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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. One patient who possessed 0.002% GPI-AP$^-$ granulocytes was judged positive because 0.026% of the patient's erythrocytes were GPI-AP$^-$ cells.

Figure S2. Changes in the proportion of +8 cells for six patients. The percentage of +8 clones revealed by G-banding increased in three patients (UPN3, 20, 33) and decreased in two patients (UPN2, 9) after successful IST.

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INTERNAL MEDICINE

CASE REPORT

Successful Treatment of Invasive Zygomycosis Based on a Prompt Diagnosis Using Molecular Methods in a Patient with Acute Myelogenous Leukemia

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Abstract

Zygomycosis is a lethal and invasive mold infection that is often associated with hematological malignancies. The keys for successful treatment include making a rapid diagnosis and appropriately administering antifungal agents. We herein report the early diagnosis of a case of zygomycosis in a patient with acute myeloid leukemia using a deoxyribonucleic acid sequence analysis. We successfully performed allogeneic hematopoietic stem cell transplantation with the use of high-dose liposomal amphotericin B and granulocyte transfusion.

Key words: zygomycosis, liposomal amphotericin B, stem cell transplantation, polymerase chain reaction

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Introduction

Zygomycosis is an invasive mold infection caused by members of the order Mucorales that frequently occurs in patients with hematological malignancies (1). Zygomycosis can present as rhinocerebral, pulmonary or disseminated disease with a rapid clinical course and lethal outcome (2). In patients with neutropenia, mortality due to zygomycosis increases significantly if antimicrobial treatment is either inappropriate or delayed. Therefore, the key aspects to treat zygomycosis include making a rapid diagnosis and appropriately administering antifungal agents. However, making a diagnosis based on histology remains challenging, as zygomycosis is rare and the causative agent has a morphology resembling that of yeast-like or filamentous fungi. We herein report a case of disseminated *Rhizomucor pusillus* infection in a patient with acute myeloid leukemia (AML). Although it is difficult to diagnose zygomycosis based on the results of a histopathological examination only, conducting a deoxyribonucleic acid (DNA) sequence analysis can lead to a rapid diagnosis and appropriate treatment of *R. pusillus*.

Case Report

A 49-year-old man was referred to our hospital with general fatigue and a history of splenectomy for idiopathic thrombocytopenia. The patient's white blood cell count was 3.3×10^9/L, with 3% neutrophils and 6% myeloblasts, and he exhibited anemia (Hb, 6.9 g/dL) and thrombocytopenia (platelet count, 2.2×10^9/L) (Table). Bone marrow aspiration revealed an increase in myeloblasts (up to 20%) with multilineage dysplasia, including a pseudo-Pelger-Huet anomaly in the myeloid lineage, megaloblastic changes in the erythroid lineage and the presence of micromegakaryocytes. The patient was diagnosed with AML with myelodysplasia-

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Table. Laboratory Findings on Admission

RBC	188×10^4 /μL	TP	7.5 g/dL
Hb	6.9 g/dL	Alb	4.3 g/dL
Plt	2.2×10^4 /μL	T-Bil	0.3 mg/dL
WBC	3230 /μL	AST	11 U/L
Neut	4.0 %	ALT	10 U/L
Lymph	85.5 %	LDH	259 U/L
Baso	1.0 %	ALP	231 U/L
Eosino	2.5 %	BUN	17.0 mg/dL
N-myelo	1.0 %	Cr	0.76 mg/dL
Blast	6.0 %	Na	141 mEq/L
		K	3.7 mEq/L
PT-TIME	12.8 sec	Cl	101 mEq/L
PT-INR	1.10 INR	CRP	1.08 g/dL
APTT	31.8 sec		
Fib	337 mg/dL		
FDP	<2.6 μg/mL		
D-dimer	0.7 μg/mL		

related changes (MRC) based on the World Health Organization 2008 classification. A cytogenetic analysis showed a complex karyotype, including monosomy 7 (Fig. 1). The administration of combined chemotherapy with idarubicin and cytarabine was immediately started as induction therapy against AML. After the completion of the chemotherapy regimen, the patient's neutropenia and fever continued despite the use of prophylactic antifungal treatment with fluconazole and broad-spectrum antibiotics. Cultures of blood, sputum, urine and stool were negative for fungi and bacteria, and the serum levels of galactomannan antigens and β-D-glucan were not elevated. However, on day 18 after the initiation of chemotherapy, the chest computed tomography (CT) findings showed a small nodular lesion in the upper right lobe of the lung. Treatment with intravenous voriconazole was initiated under a diagnosis of possible pulmonary invasive aspergillosis infection based on the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and National Institute of Allergy and Infectious Diseases Mycoses Study Group criteria (3). Despite switching the medication regimen to voriconazole, the patient's general condition deteriorated in association with the development of hypoxia (oxygen saturation: 90-92% on room air). On day 28 after the initiation of chemotherapy, the CT findings demonstrated progressive pulmonary lesions with pleural effusion in the bilateral lungs, in addition to lesions in the kidney, liver, skin and brain (Fig. 2). Although a direct examination of cultured pleural effusion fluid using lactophenol cotton blue staining showed fungus without septate hyphae, classifying the genus and species of the organism was difficult microscopically (Fig. 3). Simultaneously, a molecular analysis of organisms in the pleural effusion was performed using polymerase chain reaction (PCR) targeting the internally transcribed spacer 1 (ITS1), 5.8S ribosomal DNA (rDNA) and ITS2 of the isolated fungus, according to a previously described method (4). Amplified PCR products of approximately 600 base pairs were sequenced and used to conduct a basic local alignment search of the GenBank sequence database (www.ncbi.nlm.nih.gov/blast/). The amplicon sequences were 100% identical to the corresponding sequences of the *R. pusillus* strain ATCC 46342 (GenBank accession number GU 256738). Based on the finding of invasive *R. pusillus* infection, treatment with intravenous liposomal amphotericin B (L-AmB) was initiated at a dose of 3 mg/kg, which was promptly increased up to 10 mg/kg, along with granulocyte colony-stimulating factor (G-CSF), which resulted in improvements in the patient's symptoms, including the recovery of neutrophils (Fig. 4). The patient achieved hematological complete remission (CR) after receiving the induction therapy. Because CT scans continued to show abnormal findings, such as abscesses in the brain, liver and kidney, in addition to pneumonia, the L-AmB therapy was continued to treat disseminated zygomycosis. The severity of these symptoms made it a priority to treat the disseminated zygomycosis over the AML with MRC (Fig. 5A).

