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ORIGINAL ARTICLE

Salvage haploidentical transplantation for graft failure using reduced-intensity conditioning

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Graft failure is a major concern after cord blood transplantation (CBT) or HLA-haploidentical transplantation (haplo-SCT). As patients who undergo CBT or haplo-SCT almost always lack both matched-related and -unrelated donors, salvage transplantation would also be limited to either CBT or haplo-SCT. In this study, we assessed eight patients who received haplo-SCT as salvage therapy for graft failure. Five and three patients had received haplo-SCT and CBT, respectively, which resulted in graft failure. The median interval from the failed transplantation to salvage transplantation in six patients with primary graft failure was 33.5 days. The reduced-intensity conditioning regimen consisted of fludarabine, thiotepa, rabbit antithymocyte globulin and low-dose TBI. All eight patients achieved neutrophil engraftment, and seven patients achieved platelet recovery. The median times to neutrophil recovery and platelet recovery were 10 and 20 days, respectively. Three patients died from treatment-related causes: two from GVHD and one from rupture of carotid artery aneurysm. Five patients are alive, at a median follow-up of 946 days. The probability of overall survival at 5 years was 75%. These findings may serve as a rationale for giving precedence to haplo-SCT over CBT in salvage SCT after graft failure.
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Introduction

Graft failure is a life-threatening complication following allo-SCT. Immune rejection mediated by residual cellular immunity[1,2] or humoral immunity,[3,4] defects of the host BM microenvironment[5] and viral infections[6] are the main factors presumed to be involved in the occurrence of this complication. As immune rejection occurs as a result of the balance between residual host immunity and graft-derived immunity, the use of non-myeloablative or reduced-intensity conditioning (RIC),[7] T-cell depletion from the graft,[8] low numbers of infused progenitor cells[9,10] and immunological disparity (that is, HLA mismatch)[11] between the host and donor are known to increase the risk of graft failure. Although the overall frequency of graft failure is less than 5%, it has been reported to reach 12% for HLA-haploidentical SCT (haplo-SCT)[11] and is as high as 20% after cord blood transplantation (CBT).[12,13]

As both CBT and haplo-SCT are being increasingly performed as an alternative to HLA-matched-related or -unrelated transplantations, concerns regarding graft failure are also growing. The treatment options for graft failure are very limited. The survival rate for patients who do not receive salvage transplants are dismal (8%).[14] Salvage transplantation is generally attempted; however, the overall survival varies from 11 to 37%, with major obstacles being infections arising from prolonged neutropenia and damaged organ function as a result of previous transplantation.[14–17] Particularly, patients who undergo CBT or haplo-SCT almost always lack both matched-related and -unrelated donors during the clinically relevant period. Therefore, salvage transplantation is also limited to either CBT or haplo-SCT. We hypothesized that haplo-SCT is superior to CBT as a salvage therapy for graft failure because of the advantage of rapid neutrophil recovery, with respect to the high risk of infection in this particular setting. Therefore, we performed haplo-SCT using RIC for graft failure following CBT or haplo-SCT. Here, we describe the results for eight patients.

Patients and methods

Patients

This study is a retrospective analysis of eight consecutive patients who received a salvage transplant from an

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HLA-haploidentical related donor (2–3 Ag mismatched in the GVH vector) for primary or secondary graft failure following CBT or haplo-SCT between March 2001 and May 2010 at Osaka University Hospital or Hyogo College of Medicine Hospital. Informed consent was obtained from all the patients, and they were treated according to our institutionally approved protocols, including those for CBT and haplo-SCT.

Table 1 details the patient characteristics. Six patients had primary graft failure, whereas two had secondary graft failure. The median age of the patients was 49 years (range, 29–61 years) at the time of salvage transplantation. The stem cell sources of the previous transplantation, which failed to engraft, were cord blood in three patients, including one with double units, and haploidentical PBSC in five patients. Among these, two patients had received SCT one and two times before the failed SCT. Accordingly, they underwent salvage transplantation as their third and fourth SCT. Chimerism analysis showed no signs of donor hematopoiesis in seven patients. The remaining patient with secondary graft failure showed 100% donor chimerism in the T-cell fraction and 0% donor chimerism in the myeloid fraction.

Preparative regimen for salvage transplantation

All patients were treated with preparative regimen consisting of fludarabine 30 mg/m^2 for 3 days (days −4 to −2), thiotepa 5 mg/kg for 2 days (days −3 to −2), rabbit anti-T-lymphocyte globulin or antithymocyte globulin (ATG) and single-dose TBI 2–4 Gy. The doses of ATG and TBI in each patient are detailed in Table 2. The dose of TBI was determined according to the preparative regimen of previous transplants and the performance status of the patients at the time of salvage transplantation.

Salvage transplantation

Three of the five patients who had graft failure after haplo-SCT received salvage transplantation from the same donor. G-CSF-mobilized PBSCs were collected from the donor on days 0 and 1, with the target CD34+ cell dose of 3×10^6/kg of recipient body weight. The median number of infused CD34+ cells was 4.7×10^6/kg (range, $2.7–7.9 \times 10^6$/kg). The median interval from the failed transplantation to salvage transplantation for the six patients with primary graft failure was 33.5 days (range, 25–54 days).

Table 1 Patient characteristics

Patient no.	*Age (years)/sex*	*Diagnosis*	*Disease stage*	*No. of SCT before the failed SCT*	*SCT resulting in graft failure*				
					Stem cell source	*HLA match*		*Preparatory regimen*	*Pattern of GF*
						GVH vector	*HVG vector*		
1	29/M	MDS-AML	Refractory	0	PBSC	3/6	3/6	Flu/BU/ATG	Primary
2	54/F	CMML-AML	Refractory	2	PBSC	4/6	4/6	Flu/BU/ATG	Secondary
3	49/F	MDS-AML	Relapse after allo-SCT	1	PBSC	4/6	4/6	Flu/CA/BU/ATG	Primary
4	42/M	MDS-AML	Refractory	0	PBSC	4/6	3/6	Flu/CA/CY/TBI (8)	Primary
5	35/M	ALL	CR2	0	Double CB	5/6 5/6	5/6 4/6	CY/TBI (12)	Primary
6	57/M	MDS	RA	0	CB	4/6	4/6	Flu/CY/TBI (3)	Primary
7	61/M	MDS-AML	First relapse	0	CB	4/6	4/6	Flu/CY/TBI (3)	Primary
8	49/F	AML	Refractory	0	PBSC	4/6	3/6	Flu/CA/Mel/ATG	Secondary

Abbreviations: CA = cytosine arabinoside; CMML = chronic myelomonocytic leukemia; F = female; Flu = fludarabine; GF = graft failure; GVH = graft versus host; HVG = host versus graft; M = male; Mel = melphalan; MDS-AML = AML evolved from myelodysplastic syndrome.

Table 2 Information regarding salvage transplantation

Patient no.	*Interval from the failed SCT to salvage SCT (days)*	*Salvage transplantation*						
		Donor		*HLA match*		*Preparatory conditioning*		*CD34 ($\times 10^6$/kg)*
		Same as the failed SCT	*Relation*	*GVH vector*	*HVG vector*	*TBI dose (Gy)*	*ATG product/total dose (/kg)*	
1	25	Yes	Sibling	3/6	3/6	4	TMG/5	7.1
2	37	No	Daughter	4/6	3/6	2	TMG/2[a]	7.9
3	54	No	Daughter	4/6	3/6	4	ATG-F/10	4.0
4	31	Yes	Sibling	4/6	3/6	4	ATG-F/8	3.1
5	36	No	Mother	4/6	4/6	2	TMG/3	3.5
6	40	No	Daughter	3/6	3/6	3	TMG/3	5.5
7	31	No	Daughter	3/6	3/6	3	TMG/3	2.7
8	100	Yes	Daughter	4/6	3/6	4	TMG/3	5.3

Abbreviations: ATG-F = anti-T-lymphocyte globulin-Fresenius; GVH = graft versus host; HVG = host versus graft; TMG = thymoglobulin.
[a]Only patient no. 2 received ATG after transplantation (on days 10, 14 and 19).

GVHD prophylaxis and treatment
GVHD prophylaxis and treatment followed the institutional haplo-RIC protocol, which has been detailed elsewhere.[18] Briefly, GVHD prophylaxis consisted of continuous i.v. infusion of tacrolimus with target levels of 10–12 ng/mL and methylprednisolone 1 mg per kg per day. After patients achieved neutrophil engraftment and acute GVHD was considered absent, tacrolimus and methylprednisolone were tapered.

