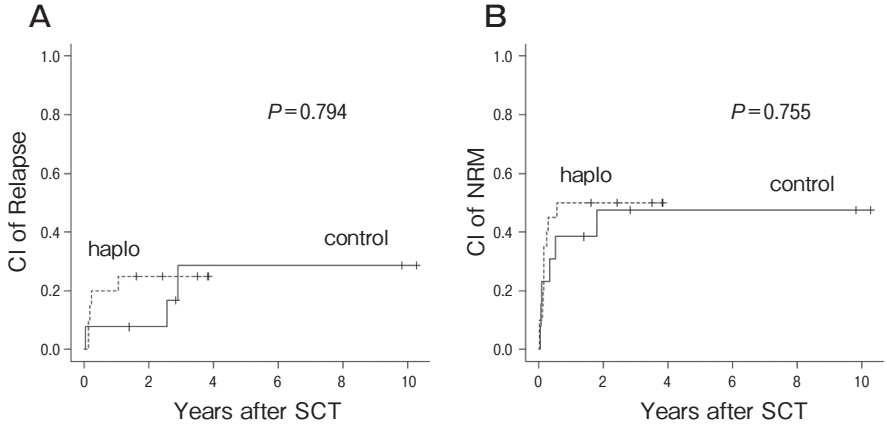

Fig. 3 **A**, Cumulative incidence of relapse after second SCT (haplo vs. control); **B**, Cumulative incidence of non-relapse mortality after second SCT (haplo vs. control).

CIR nor NRM showed a significant differences between the groups.

In the control group, there were 2 patients who relapsed more than 2 years after the second SCT. Both patients were diagnosed as Ph+ALL and they were treated with a tyrosine kinase inhibitor after the first SCT, which may have influenced their late-onset relapse. Subsequently, both patients underwent a third SCT (CBT and haplo-PBSCT) and died with pneumonia or thrombotic micro-angiopathies.

GVHD. The CIs of grades II-IV acute GVHD at 120 days were 30.0% (95% CI, 11.4-51.3%) and 46.2% (95% CI, 17.0-71.4%) for the haplo and control groups, respectively ($p=0.304$) (Fig. 4A, Table 3). The CIs of grades III-IV acute GVHD at 120 days were 20.0% (95% CI, 5.7-40.5%) and 15.4% (95% CI, 2.1-40.2%) for the haplo and control groups, respectively ($p=0.742$) (Fig. 4B, Table 3). There were no significant differences between the haplo and control groups. For chronic GVHD, 19 patients (11 in the haplo group, 8 in the control group) who survived for more than 100 days were evaluated; 3 patients (25.0%) in the haplo group and 2 (20.0%) in the control group developed extensive chronic GVHD ($p=0.348$) (Table 3).

Engraftment. The outcomes of engraftment after the second SCTs are also shown in Table 3. The hematopoietic engraftments were confirmed in 18 patients (90.0%) in the haplo group and 10 (76.9%) in the control group ($p=0.36$). The median time to neutrophil recovery (absolute neutrophil count $>0.5\times10^9$/μL) for the haplo group was 10.5 days (range 9-23 days), which was significantly shorter than that for the control group (median 16 days, range 12-21 days) ($p=0.001$). Among the 18 patients who achieved neutrophil engraftment after a haplo-SCT, 6 had no platelet recovery due to relapse or NRM. The remaining 20 patients (12 in the haplo group and 8 in the control group) achieved an unsupported platelet count of 20×10^9/μL at a median time of 16 days (range 10-82 days) in the haplo group and 20.5 days (range 14-70 days) in the control group ($p=0.06$). A cumulative incidence survey for neutrophil and platelet engraftment showed no significant difference between the haplo and control groups (neutrophil engraftment: $p=0.0609$, platelet engraftment: $p=0.414$) (Fig. 5A, B).