On day 96 after the initiation of the induction therapy, the percentage of leukemic myeloblasts in the bone marrow had increased to 15%. Despite the administration of low-dose cytarabine and aclacinon in order to decrease the leukemic burden, the patient failed to achieve CR, and cytogenetic markers indicating a poor prognosis were detected, suggesting the need for immediate allogeneic hematopoietic stem cell transplantation (allo-HSCT) to achieve a cure. Certainly, the risk of exacerbating the disseminated zygomycosis was extremely high. However, we performed allo-HSCT in addition to the administration of high-dose L-AmB (10 mg/kg). The reduced-intensity conditioning regimen consisted of fludarabine (Flu; 125 mg/m^2) and intravenous busulfan (Bu; 12.8 mg/kg). Graft-versus-host disease prophylaxis consisted of cyclosporin A and mycophenolate mofetil. The CD34$^+$ peripheral blood stem cells transplanted from a human leukocyte antigen-fully matched related donor (the patient's elderly brother) were administered at a dose of 6.6×10^6/kg. In addition, G-CSF-mobilized granulocytes were deliberately transfused from the same allo-HSCT donor on days 2 and 3 and from the patient's son on days 8 and 9 after allo-HSCT. Engraftment of neutrophils was observed on day 12 after allo-HSCT (Fig. 4), and the disseminated zygomycosis was not exacerbated during the neutropenic period of allo-HSCT (Fig. 5B).

Discussion

Zygomycosis is a lethal opportunistic fungal complication observed in immunocompromised patients, particularly those with hematological malignancies, for which therapeutic options are limited. In patients with only localized infection, performing surgical excision and debridement is fundamental for a chance of cure. However, over the past decade, several new antifungal drugs suitable for clinical use and novel strategies for treating invasive fungal infection have been developed (5). Posaconazole and L-AmB are currently available and exhibit good activity against zygomycosis (2, 6), although voriconazole is not effective, as shown in the pre-

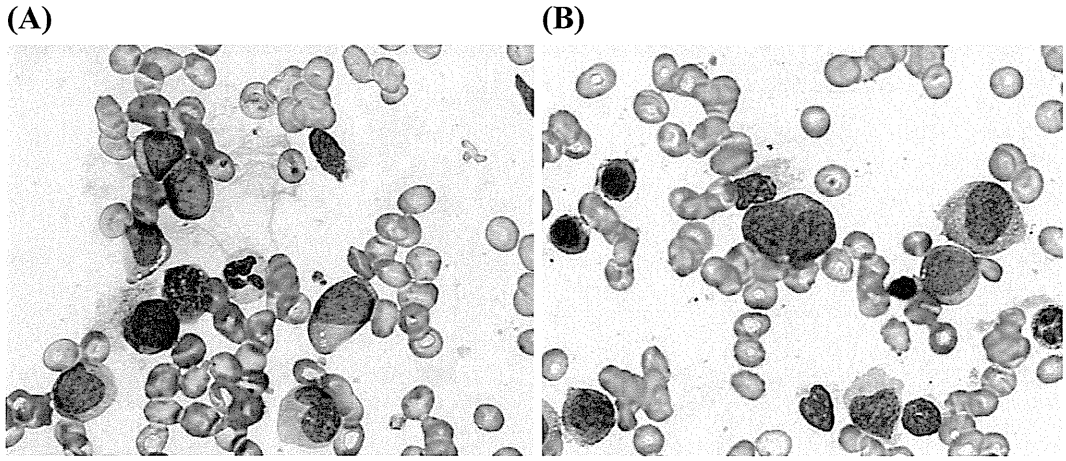


Figure 1. **Bone marrow aspirates showing hypocellular marrow with myeloblasts (A) and multilineage dysplasia (B) (Wright-Giemsa stain, ×1,000). Giemsa-banded karyogram of the bone marrow cells, as follows: 45, XY, dic(3;18)(p13;p11.2), der(5;17)(p10;q10), -7, dic(13;15)(p11.2;P11.2), add(19)(p11), +21, idic(22)(p11.2).**