Supportive care
Patients were hospitalized in single rooms ventilated with high-efficiency particulate air filtration systems. All patients received broad-spectrum antibiotics and azoles (itraconazole or voriconazole) at the time of salvage transplantation. Following engraftment, patients received trimethoprim-sulfamethoxazole or aerosolized pentamidine for prophylaxis against pneumocystis pneumonia for at least 12 months post transplantation. Acyclovir was continued at 200 mg per day until the discontinuation of immunosuppressant. Patients received i.v. Ig 100 mg/kg weekly for 2 months after transplantation. CMV was monitored weekly by a pp65 antigenemia test. In addition, human herpesvirus-6 was monitored bi-weekly by PCR for virus DNA. Documented CMV or human herpesvirus-6 reactivation was treated with either ganciclovir or foscarnet. G-CSF 300 μg/m^2 was administered from day 1 or day 5 until the neutrophil count was greater than 2500/μL for two consecutive tests.

Chimerism analysis
Donor chimerism was determined serially in the T-cell- or neutrophil-enriched cell fractions of peripheral blood and BM. The methodology used for cell separation and chimerism analysis has been detailed elsewhere.[18,19] Briefly, T cells were enriched by a negative selection system (RosetteSep; StemCell, Vancouver, Canada) to a purity of >95%, and granulocytes were recovered from the Ficoll-red blood cell interface with a purity of >99%. Chimerism analysis involved quantitative PCR of informative STRs in the recipient and donor. DNA was amplified with fluorescent PCR primers for markers that would distinguish the donor and recipient alleles. Fluorescent PCR products were separated with an Applied Biosystems 310 Genetic Analyzer (Applied Biosystems, Carlsbad, CA, USA), and GeneScan software (Applied Biosystems) was used to correlate allele peak areas with the percentage of donor or recipient DNA.

Definitions and statistical analysis
Neutrophil engraftment was defined by an ANC of at least 500/μL for three consecutive tests, whereas platelet recovery was defined by a platelet count of at least 20 000/μL without transfusion support. Primary graft failure was defined by an absence of neutrophil recovery associated with no appearance or complete loss of donor cells using STR chimerism analysis by day 18 or an absence of neutrophil recovery by day 60. Secondary graft failure was defined as a recurrent neutropenia less than 500/μL after initial recovery. Diagnosis of acute and chronic GVHD was based on standard clinical criteria,[20] with histopathological confirmation where possible. Overall survival and disease-free survival were calculated using the Kaplan–Meier method.

Results

Engraftment and chimerism
All eight patients achieved neutrophil engraftment, and seven patients achieved platelet recovery following salvage haplo-SCT (Table 3). The median times to neutrophil recovery and platelet recovery were 10 days (range, 8–11 days) and 20 days (range, 17–97 days), respectively. Chimerism analysis showed that all patients achieved complete donor chimerism in both the T-cell and myeloid fractions within 4 weeks after transplantation.

GVHD
Four patients had no clinical acute GVHD. During the tapering of immunosuppressants, two patients developed grade II GVHD, whereas two patients developed grade III GVHD. Although both patients with grade II GVHD were successfully treated with increased doses of steroid therapy, the two patients with grade III GVHD (both with stage 2 liver involvement) were resistant to steroid therapy and subsequently died. None of the evaluable six patients developed chronic GVHD clinically.

Toxicity, relapse and cause of death
In all, three of the eight patients died from treatment-related causes: two from GVHD and one from rupture of

Table 3 Outcomes of salvage transplantation

Patient no.	*Time to engraftment (days)*		*GVHD*		*Relapse*	*Current status*	*Cause of death*
	Neutrophil	*Platelet*	*Acute*	*Chronic*			
1	10	17	0	No	No	Alive, day 3468	
2	8	97	II	No	No	Dead, day 2395	Rupture of carotid artery aneurysm
3	8	35	0	No	No	Alive, day 936	
4	10	17	0	No	Yes (day 718)	Alive, day 916	
5	10	20	II	No	No	Alive, day 459	
6	9	18	0	No	No	Alive, day 246	
7	11	24	III	NE	No	Dead, day 112	GVHD
8	11	NA	III	NE	No	Dead, day 91	GVHD, leukoencephalopathy

Abbreviations: NA = not achieved; NE = not evaluable.

carotid artery aneurysm, possibly related to thrombotic microangiopathy. One patient relapsed 718 days after salvage transplantation and received a third transplantation from a haploidentical related donor.

Survival

Five patients are alive at a median follow-up of 946 days (range, 276–3498 days). The probability of overall survival and disease-free survival at 5 years was 75 and 56%, respectively.

Discussion

We showed that salvage haplo-SCT for graft failure using RIC regimen allowed rapid neutrophil engraftment in all our patients, which translated into no mortality from infectious complications and favorable long-term survival (5-year overall survival = 75%).

Recently, the result of a Japanese nationwide survey of salvage CBT for graft failure was reported by Waki *et al.*[21] Of 80 patients who received salvage CBT, 61 patients who survived for more than 28 days were evaluated for hematopoietic recovery. Among them, 45 patients (74%) achieved neutrophil engraftment at a median of 21 days, and 31 patients (51%) achieved platelet recovery. Thirteen patients developed primary graft failure again. The rate of TRM at day 100 was 45%, with 60% related to infectious complications. The probability of overall survival at 1 year after CBT was 33%. Although the number of patients in this study is too small to draw any conclusions, we found a clear advantage of haplo-RIC over CBT in terms of neutrophil engraftment. Meanwhile, the major drawback of haplo-SCT is the risk of GVHD. Although the rate of severe GVHD was limited, two patients developed fatal GVHD in this study. Optimization of GVHD prophylaxis, such as the use of higher doses of ATG, may further improve the outcome of haplo-SCT for graft failure. To date, reports describing salvage transplantation from haploidentical donors in adult patients are few.[22,23] In the pediatric setting, Lang *et al.*[24] described 11 patients who received haplo-SCT for graft failure, with findings consistent with this report with respect to rapid neutrophil engraftment at a median of day 9, associated with favorable survival (1-year event-free survival = 72%). Although the number of reported cases is limited, double-unit CBT also appears promising.[25]

This study also showed the relative safety and effectiveness of the preparative regimen, consisting of fludarabine, thiotepa, low-dose TBI and ATG. In the majority of recent studies concerning salvage transplantation for graft failure, fludarabine and either ATG or alemtuzumab were included in the preparative regimen.[26–29] These agents are highly immunosuppressive and expected to suppress host immunocompetent cells, including T and NK cells, which are involved in the mechanism of immune-mediated graft rejection. Moreover, the use of ATG or alemtuzumab reduces the risk of GVHD after salvage transplantation. Of note, the aforementioned study by Waki *et al.*[21] showed that the incidence of neutrophil engraftment was higher in patients who received alkylating agents, including melphalan, busulfan and cyclophosphamide, as part of conditioning. Furthermore, the effect of low-dose TBI in promoting donor engraftment in the settings of the first transplantation has been reported by several studies.[14,30] Collectively, the preparative regimen used in this study has a powerful potential in enabling successful donor engraftment with limited toxicity in salvage transplantation for graft failure.

Theoretically, it could be argued that the donor in salvage transplantation should be altered from the previous failed transplantation, as previous studies have shown that cytotoxic T cells targeting mismatched HLA possessed by the donor are aroused at the time of immune rejection.[1] However, in this study, all three patients who received salvage transplantation from the same donor as the previous failed transplantation achieved engraftment. Nevertheless, considering the possible risk to a healthy donor of the administration of high doses of G-CSF twice in a short period of time as well as of poor mobilization, the donor for the salvage transplantation should be chosen cautiously.

This study has several inherent limitations. First, as a retrospective review, our case series is subject to a possible selection bias. In the study period, 12 patients developed graft failure after CBT or haplo-SCT, including eight patients who received salvage haplo-SCT, and thus were analyzed in this study. Of the remaining four patients, two patients received salvage transplantation from HLA one-locus mismatched donors. The other two patients could not receive salvage SCT, as they died as early as on days 20 and 25. Thus, we do not consider this study to be biased. Second, the number of the patients was small and the duration of follow-up for some of them was short. Nevertheless, our case series suggest the usefulness of this approach, indicating the need for further clinical study.

In conclusion, we showed that salvage haplo-SCT for graft failure allowed rapid engraftment in all patients, which translated into favorable overall survival. This study may serve as a rationale for giving precedence to haplo-SCT over CBT in the settings of salvage SCT after graft failure.