Cause of death. The causes of death after the second SCTs are shown in Table 5. As mentioned above, two patients underwent a third SCT for relapse after their second SCT, and they were excluded from this analysis. In both the haplo and control groups, the main cause of NRM was infection, including sepsis, bacterial pneumonia, CMV pneumonia, invasive aspergillosis, brain abscess, and HHV-6 encephalitis. Other causes of death included disease relapse, acute GVHD, veno-occlusive disease, and acute renal failure. There were no significant differences in the causes of death between the haplo and control groups ($p=0.785$).

Discussion

Allogeneic transplantation represents the only potentially curative therapy for most patients who have relapsed after a first SCT, but it is performed infrequently because of its substantial morbidity and mortal-

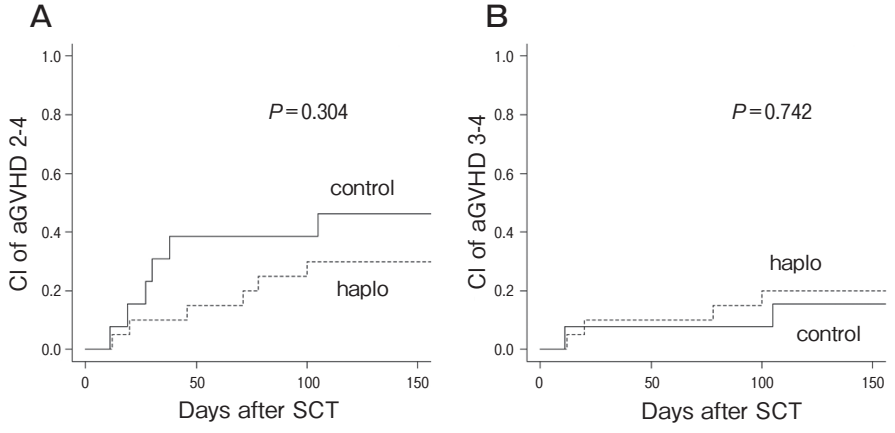

Fig. 4 A, Cumulative incidence of grade II to IV acute GVHD after second SCT (haplo vs. control); B, Cumulative incidence of grade III to IV acute GVHD after second SCT (haplo vs. control).

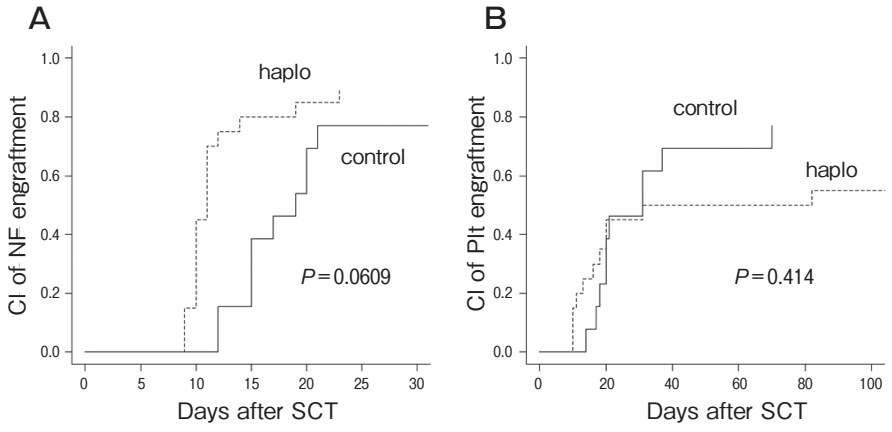

Fig. 5 **A**, Neutrophil engraftment after second SCT (haplo vs. control); **B**, Platelet engraftment after second SCT (haplo vs. control).