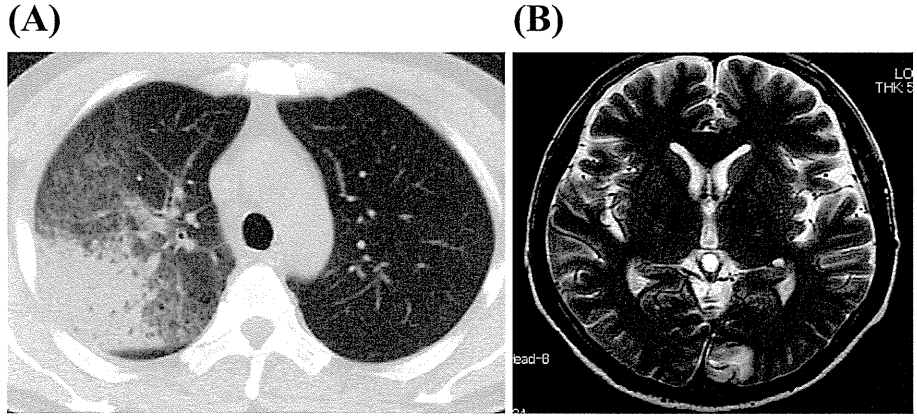


Figure 2. **(A) High-resolution chest computed tomography revealed ground-glass opacity around a consolidated area in the right lobe. (B) T2-weighted magnetic resonance imaging demonstrated cerebral infarction in the left occipital lobe.**

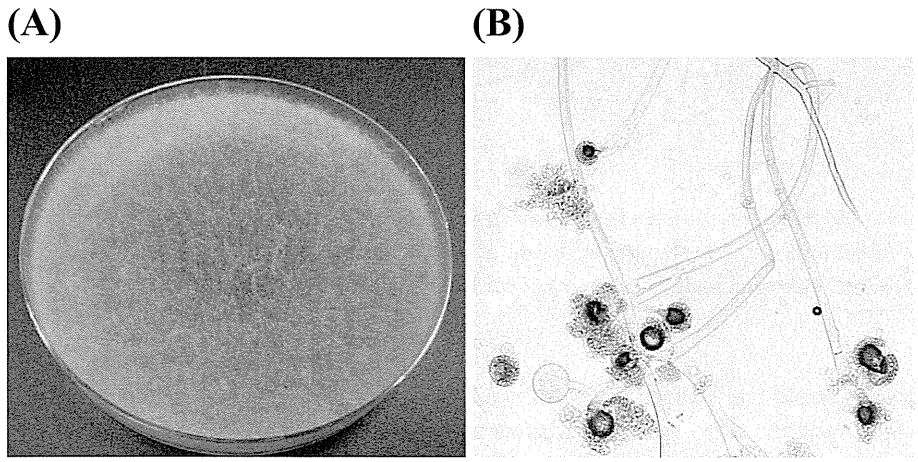
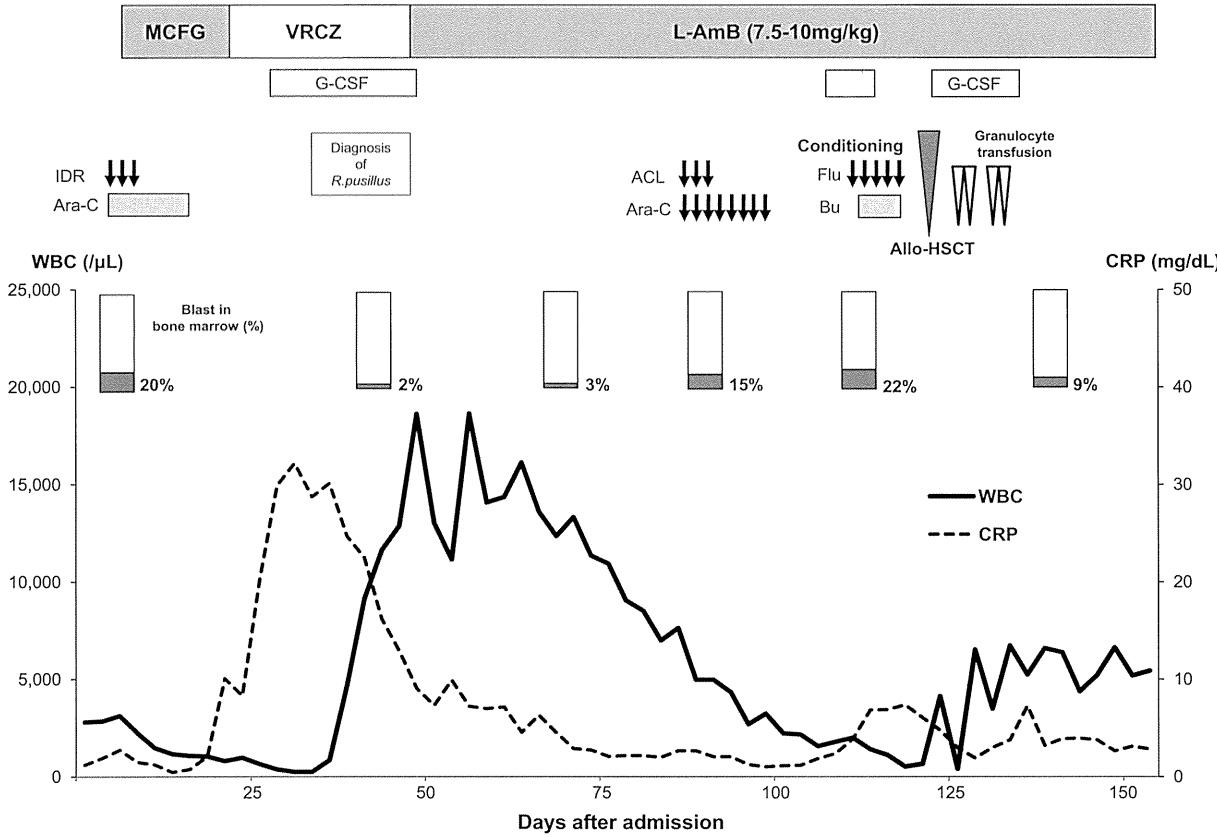