Conflict of interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

Feasibility of unmanipulated haploidentical stem cell transplantation using standard GVHD prophylaxis for HLA-homozygous patients

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Abstract HLA-haploidentical hematopoietic stem cell transplantation (haplo-SCT) in HLA-homozygous patients is accompanied by HLA mismatches only in the host-versus-graft vector, and thus theoretically could be performed with standard graft-versus-host disease (GVHD) prophylaxis. However, the risk of GVHD remains uncertain, and graft failure could be a problem. In this study, we assessed nine HLA-homozygous patients who underwent haplo-SCT. Preparative treatment was cyclophosphamide/total body irradiation-based regimen in five patients, fludarabine/busulfan-based regimen in two, and other regimens in two. GVHD prophylaxis consisted of cyclosporine and methotrexate in seven patients, cyclosporine and mycophenolate mofetil in one, and cyclosporine alone in one. Seven patients achieved neutrophil engraftment and platelet recovery. The median times to neutrophil engraftment and platelet recovery were 15 and 44 days, respectively. Two patients developed graft failure, including one who achieved engraftment with a second SCT from the same donor. Grade II GVHD was observed in half of the evaluable patients; grades III and IV were not observed. Two patients died from treatment-related causes. Five patients were alive after a median follow-up period of 563 days. The probability of overall survival at 5 years was 65 %. These findings may serve as a rationale for considering haplo-SCT as a treatment option for HLA-homozygous patients.

Keywords Haploidentical stem cell transplantation · HLA-homozygous patients · Hetero-to-homo transplantation · GVHD · Graft failure

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Introduction

The role of alternative stem cell sources in allogeneic hematopoietic stem cell transplantation (SCT) is currently expanding because of the reduced chance of finding a matched sibling donor, due to the elevation of the age limit for SCT and the low birth rates, particularly in Japan. HLA-haploidentical SCT (haplo-SCT) has substantial advantages, including the immediate availability of donors—which enables urgent SCT where necessary—and the availability of donor lymphocyte infusions after SCT [1–3]. However, earlier studies of haplo-SCT with a standard preparative regimen and graft-versus-host disease (GVHD) prophylaxis have shown high risks of graft failure and GVHD [4, 5]. Notably, Anasetti et al. demonstrated that HLA disparities in the host-versus-graft (HVG) vector and graft-versus-host (GVH) vector are correlated with the

risks of graft failure and GVHD, respectively. HLA homozygous patients inherently have no HLA mismatches in the GVH vector, whereas they usually have mismatches in the HVG vector. In fact, HLA homozygous patients who underwent 1 locus-mismatched haplo-SCT in the HVG vector had an incidence of GVHD similar to that of patients who underwent HLA-matched SCT [5]. Meanwhile, homozygous patients are predisposed to have natural killer (NK) cell alloreactivity in the GVH vector based on the killer cell immunoglobulin-like receptors (KIR) ligand incompatibility model. KIR ligand incompatibility in the GVH vector has been shown to be associated with a reduction of graft failure, GVHD, and relapse in several previous studies [6, 7].

Although these previous findings have indicated the feasibility of haplo-SCT for HLA-homozygous patients with standard GVHD prophylaxis, there have been scarce reports focusing on this treatment option. Therefore, the place of haplo-SCT in an algorithm of donor selection in homozygous patients remains unclear. One of the major reasons for the lack of the reports is probably the small number of HLA-homozygous patients. In the Japanese population, however, several haplotypes are quite common and well conserved [8–10]. Consequently, the number of homozygous patients with those common haplotypes is not negligible in Japan. Here, we describe the outcomes of 9 HLA-homozygous patients who underwent haplo-SCT from HLA-heterozygous donors ("hetero-to-homo SCT").

Subjects and methods

Patients

This study is a retrospective analysis of 9 consecutive HLA-homozygous patients who underwent haplo-SCT between May 1998 and September 2010 with a single transplant team at Osaka University Hospital (May 1998–March 2006) or Hyogo College of Medicine Hospital (January 2006–September 2010). Selection of donor source was based on its availability, disease status, and patient's request. While HLA-allele matched unrelated donors were given precedence for patients who remain in complete remission, haploidentical-related donors were given precedence for patients with active disease or those with impending relapse, which was suggested by minimal residual disease monitoring. Informed consent was obtained from all the patients, and they were treated according to our institutionally approved protocols.

The median age of the patients was 43 years (range 29–58 years) at the time of SCT (Table 1). Of 9 patients, 5 patients had acute myeloid leukemia (AML) or refractory lymphoma in no remission, including 1 who had a relapse after HLA-matched unrelated bone marrow transplantation (BMT), 3 who had AML in CR (2 had minimal residual disease), and 1 who had transfusion-dependent severe aplastic anemia.

HLA study and assessment of KIR ligand incompatibility

Generally, the patients and donors were tested for the allele type of HLA-A, B, C, and DRB1 loci. However, several patients who underwent SCT in earlier part of the study period were tested only for the serotype of HLA-A, B, and DR loci. KIR2DL ligand incompatibility in the GVH vector was scored when the KIR2DL epitope of HLA-C was present in donors and absent in recipients (that is, when recipients had Cw3 and donors had Cw3/Cw4 or Cw4/Cw4 or when recipients had Cw4 and donors had Cw3/Cw3 or Cw3/Cw4). KIR3DL ligand incompatibility in the GVH vector was scored when the HLA-Bw4 epitope including A24 was present in donors and absent in recipients. For the 5 donors or recipients who were typed only for HLA-A, B, and DR loci, those with A24-B52-DR15 were presumed to have Cw12, and those with A24-B7-DR1 were presumed to have Cw7, because Cw locus can be predicted with more than 99 % accuracy for these haplotypes in the Japanese population according to our database, which covers more than 4700 families in Japan. In patients who underwent SCT after January 2008 (no. 6–9), HLA antibodies were examined as part of the pretransplant work-up. The methodology used for the measurement of HLA antibodies was previously described [11].

Preparative regimen and stem cell sources

The preparative treatment consisted of cyclophosphamide/total body irradiation (CY/TBI, CY 60 mg/kg for 2 days and TBI 12 Gy divided in 4 fractions)-based myeloablative regimen in 5 patients, fludarabine/busulfan (Flu/BU, Flu 30 mg/m^2 for 6 days and BU 3.2 mg/kg for 4 days)-based myeloablative regimen in 2 patients, and other regimens in 2 patients (Table 2). Overall, in an attempt to overcome HLA disparity in the HVG vector, Flu was used in all 6 patients who underwent SCT after the approval of Flu in Japan. High-dose cytarabine (Ara-C, 2 g/m^2 for 4 days) was added to the CY/TBI-based regimen or to the Flu/BU-based regimen in 4 patients, mainly in an attempt to reduce tumor burden at the time of SCT. Bone marrow was used as a stem cell source in 6 patients, including all 5 who received the CY/TBI-based regimen. Peripheral blood stem cell (PBSC) were used for 3 patients, including the 2 patients who received Flu/BU-based regimen and the other patient with severe aplastic anemia, who received a reduced-intensity conditioning regimen consisting of Flu