Table 5 Cause of death

Cause of death	Haplo (n = 15)	Control (n = 7)	*P*
Relapse, n (%)	5 (33.3)	1 (14.3)	0.785
NRM			
Infection, n (%)	7 (46.7)	5 (71.4)	
Acute GVHD, n (%)	1 (6.7)	0 (0.0)	
VOD, n (%)	1 (6.7)	1 (14.3)	
Acute renal failure, n (%)	1 (6.7)	0 (0.0)	

Excluded two patients who underwent a third SCT.
NRM, non-relapse mortality; aGVHD, acute graft-vs.-host disease; VOD, veno-occlusive disease.

ity [2]. To avoid excessive toxicity, non-myeloablative conditioning has been used as a second SCT [2,15]. Another difficulty in conducting a second SCT is donor availability. Because of the disease aggressiveness and the patient's poor physical condition, a rapidly accessible donor graft is needed. Due to readily available donors, haploidentical transplants have become more popular in recent years, supported by the substantial improvements in transplant procedures such as using a relatively high dose of anti-T lymphocyte globulin (ATG) [3,16-18], post-transplantation cyclophosphamide[5-8,19,20], and *ex- vivo* T-cell depletion [21,22].

The combination of low-dose ATG and GVHD prophylaxis consisting of tacrolimus and methylprednisolone is another strategy for conducting haplo-SCT safely in patients at an advanced stage or those at high risk for relapse[3,16,23]. For these reasons, haplo-SCT for relapsed patients after a first allo-SCT is increasing in clinical practice.

In an earlier study, seven patients who experienced relapse within 1 year of their first SCT and underwent a haplo-SCT as a second SCT showed a 2-year OS rate of 42.9% [24]. However, no other studies of larger cohorts have evaluated haplo-SCTs for relapsed patients as a second SCT. In the present study, we performed a retrospective single-center review of patients who underwent a second SCT for relapse after a first allo-SCT, and we evaluated the efficacy of haplo-SCT in this setting.

According to prior studies, patients who undergo a second allo-SCT for relapsed AML have OS rates in the range of 11-42% [1,2,15,25-27]. Christopeit *et al.* performed a retrospective study of 179 patients who underwent a second SCT for relapse after a first allo-SCT, and they reported that the 2-year OS was 25% [26]. Eapen *et al.* analyzed the outcome of 279 patients who relapsed after HLA-identical sibling transplantation and underwent a second allo-SCT, and they reported a 5-year OS of 28% [27]. Bosi *et al.* performed a retrospective study of 170 patients who underwent a second SCT for acute leukemia and experienced relapse after their first SCT, and they reported 5-year OS and PFS rates of 26% and 25%, respectively [25]. The OS and PFS of our patients seem comparable to these earlier reports.

It has been suggested that good-risk prognostic factors for the outcome of second SCTs include younger patient age, longer duration between the first SCT and relapse, CR status at the time of the second SCT, RIC for the second SCT, and the use of an HLA-identical related donor for the first SCT [1,2,15,25-27]. We therefore evaluated these factors among our patients in

univariate and multivariate analyses, and the results confirmed that non-CR status at the second SCT, relapse earlier than 1 year after the first SCT, and a poor HCT-CI score were significantly associated with shorter PFS. These factors might reflect the aggressiveness of our patients' disease.

Our comparison of the haplo-SCTs and conventional SCTs showed equivalent outcomes in the OS and PFS of the recipients. The results of our analysis also demonstrated equivalent cumulative incidences of relapse and NRM between the haplo and control groups. The comparison of the patients' characteristics showed that the time from the first SCT to relapse in the haplo group was significantly shorter than that in the control group (median 232.5 days vs. 667 days, $p = 0.009$). As we mentioned, a shorter-period relapse after the first SCT contributed to the poor outcomes after the second SCTs. Although the disease status at the first and the second SCTs were not significantly different between the haplo and control groups, this earlier relapse suggests that the haplo-SCT group might have included patients with more aggressive relapsed disease compared to the control group. Thus, the equivalence in basic clinical outcomes after haplo-SCT and conventional SCT may suggest a relatively higher potential for GVH effects in haplo-SCT.