Figure 3. **(A) Colonies formed from the pleural effusion fluid cultured on Sabouraud agar at 37˚C for three days. (B) Photomicrograph of lactophenol cotton blue-stained fungi.**



Micafungin (MCFG), voriconazole (VRCZ), liposomal amphotericin B (L-AmB), idarubicin (IDR), cytarabine (Ara-C), fludarabine (Flu), busulfan (Bu), Aclacinon (ACL), *Rhizomucor pusillus* (*R. pusillus*),
granulocyte colony-stimulating factor (G-CSF), allogeneic hematopoietic stem cell transplantaion (Allo-HSCT), white blood count (WBC), C-reactive protein (CRP)

Figure 4. **Clinical course of the anti-fungal therapy for zygomycosis during chemotherapy and allogeneic hematopoietic stem cell transplantation.**

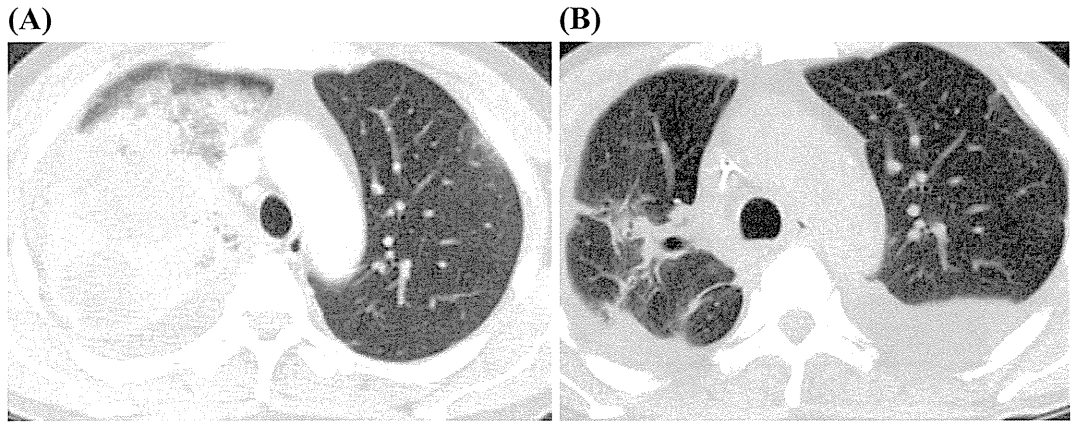


Figure 5. **(A) High-resolution chest computed tomography (HRCT) disclosed consolidation and pleural effusion on day 63 after the initiation of induction therapy. (B) HRCT performed on day 37 after allo-HSCT showed an improvement of the lesion in the right lobe.**

sent case. Because posaconazole has not yet been approved for use in Japan, L-AmB was the only choice of treatment in this case. L-AmB is available with less nephrotoxicity than conventional AmB; therefore, L-AmB can be administered safely at much higher doses (10 mg/kg), even in the setting of allo-HSCT. In addition, the transfusion of granulocytes likely decreases the risk of exacerbated invasive zygomycosis during the period of neutropenia that occurs after allo-HSCT (7, 8). Nevertheless, unlike invasive aspergillosis, zygomycosis is extremely rare, and the outcome of invasive zygomycosis treatment remains disappointing due to its association with a high mortality rate in immunocompromised patients (9).

In the present case, combined treatment consisting of the

transfusion of high-dose L-AmB (2) and granulocytes (8) may account for the positive treatment outcome. However, the primary key to the successful treatment of disseminated zygomycosis was the rapid diagnosis of *R. pusillus* using the PCR method (10), which enabled the patient to undergo allo-HSCT in association with directed treatment comprising high-dose L-AmB, improving the prognosis and chance of survival (11). In general, diagnosing zygomycosis is more difficult than other fungal infections, as there are no specific clinical signs or biomarkers for identifying this fungal species (5). The serum levels of Aspergillus GM antigens or β-D-glucan are usually not elevated in patients with invasive zygomycosis. Therefore, conducting an early examination upon the suspicion of zygomycosis is very important, and tissue biopsies, the gold standard for diagnosis, should be performed in order to document zygomycosis when possible. Nevertheless, the rate of positive fungal cultures is usually low (12), and it is difficult to morphologically classify fungi to the species level. In this case, *R. pusillus* was detected in the pleural effusion, which is usually sterile, using PCR. We believe that PCR-based methods are useful for promptly confirming the classification of zygomycosis at the species level (13). In order to appropriately select antifungal agents, differentiating zygomycosis from more common opportunistic molds, such as Aspergillus, is also very important. Voriconazole, which is used as a first-line treatment for invasive pulmonary aspergillosis, does not exhibit good activity against zygomycosis (3). *Cunninghamella* species, which can cause zygomycosis with an aggressive clinical course, must be treated with high-dose L-AmB (14). Because molecular techniques are not widely clinically validated, they should be used to make a definitive diagnosis with caution in combination with morphological methods.

In conclusion, the correct identification and early diagnosis of invasive zygomycosis is necessary to initiate timely and appropriate antifungal therapy, as invasive zygomycosis can have a fatal outcome. The PCR method is useful for obtaining the appropriate diagnosis; however, further validation is required before this method can be routinely used in the clinical setting.

The authors state that they have no Conflict of Interest (COI).