Table 1 Patients characteristics

Patient no.	UPN	Age (years)/ sex	Diagnosis	Disease stage	Donor	HLA typing		No. of HLA mismatch[a]		KIR ligand mismatch		
						Recipient HLA	Donor HLA	GVH vector	HVG vector	GVH vector		HVG vector
										KIR2DL ligand	KIR3DL ligand	
1	174	43/F	AML	CR1	Daughter	A24-B52-(Cw12)-DR15	A24-B52-(Cw12)-DR15 A24-B7-(Cw7)-DR1	0	2	No	No	No
2	209	51/F	DLBCL	Relapse after auto-SCT	Daughter	A24-B7-Cw7-DR1	A24-B7-Cw7-DR1 A2-B13-Cw10-DR12	0	3	No	No	No
3	312	36/F	AML	CR1 (MRD positive)	Sibling	A*02:06-B*40:02-DRB1*14:05 A*02:01-B*40:01-DRB1*14:05	A*02:06-B*40:02-DRB1*14:05 A*31:01-B*40:01-DRB1*04:03	0	2	Not evaluable	No	No
4	444	44/M	FL (grade 3)	Refractory	Sibling	A24-B52-(Cw12)-DR15	A*24:02-B*52:01-Cw*12:02-DRB1*15:02 A*26:01-B*56:03-Cw*01:02-DRB1*12:01	0	3	No	No	No
5	490	50/M	DLBCL	Relapse after auto-SCT	Sibling	A*31:01-B*15:07-Cw*03:03-DRB1*04:05 A*31:01-B*15:07-Cw*03:04-DRB1*04:05	A*31:01-B*15:07-Cw*03:03-DRB1*04:05 A*24:02-B*55:02-Cw*01:02-DRB1*09:01	0	3	No	Yes (A24 = Bw4)	No
6	536	35/F	AML	Relapse after uBMT	Sibling	A24-B52-(Cw12)-DR15	A*24:02-B*52:01-Cw*12:02-DRB1*15:02 A*26:02-B*15:01-Cw*03:03-DRB1*14:06	0	3	No	No	No
7	617	58/M	MDS-AML	No treatment	Daughter	A*02:01-B*54:01-Cw*01:02-DRB1*04:05	A*02:01-B*54:01-Cw*01:02-DRB1*04:05 A*24:02-B*07:02-Cw*07:02-DRB1*01:01	0	3	No	Yes (A24 = Bw4)	No
8	654	36/M	AML	CR2 (MRD positive)	Sibling	A*24:02-B*07:02-Cw*07:02-DRB1*01:01	A*24:02-B*07:02-Cw*07:02-DRB1*01:01 A*02:06-B*54:01-Cw*08:03-DRB1*04:05	0	3	No	No	No
9	681	29/M	AA	Severe	Sibling	A*24:02-B*52:01-Cw*12:02-DRB1*15:02	A*24:02-B*52:01-Cw*12:02-DRB1*15:02 A*02:06-B*35:01-Cw*03:03-DRB1*15:01	0	2	No	No	No

UPN unique patient number, *GVH* graft-versus-host, *HVG* host-versus-graft, *AML* acute myeloid leukemia, *DLBCL* diffuse large B-cell lymphoma, *FL* follicular lymphoma, *MDS-AML* AML evolving from myelodysplastic syndrome, *AA* aplastic anemia, *auto-SCT* autologous stem cell transplantation, *MRD* minimal residual disease

[a] Number of serological mismatches in A, B, or DR loci

Table 2 Transplantation protocols and grafts

Patient no.	Transplant no.	Preparative regimen	Stem cell source	Infused cell dose		GVHD prophylaxis
				NCC ($\times 10^8$/kg)	$CD34^+$ ($\times 10^6$/kg)	
1	1	CY/TT	BM	3.5	–	CsA/MTX
2	2	CY/TBI (12)	BM	2.2	–	CsA/MTX
3	3	Flu/CY/TBI (12)	BM	4.8	–	CsA/MTX
4	4	Flu/CA/CY/TBI (12)	BM	2.2	–	CsA
5	5	Flu/CY/TBI (12)	BM	2.4	–	CsA/MTX
6	6-1	Flu/CA/BU4	PBSC	–	8.9	CsA/MTX
	6-2	TBI (2)	BM	1.2		CsA
7	7	Flu/CA/BU4/TBI (4)	PBSC	–	3.3	CsA/MTX
8	8-1	Flu/CA/CY/TBI (12)	BM	2.2	–	CsA/MTX
	8-2	Flu/CY/ATG	PBSC	–	7.0	CsA
9	9	Flu/CY/TBI (3)/ATG	PBSC	–	3.1	CsA/MMF

CY cyclophosphamide, *TT* thiotepa, *TBI (12)* total body irradiation 12 Gy, *Flu* fludarabine, *BU* busulfan, *CA* cytosine arabinoside, *BU4* once-daily BU for 4 days, *ATG* antithymocyte globulin, *MMF* mycophenolate mofetil

(30 mg/m^2 for 6 days), CY (50 mg/kg for 2 days), TBI (3 Gy), and antithymocyte globulin (thymoglobulin, 1 mg/kg for 4 days). Granulocyte-colony stimulating factor-mobilized PBSC were collected from the donor for 3 days, on days 0–2 when possible, to obtain as many stem cells as possible. The median number of infused nuclear cells in BMT was 2.3 $\times$ 10^8/kg (range 2.2–4.8 $\times$ 10^8/kg), and the median number of infused $CD34^+$ cells in peripheral blood stem cell transplantation (PBSCT) was 5.2 $\times$ 10^6/kg (range 3.1–8.9 $\times$ 10^6/kg).

GVHD prophylaxis and treatment

GVHD prophylaxis consisted of cyclosporine and short-term methotrexate on days 1, 4, and 8 in 7 patients; cyclosporine and mycophenolate mofetil (15 mg/kg/day) in 1 patient; and cyclosporine alone in 1 patient who had a bulky lymphoma at the time of SCT (Table 2). In the second transplantation following primary graft failure in 1 patient (no. 8), cyclosporine alone was used as GVHD prophylaxis.

Supportive care

Patients were hospitalized in single rooms ventilated with high-efficiency particulate air filtration systems. All patients received broad-spectrum antibiotics and either amphotericin B or azoles (itraconazole or voriconazole) during the neutropenic period before and after SCT. Following neutrophil engraftment, patients received trimethoprim–sulfamethoxazole or aerosolized pentamidine for prophylaxis against pneumocystis pneumonia for at least 12 months post-transplantation. Acyclovir (200 mg/day) was continued until the discontinuation of immunosuppressant. Patients received intravenous immunoglobulin (100 mg/kg) weekly for 2 months after transplantation. Cytomegalovirus was monitored weekly by a pp65 antigenemia test. Documented cytomegalovirus reactivation was treated with either ganciclovir or foscarnet. Granulocyte-colony stimulating factor (300 μg/m^2) was administered from days 1 or 5 until the neutrophil count was greater than 2500/μL for 2 consecutive tests.

Chimerism analysis

In patients who underwent SCT after April 2005 (no. 4–9), donor chimerism was examined serially in the T-cell- or neutrophil-enriched cell fractions of peripheral blood and bone marrow. The methodology used for cell separation and chimerism analysis has been detailed elsewhere [12, 13]. Briefly, T cells were enriched by a negative selection system (RosetteSep; StemCell, Vancouver, Canada) to a purity of >95 %, and granulocytes were recovered from the Ficoll-red blood cell interface with a purity of >99 %. Chimerism analysis involved quantitative polymerase chain reaction (PCR) of informative short tandem repeats in the recipient and donor. DNA was amplified with fluorescent PCR primers for markers that would distinguish the donor and recipient alleles. Fluorescent PCR products were separated with an Applied Biosystems 310 Genetic Analyzer (Applied Biosystems, Carlsbad, CA, USA), and GeneScan software (Applied Biosystems) was used to correlate allele peak areas with the percentage of donor or recipient DNA.

Definitions and statistical analysis

Donor-specific HLA antibodies were defined as HLA antibodies that correspond to the mismatched donor HLA antigen with median fluorescence intensity >5000 in the LABScreen Single Antigen analysis (One Lambda, Canoga Park, CA, USA). Neutrophil engraftment was defined by an absolute neutrophil count of at least 500/μL for 3 consecutive tests,

whereas platelet recovery was defined by a platelet count of at least 20,000/μL without transfusion support. Primary graft failure was defined by an absence of neutrophil recovery associated with no appearance or a decrease of donor cells in chimerism analysis by day 18 or an absence of neutrophil recovery by day 60. Diagnosis of acute and chronic GVHD was based on the standard clinical criteria [14], with histopathologic confirmation where possible. Overall survival was calculated using the Kaplan–Meier method.

Results

HLA and KIR ligand incompatibility

Four patients (no. 1, 4, 6, 9) were homozygous for the most common haplotype in Japan (HLA A*24:02-B*52:01-Cw*12:02-DRB1*15:02), which is possessed by approximately 8.4 % of the Japanese population (Table 1). Two patients (no. 2, 8) were homozygous for the third most common haplotype in Japan (A*24:02-B*07:02-Cw*07:02-DRB1*01:01), which is possessed by approximately 3.5 % of the Japanese population. In 2 patients (no. 3, 5), the haplotypes were serologically identical, but genotypically different. Thus, these patients were not homozygous, as stringently defined, but were included in this analysis because they also had no serological mismatches in the GVH vector. The other patient (no. 7) was homozygous for a less-frequent haplotype, which is possessed by approximately 0.42 % of the Japanese population. In 6 patients, the donors were siblings, and in 3 patients, the donors were daughters. The number of HLA mismatches in the HVG vector in A, B, and DR loci was 2 in 3 patients and 3 in the remaining 6 patients. KIR ligand incompatibility in the GVH vector was found in only 2 (no. 5, 7) of 8 evaluable patients. Both were KIR3DL-ligand incompatible, with A24 present in the donors and absent in the recipients. None of the 4 evaluated patients (no. 6–9) had donor-specific HLA antibodies.