On the other hand, the median time from the relapse to the second SCT was not significantly different between the haplo and control groups. We could not directly confirm a more rapid preparation of haplo-donors compared to other conventional donors, but as we observed at the period of the second SCT, the number of patients who undergoing a haplo-SCT is increasing. The accessibility of haplo-donors may contribute to this expansion of second SCTs for relapsed patients.

In this study, the cumulative incidences of acute GVHD and chronic GVHD were comparable between the haplo-SCT recipients and conventional-SCT recipients. Studies of second conventional-SCTs for relapsed leukemia patients after their first SCTs have reported cumulative incidences of grade II-IV acute GVHD of 22.5-53% [25-27]. These data are similar to those of our cohorts. The primary concern about haploidentical transplantation is the intense bidirectional alloreactivity, which potentially results in high incidences of graft failure and severe GVHD. However, haplo-SCT was reported to be associated with a low incidence of acute GVHD (in the range of 14-41%), which is comparable to the incidence observed in conventional SCTs [5-8, 16, 17, 20]. In our cohort, even in the second SCT setting, there was no increase in the incidence of severe acute GVHD after haplo-SCT. *In vivo* T-cell depletion by ATG and the early use of methylprednisolone as GVHD prophylaxis might have contributed to the decrease of severe acute GVHD.

Haplo-SCT using T cell-depleted grafts and post-transplant cyclophosphamide (PTCy) are associated with a lower incidence of chronic GVHD compared to transplants from conventional donors. [5, 7, 20] In general, the use of peripheral blood stem cells (PBSCs) is a risk factor for chronic GVHD; however, several studies that have used PBSCs alone as a transplant graft have indicated that the incidence of moderate to severe chronic GVHD in haplo-SCT recipients is still 25-28% [6, 7]. A study of haplo-SCTs with low-dose ATG revealed a 20% incidence of chronic extensive GVHD [16]; our results are similar. These findings suggest that haplo-SCT is sufficient to avoid symptomatic chronic GVHD.

We observed that the cumulative incidence of neutrophil and platelet engraftment after SCT was equivalent in the haplo and control groups. In addition, haplo-SCT was associated with rapid hematopoietic recovery; the median times for neutrophil recovery was 10.5 days, which is similar to other studies of haplo-SCT with low-dose ATG [3, 16]. By contrast, patients who underwent a haplo-SCT with PTCy have been reported to show slower neutrophil recovery than those who underwent HLA-matched unrelated transplantation [5-8]. Even in cases in shich a PB graft is used, the neutrophil and platelet recovery after haplo-SCT with PTCy were delayed (median time to neutrophil engraftment, 16 days, median time to platelet engraftment, 22-26 days) [7, 8]. The rapid achievement of engraftment after transplantation is very important to avoid serious bacterial or fungal infections. Although there have been no direct comparisons, a haplo-SCT with low-dose ATG might be preferable to haplo-SCT with PTCy, especially for patients with active infection at the second SCT.

Our study has several limitations. As a retrospective acquisition of data, the possibility of selection bias cannot be completely excluded. In addition, statistically significant differences were not detected due to the small sample size and potential lack of power. Moreover, our median follow-up time for survivors was

too short to draw conclusions. Despite these limitations, our study is the first analysis to compare transplantation outcomes between haploidentical versus conventional SCTs for relapsed patients after a first allo-SCT at a single-institute during the same period of time. Our results suggest that patients with indications for transplantation may undergo a transplantation using a haploidentical donor as safely as transplantation using a conventional matched donor.

Our analysis revealed that the outcomes of patients with relapse after s first SCT treated with transplants from HLA-haploidentical donors are comparable to the outcomes of patients treated with conventional transplants. These results need further confirmation in a longer follow-up and a larger-scale study. As it is usually easier to obtain a second graft from a family member than to activate an unrelated donor graft, SCT from a haploidentical donor could provide a promising alternative strategy in clinically difficult and time-constrained situations. Further improvements in the reduction of relapse and other complications of haplo-SCT with low-dose ATG in the near future will broaden the treatment options for better clinical outcomes.

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