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LETTER TO THE EDITOR

Monitoring mycophenolate mofetil is necessary for the effective prophylaxis of acute GVHD after cord blood transplantation

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Use of mycophenolate mofetil (MMF), a novel immunosuppressant that inhibits T- and B-cell proliferation, together with a calcineurin inhibitor (CNI) has recently increased in cord blood transplantation (CBT) to reduce severe GVHD and pre-engraftment syndrome (PES).[1,2] Wide interpatient variability has been reported in the plasma levels of mycophenolate (MPA), an active form of MMF, even after the same MMF exposure; however, few reports have performed therapeutic drug monitoring in CBT.[3] Therefore, we performed a prospective cohort study to (1) determine the correlation between MPA concentration and incidence of severe PES or acute GVHD (aGVHD) after CBT and (2) compare the incidence of these complications with the historical cohort using CNI alone for GVHD prophylaxis after CBT.

We continuously enrolled 24 adult patients who underwent single-unit CBT with GVHD prophylaxis using CNI and MMF at our department between September 2011 and December 2013. The protocols were approved by the Ethics Committee of Kyoto University. The historical cohort consisted of 38 patients who received single-unit CBT with GVHD prophylaxis using CNI alone between June 2003 and August 2011. Patient characteristics and CBT procedures are summarized in Table 1, and definition of transplantation risk and myeloablative conditioning regimens were in accordance with previous studies.[4,5]

MMF was orally administered at 10 mg/kg, three times a day (i.e. total dose=30 mg/kg/day) (with the exact intervals of 8 h), from Day 0 to Day 30. The MMF dosage was not modified during the clinical course after CBT. PES was defined as noninfectious fever with an unexplained skin rash before neutrophil engraftment.[6] Severe PES was defined according to a previous report.[2]

Blood samples were collected immediately before and 1, 2 and 4 h after the morning administration of MMF on Day 7 (first week) and Day 21 (third week) after CBT. Total MPA levels in the plasma were measured using the enzyme multiplied immunoassay technique (EMIT)[7] (C_0, C_1, C_2 and C_4, respectively), and C_8 (which means the trough level of the next administration) was assumed to be equal to C_0 values based on results of our preliminary analysis ($n=12$ both at the first and third weeks) and other studies.[3,8,9] Area under the curve $(AUC)_{0-8\,h}$ was determined using the linear trapezoidal method, and the $AUC_{0-24\,h}$ was calculated as $3 \times AUC_{0-8\,h}$. This method is one of the standards in the setting of MMF administration three times a day (every 8 h),[3,8] because MPA concentrations reached to the peak within 2 h from administration, and decreased linearly after 4 h.[9] Patients whose $AUC_{0-24\,h}$ was below the lower quartile were categorized in 'the lower concentration group' at each time point (first and third week). We set our threshold at the lower quartile in order to find out the minimally required concentration of MPA $AUC_{0-24\,h}$ to prevent PES and aGVHD effectively.

Cumulative incidence of PES and aGVHD was calculated in each cohort using Gray's method, and relapse or early death was considered a competing risk. Statistical analyses were performed using R (The R Foundation for Statistical Computing, version 2.13.0). The alpha level of all the tests and the *P*-value were set at 0.05.

MPA measurements revealed that C_1 showed the highest values among the four points (mean ± s.d. in the first and third week; C_0, 0.92 ± 0.87 and 0.92 ± 0.84 μg/mL; C_1, 5.96 ± 3.88 and 5.47 ± 4.11 μg/mL; C_2, 4.18 ± 1.87 and 3.63 ± 1.99 μg/mL; and C_4, 1.56 ± 0.81 and 1.52 ± 0.97 μg/mL). The $AUC_{0-24\,h}$ was 54.3 ± 21.9 μg h/mL (mean ± s.d.) in the first week and 50.0 ± 22.2 μg h/mL in the third week. The threshold of lower or higher MPA $AUC_{0-24\,h}$ levels was set at 40 μg h/mL in both the first and third weeks, because the lower quartile values were 38.7 and 35.6 μg h/mL, respectively.

As per results, severe PES was observed in two patients, whose $AUC_{0-24\,h}$ in the first week was 23.0 μg h/mL (the lowest in the MMF cohort) and 84.0 μg h/mL (the third highest), indicating that severe PES may occur regardless of the MPA concentration. Actually, there was no statistical difference in the incidence of severe PES between the two groups in the first week (lower vs higher concentration groups; $n=7$ vs $n=17$; incidence 14.3% vs 5.9%, $P=0.53$, respectively). The incidence of severe PES for the entire MMF group (8.3%) was significantly lower than that of the historical control cohort (CNI alone; 31.6%, $P=0.02$) (Figure 1a). On the other hand, the incidence of aGVHD (grade 2–4) was significantly higher in the lower concentration group ($n=8$) compared with the higher cohort in the third week ($n=16$; 75.0% vs 31.2% on Day 100, $P=0.02$; Figure 1b). This difference was still significant after adjusting for various confounding factors between the two groups (age, transplantation risk, conditioning regimens, donor-recipient sex and ABO mismatch and prior severe PES), and the relative risk was 8.05 (95% confidence interval 1.09–59.7, $P=0.04$). The incidence of aGVHD in the whole MMF group (45.8%) was similar to the historical cohort (39.5%, $P=0.72$; Figure 1c).

The relapse rate was unrelated to the MPA concentration (the higher AUC group, 22.8%; the lower group, 16.7% at 1 year; $P=0.76$), and that rate was almost the same both in the entire MMF group and in the historical cohort (MMF group, 20.6%; historical cohort, 28.6% at 1 year, $P=0.54$). As for infection, the incidence of severe bacterial infection (documented bacteremia up to Day 100) was 25.0% in the MMF group, similar to that in the historical cohort (31.6%, $P=0.62$). The incidence of human herpes virus (HHV)-6 reactivation was higher in the MMF group (53.6% at Day 100) than in the historical cohort (34.2%, $P=0.15$). There was no increase in the incidence of other viral or fungal infections.