Engraftment

Of 9 patients, 7 achieved neutrophil engraftment and platelet recovery. The median times to neutrophil engraftment and platelet recovery were 15 days (range 11–30 days) and 44 days (range 15–189 days), respectively. Two patients (no. 6, 8) developed primary graft failure.

One patient (no. 6), who underwent haplo-SCT as second SCT, showed no signs of neutrophil recovery, and donor chimerism in the T cell fraction started to decline by day 17. Salvage SCT (BMT) from the same donor following low dose TBI (2 Gy) was performed 21 days after haplo-SCT, followed by donor lymphocyte infusion, including 1.8×10^7 $CD3^+$ cells/kg. However, donor chimerism in the T cell fraction continued to decline and completely disappeared 31 days after the first haplo-SCT. The patient died with bacterial pneumonia 36 days after first haplo-SCT, as a consequence of prolonged neutropenia.

Another patient (no. 8) also showed a gradual increase of donor chimerism in the T cell fraction, up to 88.3 % on day 13. However, following a high fever beginning on day 11 and a 10-fold elevation of serum soluble interleukin 2 receptor levels from the baseline (from 548 U/ml on day 2 to 5163 U/ml on day 14), donor chimerism in the T cell fraction was suddenly completely lost on day 17. Consequently, the lymphocyte count rapidly increased from 10 cells/μl on day 16 to 440 cells/μl on day 20. Based on the diagnosis of graft failure with the mechanism of immune rejection, a second SCT (PBSCT) from the same donor with a highly immunosuppressive nonmyeloablative conditioning regimen (Flu 30 mg/m^2 for 4 days, CY 50 mg/kg for 1 day, Thymoglobulin 2 mg/kg for 3 days) was performed 26 days after the first SCT and achieved donor engraftment on day 12 after the second SCT. Chimerism analysis on day 12 showed complete donor chimerism in both the T cell and myeloid fractions. Chimerism analysis in all 4 patients who were evaluated for chimerism serially and achieved engraftment showed complete donor chimerism in both the T cell and myeloid lineages by 4 weeks after SCT.

GVHD

Of 8 evaluable patients, including 1 who achieved engraftment after a second SCT, 4 patients (50 %) developed grade II acute GVHD. One patient developed grade I GVHD, and the remaining 4 patients had no clinical GVHD. None of the evaluable patients died from acute GVHD-related complications. Chronic GVHD was observed in 4 patients (extensive type in 3 and limited type in 1 patient). Of the 2 patients with KIR ligand incompatibility in the GVH vector, 1 patient developed grade I acute GVHD and extensive chronic GVHD, and the other patient developed grade II acute GVHD but had no signs of chronic GVHD.

Outcomes

The outcomes of the patients are shown in Table 3. In all, 2 of the 9 patients died from treatment-related causes: 1 from *Pneumocystis jirovecii* pneumonia and 1, who had primary graft failure, from bacterial pneumonia. One patient died more than 9 years after SCT from repeated pancreatitis and encephalopathy of unknown etiology. One patient had a relapse of lymphoma 77 days after SCT and died with disease progression. Five patients were alive at a median

Table 3 The outcomes of haplo-SCT in HLA-homozygous patients

Patient No.	Transplant No.	Donor engraftment	Time to engraftment (days)		GVHD		Relapse	Current status	Cause of death
			Neutrophil	Platelet	Acute	Chronic			
1	1	Yes	23	139	0	Extensive	No	Dead, day 286	*Pneumocystis jirovecii* pneumonia
2	2	Yes	19	189	II	Extensive	No	Dead, day 3532	Pancreatitis, encephalopathy
3	3	Yes	15	15	0	No	No	Alive, day 2822	
4	4	Yes	30	60	II	No	Yes (day 77)	Dead, day 172	Relapse
5	5	Yes	15	28	I	Extensive	No	Alive, day 1331	
6	6-1	No	NA	NA	NE	NE	NE		
	6-2	NE	NA	NA	NE	NE	NE	Dead, day 36[a]	Bacterial pneumonia
7	7	Yes	13	141	II	No	No	Alive, day 563	
8	8-1	No	NA	NA	NE	NE	NE		
	8-2	Yes	12	23	II	Limited	No	Alive, day 365[a]	
9	9	Yes	11	19	0	No	No	Alive, day 221	

NE not evaluable, *NA* not achieved

[a] Counted from the date of first SCT

follow-up of 563 days (range 221–2822 days). The probability of overall survival at 5 years was 65 %.

Discussion

The present study had several significant findings regarding the feasibility of unmanipulated haplo-SCT for HLA-homozygous patients. First, we found that primary graft failure remains a major obstacle for those patients; 2 of 9 patients developed primary graft failure. Two major mechanisms are thought to be involved in primary graft failure after HLA-mismatched SCT: T cell-mediated cellular immune rejection [15, 16] and HLA antibody-mediated humoral immune rejection [11, 17–19]. Because donor-specific HLA antibodies were absent, the latter mechanism was unlikely to be involved in the 2 patients who had graft failure in the present study. The former mechanism occurs as a result of the balance between residual host T cells and donor-derived T cells. Several previous studies have supported this mechanism by demonstrating that host T cells that recognize donor HLA antigens emerge at the time of graft failure [20, 21]. In this respect, haplo-SCT in homozygous patients from heterozygous donors is inherently predisposed to cellular immune rejection, because T cell-derived alloreactivity occurs only in the HVG direction. In fact, the clinical course of patient no. 8—who developed a high fever simultaneous with the rapid decline of donor chimerism in the T cell fraction, followed by an increase of lymphocytes—suggested the emergence of host-derived alloreactive T cells during the process of immure rejection.

Because the preparative regimen affects only the residual host immunity (with the exception of antithymocyte globulin or alemtuzumab), it promotes engraftment by changing the balance between host residual T cells and donor-derived T cells. One of the 2 patients who failed to achieve engraftment underwent haplo-SCT (PBSCT) as a second SCT for relapse after unrelated BMT. Because the patient had received the conventional dose of TBI (12 Gy) at the time of unrelated BMT, haplo-SCT was performed with a non-TBI regimen consisting of Flu, BU (4 days), and Ara-C. One of the previous studies in the settings of cord blood transplantation has shown that Flu/BU regimen provided donor-derived neutrophil engraftment in only 2 of 10 patients [22]. This suggests that Flu/Bu regimen is less immunosuppressive than regimens containing CY or TBI and has less potential to facilitate engraftment. The other patient, who developed primary graft failure despite a highly myeloablative and lymphoablative conditioning regimen with Flu, CY, TBI, and Ara-C, was used BM as a stem cell source. Collectively, considering the substantial

risk of graft failure, a combination of a highly lymphoablative regimen (such as Flu/CY with low or conventional dose of TBI) and PBSCT should be used for future studies.

Our second major finding was that the incidence of GVHD with haplo-SCT in homozygous patients using standard GVHD prophylaxis was comparable to that with HLA-matched SCT. Although grade II GVHD was observed in half of the evaluable patients, none had grade III or IV GVHD, and there were no GVHD-related mortalities. These findings support the hypothesis that haplo-SCT in HLA-homozygous patients generates a GVH response comparable to that from HLA-matched SCT.

Our third finding was that the incidence of KIR ligand incompatibility in the GVH vector was low in the Japanese population, even in the combination of HLA-heterozygous donors and HLA-homozygous patients. In fact, only 2 of 8 evaluable patients had incompatibility in the present study. This is probably attributable to the remarkably biased frequency of the HLA-Cw groups in Japanese population (92.4 % of the population has the Cw3 group and 7.6 % has the Cw4 group) [23]. KIR ligand incompatibility in the GVH vector has been shown to be associated with a reduction of graft failure, GVHD, and relapse in patients who underwent T-cell-depleted haplo-SCT with CD34 positive cell selection [6, 7]. These favorable effects are delivered by alloreactive NK cells that are differentiated from the engrafted stem cells [24]. However, several studies in the settings of on unmanipulated unrelated BMT have shown KIR ligand incompatibility in the GVH vector to be associated with a high incidence of GVHD and poor overall survival [23, 25]. The use of antithymocyte globulin and/or the T-cell depletion was suggested to be a major reason for the discrepancy [19]. In this respect, KIR ligand incompatibility in the GVH vector could negatively affect outcomes in our transplant settings, although this was not evaluable due to the small number of patients with this incompatibility.