In the present prospective cohort study, we demonstrated that (1) a certain level of MPA is necessary to effectively prevent aGVHD after CBT and (2) severe PES can be reduced with the addition of MMF to CNI as GVHD prophylaxis. First, we observed that MPA levels and the $AUC_{0-24\,h}$ widely fluctuated among patients and time points after CBT because the kinetics of MMF are drastically influenced by gastrointestinal damages caused by preparative regimens, loss of appetite and decreased oral intake or other drug interactions.[10] However, appropriate concentrations are still not confirmed in patients who undergo CBT.[1] We suggest

Table 1. Patient characteristics

Variables		*CNI+MMF (n = 24)*	*Historical cohort (CNI alone, n = 38)*	P-*value*
Sex	Male/female	17/7	20/18	0.19
Age (years)	Median (range)	48.5 (19–65)	49 (18–65)	0.89
Disease	Leukemia/lymphoma	22/2	27/9	0.14
Transplantation risk	High/standard	14/10	21/17	1.00
Donor-recipient sex mismatch	Y/N	8/16	22/16	0.07
Donor-recipient ABO mismatch	Major/minor/both/none	10/4/2/8	14/2/10/12	0.21
NCC (×10E7 cells/kg)	Median (range)	2.47 (1.90–3.76)	2.96 (1.51–5.40)	0.12
Conditioning	MAC/RIC	8/16	9/29	0.56
TBI	Y/N	20/4	32/6	0.94
GVHD prophylaxis	FK506/CsA	23/1	36/2	1.00

Abbreviations: CNI = calcineurin inhibitor; MAC = myeloablative conditioning; MMF = mycophenolate mofetil; N = no; NCC = nuclear cell count; RIC = reduced-intensity conditioning; Y = yes.

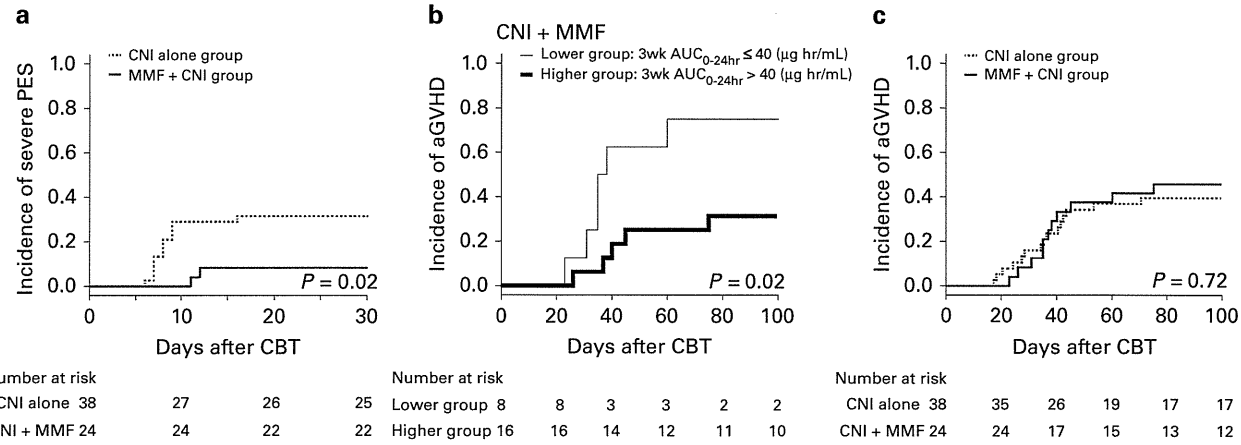

Figure 1. Incidence of severe PES and aGVHD (grade 2–4) in each cohort. (**a**) Incidence of severe PES. The MMF group (including the lower and higher concentration groups) had a significantly lower incidence of severe PES compared with the historical cohort (CNI alone; $P = 0.02$). (**b** and **c**) Incidence of aGVHD (grade 2–4). (**b**) The lower concentration group had a significantly higher incidence of aGVHD than the higher group ($P = 0.02$). (**c**) The incidence was the same in the historical cohort (CNI alone) and the entire MMF group ($P = 0.72$).

that an $AUC_{0-24\,h}$ of 40 μg h/mL in the third week should be the minimal requirement. In contrast, another group calculated $AUC_{0-24\,h}$ just as the same strategy with ours, and reported that even $AUC_{0-24\,h} < 30$ μg h/mL was sufficient to decrease the incidence of aGVHD in a small cohort study.[3] Their patient characteristics, transplant procedures and strategies of MMF administration and AUC calculation were almost the same as those used for our patients. Therefore, these controversial data should be validated in large-scale prospective studies.

Next, we demonstrated that even lower concentrations of MMF could reduce the incidence of severe PES compared with CNI alone. Severe PES may increase the risk of infection and organ dysfunction, leading to high rates of TRM.[2] A previous study demonstrating that CBT after reduced-intensity conditioning regimens decreased the incidence of severe PES with GVHD prophylaxis using MMF plus CNI compared with CNI alone support our results.[2] An increase in relapse was not shown in the MMF cohort, and the infection rate was almost the same except for HHV-6 reactivation. These data suggest that GVHD prophylaxis using MMF and CNI may be a more advantageous strategy compared with CNI alone.

In summary, drug monitoring of MMF is necessary for the effective prophylaxis of aGVHD after CBT. This study has the limitation of a small patient number, so large-scale prospective studies are needed to substantiate our results and to determine the optimal GVHD prophylaxis regimen. Moreover, measuring the levels of the unbound form of MPA[11] or the activity of inosine monophosphate dehydrogenase isoenzymes[12] may be necessary to analyze the direct effects of MMF on lymphocytes after CBT.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Comparison of Cord Blood Transplantation with Unrelated Bone Marrow Transplantation in Patients Older than Fifty Years

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A B S T R A C T

We retrospectively compared the transplantation outcomes for patients 50 years or older who received umbilical cord blood transplantation (UCBT) with those who received unrelated bone marrow transplantation (UBMT) for hematologic malignancies. A total of 1377 patients who underwent transplantation between 2000 and 2009 were included: 516 received 8/8 HLA allele-matched UBMT, 295 received 7/8 HLA allele-matched UBMT, and 566 received 4/6 to 6/6 HLA-matched UCBT. Adjusted overall survival (OS) was significantly lower in those who underwent UCBT than those who underwent 8/8 HLA–matched UBMT but was similar to that of 7/8 HLA–matched UBMT (the 2-year OS after 8/8 HLA–matched UBMT, 7/8 HLA–matched UBMT, and UCBT were 49% [95% confidence interval (CI), 45% to 55%], 38% [95% CI, 32% to 45%], and 39% [95% CI, 34% to 43%], respectively). However, adjusted OS was similar between 8/8 HLA–matched UBMT and UCBT receiving $\geq .84 \times 10^5$ $CD34^+$ cells/kg among those with acute myeloid leukemia and those with acute lymphoblastic leukemia (the 2-year OS was 49% [95% CI, 43% to 55%], and 49% [95% CI, 41% to 58%], respectively). These data suggest that UCB is a reasonable alternative donor/stem cell source for elderly patients with similar outcomes compared with UBM from 8/8 HLA–matched unrelated donors when the graft containing $\geq .84 \times 10^5$ $CD34^+$ cells/kg is available.

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INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is a curative treatment for patients with high-risk hematologic malignancies. The frequency of adverse cytogenetic abnormalities is higher in elderly patients with acute myeloid leukemia (AML) or acute lymphoblastic leukemia (ALL) than in younger patients, and overall survival (OS) after intensive chemotherapy in elderly patients is shorter than that in younger patients [1,2]. Inductions of reduced-intensity and nonmyeloablative stem cell transplantations allow elderly patients to receive allogeneic HSCT [3,4], and these patients have increasingly received this type of transplantation [5]. Only approximately 30% of patients

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have an HLA-identical sibling, and some elderly patients have siblings who cannot serve as a donor because of their age or underlying comorbidities; in such cases, an alternative donor is needed.

HLA-matched unrelated bone marrow or peripheral blood stem cells have been used as an alternative to an HLA-identical sibling donor. Umbilical cord blood has been used more frequently over the past decade, and several studies and meta-analyses have compared the outcomes of umbilical cord blood transplantation (UCBT) with that of unrelated bone marrow transplantation (UBMT) or unrelated peripheral blood stem cell transplantation (UPBSCT) [6-15]. However, the findings of those reports varied, and most of those studies included a small number of elderly patients. To the best of our knowledge, there has been no report that compared the outcomes of elderly patients who received UCBT with those who received UBMT or UPBSCT. Therefore, the main objective of this study was to compare the outcomes of patients 50 years or older who received UCBT with those who received UBMT using the Japanese nationwide registry data.

METHODS

Data Collection

Data regarding transplantations were extracted from the Transplant Registry Unified Management Program system of the Japan Society for Hematopoietic Cell Transplantation [16]. A total of 171 transplantation centers performed unrelated HSCT for adults and reported transplantation data to Japan Society for Hematopoietic Cell Transplantation between 2000 and 2009. All patients gave written informed consent at each transplantation center. The trial was conducted in accordance with the Declaration of Helsinki.

Patients with acute leukemia or myelodysplastic syndrome (MDS) who were 50 years or older and who received unrelated HSCT between 2000 and 2009 were included. Because the bone marrow was exclusively harvested from volunteer unrelated donors in Japan, cases of peripheral blood stem cell transplantation were not included in this analysis. Only 7 patients received double UCBT; therefore, these patients were also excluded. For the bone marrow recipients, recipients whose HLA matched 8/8 or 7/8 with their donor at the allelic level for HLA-A, HLA-B, HLA-C, and HLA-DRB1 were included. For UCBT, recipients whose HLA matched 4/6 to 6/6 with their donor at the antigen level for HLA-A and HLA-B and at the allelic level for HLA-DRB1, and who received a single unit of umbilical cord blood containing 2.0×10^7 or more total nucleated cells per kilogram of recipient's body weight at cryopreservation were included. Patients who had previously received autologous or allogeneic transplantation were excluded.