The present study had several inherent limitations. First, as a retrospective review, our case series was subject to a possible selection bias. Second, the number of patients was small, and the duration of follow-up was short in some patients. Nevertheless, our case series suggests the usefulness of this approach, which warrants further clinical study.

In conclusion, we showed the feasibility of unmanipulated haploidentical transplantation for HLA-homozygous patients using standard GVHD prophylaxis. While HLA-allele matched unrelated donors can be found in the majority of the HLA-homozygous patients, the major drawback associated with unrelated transplantation is a delay in provision of unrelated donor [26]. Previous studies have indeed shown that significant proportion of the patients became medically unfit while waiting for an unrelated transplantation [27]. Taken together with our findings, haploidentical transplantation can be considered to be a viable treatment option particularly for patients in need of an urgent transplant.

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Conflict of Interest The authors declare no conflict of interest.

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Extramedullary Relapse of Acute Myeloid Leukemia after Allogeneic Hematopoietic Stem Cell Transplantation: An Easily Overlooked but Significant Pattern of Relapse

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Acute myeloid leukemia may manifest as myeloid sarcoma in a variety of extramedullary (EM) tissues at diagnosis or at relapse. Although EM relapse after allogeneic hematopoietic stem cell transplantation (alloSCT) has been considered to be rare, recent studies have suggested that it occurs in 5% to 12% of patients who receive alloSCT, accounting for 7% to 46% of total relapses. The incidence of EM relapse after immunomodulation (eg, donor lymphocyte infusion) or a second SCT is even higher. Moreover, patients with EM relapse are more likely to have had preceding acute graft-versus-host disease or chronic graft-versus-host disease relative to those with bone marrow relapse. Collectively, these observations suggest that the preferential occurrence of the graft-versus-leukemia effect underlies the pathogenesis of EM relapse. Establishing an early diagnosis of EM relapse has been challenging because of the immense diversity in the relapse sites; however, recent studies have suggested the usefulness of ^{18}F-fluorodeoxyglucose positron emission tomography scans in the detection of EM relapse. As a treatment for EM relapse, a combination of local and systemic therapy should be considered, because local therapy alone often results in subsequent systemic relapse. The prognosis for patients who develop EM relapse after SCT remains poor but is slightly better than that after bone marrow relapse. In addition to an early diagnosis with new modalities, clinical studies using new agents that may offer systemic activity while preserving the graft-versus-leukemia effect are warranted as part of an effort to improve the clinical outcome.

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KEY WORDS: Acute myeloid leukemia, Extramedullary relapse, FDG-PET/CT, Graft-versus-leukemia effect

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (alloSCT) has been established as an effective treatment modality in patients with acute myeloid leukemia (AML). AlloSCT improves survival in patients with unfavorable-risk AML in remission by reducing the risk of relapse [1] and can also provide long-term survival in a certain proportion of patients with advanced AML [2]. Because recent improvements in supportive care and graft-versus-host disease (GVHD) prophylaxis regimens have reduced treatment-related mortality, disease relapse has now emerged as the principle cause of treatment failure after alloSCT [3].

AML may manifest as myeloid sarcoma in a variety of extramedullary (EM) tissues at diagnosis or at relapse [4]. Although the incidence of EM relapse after alloSCT has been considered to be rare, recent studies have suggested that EM relapse accounts for a significant proportion of relapses, particularly in situations where the graft-versus-leukemia (GVL) effect is induced. In this review, we outline clinically important issues regarding EM relapse, including advances in diagnostic modalities and its management.

Incidence and Risk Factors

The incidence of EM relapse in reported studies is summarized in Table 1. In a large retrospective study from the European Group for Blood and Marrow Transplantation, the incidence of EM relapse after SCT was 0.65% for patients with AML (20 of 3,071 patients) [5]. However, the incidence in this cohort may have been underreported [6]. Other studies, including recent ones, have shown that EM relapse of

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Table 1. Incidence of EM Relapse after SCT

Author and Citation	Disease	Disease Status at SCT	Donor	Conditioning	Incidence of EM Relapse	EM Relapse/Total Relapse
Békássy et al. [5]	AML (n = 3,071)	ND	ND	ND	0.65%	ND
Simpson et al. [7]	AML (n = 81)	CR 64% Non-CR 36%	MRD 94% MMRD 4%	BU/CY	12%	45%
Lee et al. [9]	AML (n = 78)	CR 92% Non-CR 8%	MRD 83% MUD 15% MMUD 1%	BU/CY 95% Flu/BU/ATG 5%	10%	33%
Blum et al. [10]	AML (n = 228)	CR 54% IF 17% Rel 29%	Allo 84% Auto 16%	BU/CY-based 89% BU/VP16 11%[a]	7%	19%
Shimoni et al. [11]	MDS/AML (n = 277)	CR1 68%[b] Other 32%[b]	MRD 54%[b] Unrelated 39%[b] Haplo/cord 7%[b]	Myeloablative 68%[b] RIC 32%[b]	ND	8%
Harris et al. [12] and Porter et al. [13]	AML (n = 257)	ND	ND	ND	10%	26%
Kogut et al. [14]	AML (n = 246)	CR1/2 56% IF 16% Rel 28%	MRD 44% MUD 56%	RIC (Flu/Mel 92%)	10.4%	18%
Yoshihara et al. [15]	AML (n = 57; 38 in 1st and 19 in 2nd SCT)	CR 9% Non-CR 91%	Haploidentical	Myeloablative 25% RIC 75%	1st SCT: 11% 2nd SCT: 32%	1st SCT: 21% 2nd SCT: 51%
Solh et al. [16]	AML (n = 436)	ND	ND	ND	5.70%	20%

EM indicates extramedullary; SCT, stem cell transplantation; AML, acute myeloid leukemia; ND, not described; CR, complete remission; MRD, matched related donor; MMRD, mismatched related donor; BU/CY, busulfan and cyclophosphamide; MUD, matched unrelated donor; MMUD, mismatched unrelated donor; Flu/BU/ATG, fludarabine, busulfan, and antithymocyte globulin; IF, induction failure; Rel, relapse; Allo, allogeneic; Auto, autologous; MDS, myelodysplastic syndrome; Haplo/cord, haploidentical or cord blood; RIC, reduced-intensity conditioning; Flu/Mel, fludarabine and melphalan.

[a]All are patients who received auto-SCT.

[b]Patients with acute lymphoblastic leukemia (n = 79) were included in the analysis.

AML occurs in 5% to 12% of patients who receive alloSCT, accounting for 7% to 46% of total relapses [7-16]. In a study that examined data from the European Bone Marrow Transplant Registry database, the incidence of EM relapse was statistically higher in patients who underwent allogeneic bone marrow (BM) transplantation than in those who underwent autologous BM transplantation (11% vs 6%; $P = .02$) [17].

The incidence of EM relapse after immunomodulation (eg, donor lymphocyte infusion [DLI]) or a second SCT is even higher. Choi et al. [18] reported that among 16 patients with AML who received chemotherapy + DLI, 10 achieved complete remission (CR). Among these 10 patients, 5 patients relapsed; all these patients relapsed at EM sites in the presence of continuous remission in the BM. The results of several other studies also indicate that EM relapse may commonly occur after immunomodulatory therapy for postSCT AML relapse [19,20]. Moreover, Yoshihara et al. [15] reported that the cumulative incidence of EM relapse among 19 patients who received haploidentical SCT as their second SCT was 32%, whereas the incidence among 38 patients who received haploidentical SCT as their first SCT was 11%.

Factors that are reported to be associated with EM relapse after SCT include younger age, EM involvement before SCT, advanced disease at SCT, unfavorable cytogenetics, and the M4/M5 French, American, British subtypes [9,12-14]. Although chromosomal abnormalities such as t(8;21) and inv(16), and CD56 expression in leukemic cells have been suggested to be associated with EM infiltration of AML at diagnosis or at relapse after chemotherapy [4,21-26], the significance of these factors in EM relapse in the SCT settings remains unclear. Meanwhile, Simpson et al. [7] reported the incidence of EM relapse was higher after busulfan-based conditioning than the reported incidence after total body irradiation (TBI)-based conditioning [27] and thus hypothesized that the conditioning regimen affects the incidence of EM relapse. However, the role of TBI in preventing EM relapse remains uncertain, because recent studies have shown a similar incidence of EM relapse after TBI-based and non–TBI-based conditioning regimens [10,16]. Further studies are also needed to clarify the role of other factors in EM relapse, such as the use of T cell depletion of grafts, stem cell sources, HLA disparities and the kinetics of T cell chimerism, all of which have been reported to be associated with the risk of BM relapse.

Clinical Manifestation

EM relapse of AML after SCT can occur not only in immunological sanctuary sites (eg, the central nervous system [CNS], testis, and ovary) but also in any organ or site in the body, including the skin, muscle, bone, nasal sinuses, mammary glands, peritoneal cavity, pancreas, adrenal glands, gastrointestinal tract,

kidney, and urinary tract [5,28-30]. EM relapse occurring close to the surface of the body (eg, skin, muscle, testis, and mammary glands) can manifest as a soft tissue mass. Other clinical symptoms are various and nonspecific, including anorexia, jaundice, ascites, lumber pain, abdominal pain, and hematuria. The site of involvement can be localized but often includes multiple locations or is diffusely infiltrated, possibly because of the delayed diagnosis due to nonspecific symptoms.

BM relapse and EM relapse show significant differences in their clinical manifestations. The median time from SCT to EM relapse has been reported to be at 10 to 17 months, which is longer than the median time from SCT to BM relapse at 3 to 7 months [9,11,12,14,16,29]. Interestingly, patients with EM relapse have been reported to be more likely to have had preceding acute graft-versus-host disease (aGVHD) and/or chronic GVHD (cGVHD) compared to those with BM relapse [8,11,16].

Pathogenesis

Although both BM and EM relapses may occur as a result of immune escape from the GVL effect via various mechanisms [31], the above-mentioned observations highlight a role for the preferential occurrence of the GVL effect in the pathogenesis of EM relapse. These above-mentioned observations include (1) a higher incidence of EM relapse after alloSCT than after autologous SCT; (2) an increased likelihood that patients with EM relapse have had preceding aGVHD and/or cGVHD when compared to those with BM relapse; (3) a longer time from SCT to EM relapse than the time to BM relapse; (4) a high incidence of EM relapse in patients who received post-transplantation immunomodulatory treatment, such as DLI; and (5) a high incidence of EM relapse after second SCT. Lee et al. [32] indicated that the GVL effect associated with an occurrence of GVHD is less effective in preventing an EM relapse than a BM relapse, by showing that the occurrence of aGVHD was significantly associated with better BM relapse-free survival but that EM relapse-free survival was similar in patients with or without aGVHD.

Although the precise mechanism for the difference in the GVL effect between BM and EM tissues remains to be clarified, several possible hypotheses have been suggested. Major effector cells for the GVL response—that is, CD8-positive T cells and natural killer cells—are present in much higher numbers in BM than in EM tissues [33]. In addition, the recruitment of the accessory cells necessary to achieve efficient local anti-leukemic activity may be deficient at the sites of EM relapse [34]. In other words, the GVL effect could be regarded as a "lymphohematopoietic GVHD response." Chakraverty et al. [35] showed that the presence of inflammation within tissues targeted in GVHD controls the trafficking level of activated T cells to the affected sites. If the trafficking of T cells is the determining factor separating GVL from GVHD, the EM sites should inherently be less susceptible to the GVL effect. In addition, Stölzel et al. [36] suggested that HLA loss in leukemic cells, which has been reported in patients with BM relapse after HLA-mismatched SCT [37,38], may also be involved in the mechanisms of EM relapse after matched related alloSCT [36]. They reported a patient presenting with a partial loss of HLA genes in leukemic cells taken from EM relapse, which was absent in leukemic cells from BM at diagnosis. They hypothesized that the partial loss of several HLA class I genes resulted in reduced presentation of minor histocompatibility antigens [39] and reduced ligation of activating natural killer cell receptors [40], both of which lead to the loss of GVL response and subsequent EM relapse in tissues with reduced immunologic surveillance.

In addition to the preferential occurrence of the GVL effect in BM, the intrinsic characteristics of leukemic cells, such as CD56 expression, may also be involved in the pathogenesis of EM relapse, particularly in the process of the homing of leukemic cells to the sites of relapse. The CD56 antigen has been identified as an isoform of the neural cell adhesion molecule and mediates cell-to-cell interactions via homophilic adhesion [41]. CD56/neural cell adhesion molecule has been reported to be highly expressed in various tissues, including neural tissues, gut, pancreas, thyroid gland, adrenal gland, testis, ovary, visceral smooth muscle, and cardiac muscle [42]. Thus, EM involvement at these sites may result from the homing of leukemic cells to these sites via homophilic adhesion of CD56 antigens.

Diagnostic Modalities

No standardized strategy has been established for the surveillance of EM relapse after alloSCT. The most significant challenge in the early diagnosis of EM relapse is the above-mentioned diversity in relapse sites. Thus, the diagnosis of an EM relapse is often delayed until patients develop the symptoms of a large mass, such as abdominal pain or constipation. However, a number of recent reports have shown that EM infiltration of AML can be detected by ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET) or FDG-PET/computed tomography (CT) [43-53]. Stölzel et al. [54] recently reported the results of FDG-PET/CT in 10 patients with AML with histologically proven EM disease at diagnosis (n = 5), relapse after alloSCT (n = 4), or relapse after chemotherapy (n = 1). The scans were able to detect known EM lesions in 9 patients (90%) and successfully detected additional EM sites in 6 patients (60%). Although these results strongly indicate the usefulness

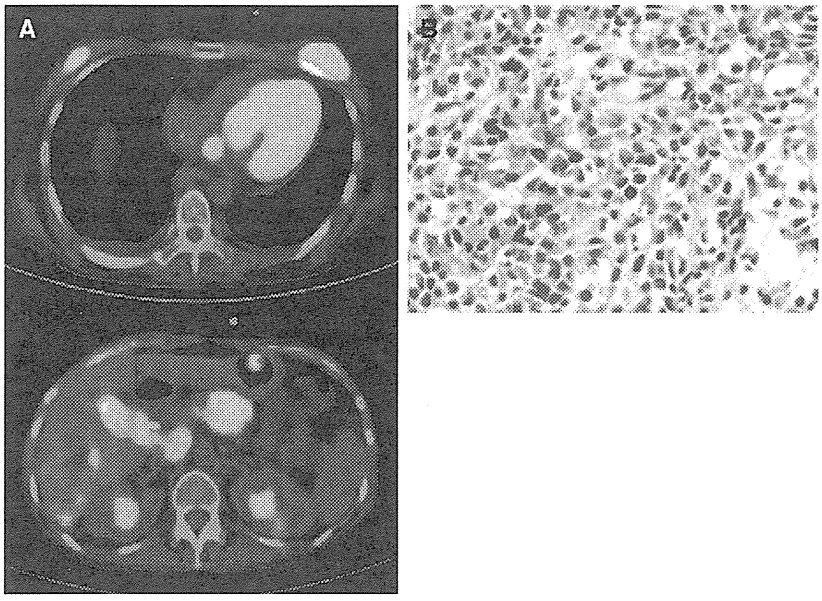

Figure 1. Fluorodeoxyglucose-positron emission tomography (FDG-PET)/computed tomography (CT) images and histologic examination of a biopsy specimen in a patient with extramedullary (EM) relapse after allogeneic stem cell transplantation (allo-SCT). The FDG-PET/CT images indicate multiple sites of FDG uptake, including breasts, bile ducts, the pancreas, the mediastinum, the para-aorta, the mesenterium, the stomach, and the left kidney (A). Histologic examination of the breast tumor showed the diffuse infiltration of medium- to large-sized blast cells (H&E stain, ×400) (B).

of FDG-PET/CT as a tool for the systemic surveillance of EM relapse, this method has several limitations [54]. First, because FDG uptake is not specific for leukemic infiltration, histological confirmation is necessary for the diagnosis of EM relapse. Second, the detection of EM relapse in areas with a physiologically high background of FDG uptake (eg, CNS, heart, and urinary tract) is difficult. However, the detection of EM relapse in these areas might be possible with alternative modalities, such as a lumbar puncture, echocardiography, and urine tests. Third, cost-effectiveness may be an issue. Because the median onset of EM relapse is approximately 1 year after SCT, monitoring with FDG-PET/CT may be practical only in high-risk patients.

In this regard, it is notable that several recent reports have suggested that minimal residual disease monitoring using a chimeric gene or a WT1 transcript assay using real-time quantitative PCR may predict EM relapse of AML [55]. Tamaki et al. [56] reported that a patient with AML with t(8;21) showed high AML1-MTG8 expression levels in the peripheral blood (PB) at the time of EM relapse [56]. Interestingly, the AML1-MTG8 expression levels in the BM remained lower than those in the PB, which is extremely unusual in patients with BM relapse [57]. Similarly, Kwon et al. [58] reported that 3 patients with EM relapse showed high WT1 levels in the PB, but the WT1 level in the BM remained negative. These results suggest that minimal residual disease monitoring in the PB can predict EM relapse after alloSCT. Moreover, minimal residual disease monitoring in both the PB and BM can possibly discriminate between BM relapse and EM relapse [55]. The reason for the pattern of increased AML1-MTG8 and WT1 expression in the PB relative to the BM may be attributable to the capability of detecting circulating leukemic cells derived from EM sites.

In summary, minimal residual disease monitoring using a chimeric gene or WT1 transcripts in the PB may be useful for the prediction of relapse, and FDG-PET/CT may be useful for detecting the sites of relapse. Thus, the combined use of minimal residual disease monitoring and FDG-PET/CT might be a useful strategy, as suggested in a patient we briefly present here. A 20-year-old female patient underwent a third alloSCT from a haploidentical related donor for posttransplantation relapse of AML at our institute. The monitoring of WT1 in the PB showed a steady increase in its levels, whereas the WT1 levels in the BM were normal. FDG-PET/CT showed multiple FDG uptake sites in the bilateral breast, para-aorta, bile duct, pancreas, stomach, and left kidney (Figure 1A). The diagnosis of an EM relapse of AML was confirmed with a core-needle biopsy from a breast tumor (Figure 1B).

Management

An optimal treatment strategy for patients with EM relapse has yet to be established. Although several reports have shown that local therapy, including surgical excision and radiotherapy, can offer some patients long-term survival [5,59], most patients develop systemic relapse [60]. Thus, systemic therapy, such as

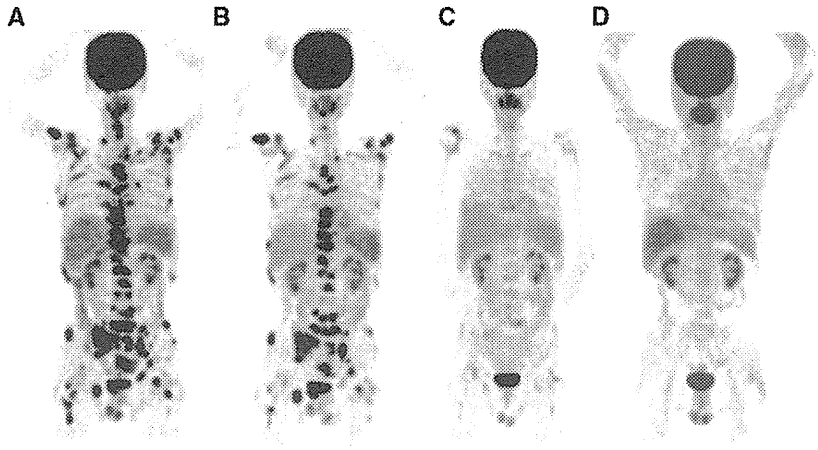

Figure 2. Fluorodeoxyglucose-positron emission tomography (FDG-PET) images of a patient who received gemtuzumab ozogamicin as a treatment for extramedullary (EM) relapse after allogeneic stem cell transplantation (allo-SCT) (partly reproduced from Ando et al. [71] with permission from Tohoku University Medical Press). Multiple EM relapse sites were detected by FDG-PET (A). Two weeks after the discontinuation of immunosuppressants, the number of EM lesions had slightly decreased in number (B). Complete remission was achieved 3 weeks after a single dose of gemtuzumab ozogamicin (C) and was maintained 28 months after the administration of gemtuzumab ozogamicin, without further treatments (D).

chemotherapy, DLI, second SCT, or a combination of these modalities, should be considered, particularly in patients with a better performance status. Solh et al. [16] reported that patients who received systemic or combined modality therapy (ie, systemic + local therapy) for an EM relapse had a better 6-month overall survival when compared with those treated with local therapy only. Moreover, a recently reported study, which reviewed the outcome of patients with EM relapse presenting as breast tumors in both SCT and non-SCT settings, showed that a combination of local therapy and chemotherapy resulted in a superior outcome relative to chemotherapy alone [61]. Taking these findings together, a combination of local and systemic therapy should be considered in patients with EM relapse with a limited number of involved sites.

In terms of immunomodulatory treatments, DLI has a limited efficacy [31], and a second SCT often results in repeated EM relapse [15], as expected from the above-discussed mechanism. Interestingly, Neumann et al. [61] examined the feasibility of an intrathecal application of donor lymphocytes in 3 patients who had isolated CNS relapse of CML or AML after SCT. No acute or delayed toxic side effects were observed. The efficacy of this strategy remains unclear due to the combined systemic treatment. Further studies are warranted to establish a strategy for achieving an efficient GVL effect through the delivery of donor lymphocytes to the sites of relapse.

Based on the above observations and the pathogenesis of EM relapse, systemically active agents that do not abrogate the GVL effect would be desirable. All-trans retinoic acid and arsenic trioxide have been reported to be effective for the treatment of EM relapse of acute promyelocytic leukemia [62,63]. Other examples include hypomethylating agents such as 5-azacytidine and decitabine. These agents have been shown to induce leukemic cell differentiation and to increase the expression of HLA antigens and tumor-associated antigens [64-67]. Thus, the use of hypomethylating agents might enhance the GVL effect. Jabbour et al. [68] reported the efficacy of 5-azacytidine as a treatment in 9 patients with AML relapse after alloSCT. Two of the 3 patients with EM relapse responded to the treatment. Singh et al. [69] reported on a patient who achieved durable CR of EM relapse with decitabine.

Another example of an agent that may offer systemic activity while preserving the GVL effect is gemtuzumab ozogamicin, an anti-CD33 monoclonal Ab conjugated to the antitumor antibiotic calicheamicin [70,71]. Although gemtuzumab ozogamicin was withdrawn in the United States and Europe based on the results of several studies, including one that showed an excess of deaths during induction therapy with no beneficial effects [72], a recently reported study has shown the usefulness of this agent, which may suggest a rationale for re-evaluating the place for this agent [73,74]. Ando et al. [71] reported the clinical course of a patient who received gemtuzumab ozogamicin for the treatment of posttransplantation multiple EM relapses in the soft tissue and bone (Figure 2). A biopsy specimen from soft tissue revealed diffuse infiltration of AML cells and CD8+ lymphocytes, possibly reflecting the occurrence of a GVL response. Discontinuation of immunosuppressants resulted in a mild reduction in the number of EM lesions. A single dose of gemtuzumab ozogamicin at 9 mg/m^2 resulted in CR, which has been maintained now for more than 3 years. The clinical course of the

patient may suggest the effectiveness of the GVL effect when the effector-to-tumor cell ratios are improved by targeted chemotherapy that results in the preservation of effector cells. In this context, the CD56 monoclonal Ab conjugated with a toxin or a radioisotope [75,76] is also a good candidate for future studies of EM relapse involving CD56-positive leukemic cells.

Prognosis

With current diagnostic and treatment strategies, the prognosis for patients who develop EM relapse after SCT remains poor. However, several previous studies have shown that survival after the occurrence of EM relapse is slightly better than that after BM relapse [5,8,11,16]. Solh et al. [16] reported that the probability of survival 6 months after the diagnosis of a relapse was significantly better in patients with isolated EM relapse (69%) than in those with combined EM and BM relapses (8%) or with a BM relapse alone (27%; $P < .01$) [16]. In addition, the type of treatment (local, systemic, or combined) and the response to the treatment were significantly associated with survival after EM relapse. Further studies are needed to clarify the significance of other factors, such as patient age, sites of relapse, and conditioning for a second SCT.

SUMMARY

Recent studies have revealed that the EM relapse of AML has been underreported and represents a significant pattern of relapse. Awareness of EM relapse has become particularly important, because recent advances in diagnostic modalities, including FDG-PET/CT, have enabled the early diagnosis of EM relapse. The possibility of an EM relapse should be taken into consideration, particularly in patients who have had aGVHD and/or cGVHD or in those who have received immunomodulatory therapy or second transplantation. A combination of local and systemic therapy should be considered for the treatment of EM relapse, because local therapy alone often results in subsequent systemic relapse. The prognosis for patients who develop EM relapse after SCT is slightly better than that after BM relapse but remains poor. In addition to an early diagnosis with new modalities, clinical studies using new agents that may offer systemic activity while preserving the GVL effect are warranted in the effort to improve the clinical outcome.

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