A myeloablative conditioning (MAC) regimen was defined as a total busulfan dose of more than 8 mg/kg, total melphalan dose of more than 140 mg/kg, fractionated total body irradiation (TBI) of 8 Gy or more, or single TBI of 5 Gy or more [17,18]. Other conditioning regimen was defined as reduced-intensity conditioning (RIC). Acute leukemia in the first complete remission (CR), refractory anemia with or without ringed sideroblasts, and refractory cytopenia with multilineage dysplasia for MDS were defined as early phase; acute leukemia in the second or subsequent CR were defined as intermediate phase; and all other statuses were defined as advanced phase. The karyotype at diagnosis for AML, ALL, and MDS were classified as previously reported [2,19,20]. The year of transplantation was divided into 2 groups: 2000 to 2004 was defined as the early period and 2005 to 2009 was defined as the recent period. Neutrophil recovery was defined as the first 3 consecutive days in which absolute neutrophil counts rose to greater than or equal to 500/mm^3. Acute graft-versus-host disease (GVHD) was evaluated based on standard criteria [21]. Chronic GVHD was defined according to the classical classification [22]. Relapse was defined as disease recurrence detected by hematological examination or detected by cytogenetic or molecular examination and requiring any treatment. Patients who did not obtain CR after HSCT were defined as patients who had a relapse the next day after HSCT. Nonrelapse mortality (NRM) was defined as death without relapse. OS was defined as the survival time from the date of transplantation to death from any cause or the last follow-up.

Statistical Analysis

The demographic factors and disease characteristics were compared between patients who underwent transplantation with 8/8 HLA–matched unrelated bone marrow, 7/8 HLA–matched bone marrow, and umbilical cord blood using Fisher's exact test for the categorical data and the Mann-Whitney U test for the continuous variables. OS was calculated from the date of transplantation to death from any cause or last follow-up and was estimated by the Kaplan-Meier method. Cox proportional hazards regression model was used for the multivariate analyses. Adjusted comparison of the stem cell source on OS was performed using the Cox proportional hazards regression model. Gray's test was employed for the comparison of cumulative incidence curves for relapse, NRM, neutrophil and platelet recoveries, and GVHD [23]. NRM and relapse were the competing event for each other. For neutrophil and platelet recovery, death before neutrophil or platelet recovery was the competing event; for GVHD, death without GVHD was the competing event. Fine and Gray's proportional hazard regression model was employed for multivariate analyses with competing risks [24]. Multivariate analyses to compare the effect of stem cell source on transplantation outcomes were performed with the consideration of other significant clinical variables in the final models, which were built with the significant variables ($P < .10$) from the univariate analysis, which were then deleted in a stepwise fashion from the model when a variable was not statistically significant ($P > .05$). The stem cell source was added in the final model. The following variables were considered: patient age at transplantation, sex, primary disease (AML versus ALL versus MDS), karyotype at diagnosis (favorable versus intermediate versus adverse), disease status at transplantation (early phase versus intermediate phase versus advanced phase), year of transplantation (early period versus recent period), conditioning regimen (MAC versus RIC), use of TBI, and GVHD prophylaxis (cyclosporine alone versus cyclosporine and other agent versus tacrolimus alone versus tacrolimus and other agent versus other). All tests were 2-sided, and $P < .05$ was considered to indicate statistical significance. Analyses were performed with EZR version 1.20 (Saitama Medical Center, Jichi Medical University) [25], which is a graphical user interface for R version 3.0.2 (R Development Core Team, Vienna, Austria).

RESULTS

Patients and Transplantation Characteristics

Patients and transplantation characteristics are shown in Table 1. A total of 1377 patients were included in this analysis, and of those, 516 patients received 8/8 HLA allele-matched UBMT, 295 patients received 7/8 HLA allelic-matched UBMT, and 566 patients underwent transplantation from 4/6 to 6/6 HLA–matched UCBT. The UCBT recipients were significantly older than the 8/8 or 7/8 HLA–matched UBMT recipients ($P < .001$), and more UCBT recipients underwent RIC or nonmyeloablative transplantation ($P < .001$) and received a TBI-containing conditioning regimen than did the 8/8 or 7/8 HLA–matched UBMT recipients ($P < .001$). More UCBT recipients had advanced phase disease ($P < .001$). Female donor to male recipient transplantation was included in UCBT more than in UBMT ($P < .001$). Compared with those receiving UBMT, more UCBT recipients had AML ($P < .001$) and received GVHD prophylaxis with a single-agent regimen ($P < .001$). The distribution of karyotype at diagnosis was similar (Supplemental Tables 1–3). The distribution of recipients' sex and year of transplantation were similar among the 3 groups. The median duration of follow-up for the surviving patients who underwent transplantation with 8/8 HLA–matched UBMT, 7/8 HLA–matched UBMT, and 4/6 to 6/6 HLA-matched UCBT was 23.7 months (range, 1.8 to 125.2 months), 18.6 months (range, 1.6 to 94.0 months), and 22.3 months (range, .1 to 107.5 months), respectively.

Hematopoietic Recovery

The median time from transplantation to neutrophil recovery in patients who underwent 8/8 HLA–matched UBMT, 7/8 HLA–matched UBMT, and 4/6 to 6/6 HLA–matched UCBT was 17 days (range, 1 to 100 days), 17 days (range, 4 to 169 days), and 24 days (range, 0 to 95 days), respectively. Neutrophil recovery was faster in recipients with early phase disease or intermediate phase disease than in those with advanced phase disease ($P < .001$). MAC was an independent negative predictor for neutrophil engraftment ($P = .007$). The

Table 1
Patients, Disease, and Transplantation Characteristics

Characteristic	Total	8/8 HLA–Matched Bone Marrow	7/8 HLA–Matched Bone Marrow	Umbilical Cord Blood	*P* Value
Number	1377	516	295	566	
Sex (male)	816 (59%)	310 (60%)	188 (64%)	318 (56%)	.091
Age, median (range), yr	57 (50-82)	56 (50-70)	57 (50-71)	58 (50-82)	<.001
50-59	892 (65%)	376 (73%)	198 (67%)	318 (56%)	
60-69	468 (34%)	138 (27%)	96 (33%)	234 (41%)	
70 or older	17 (1%)	2 (<1%)	1 (<1%)	14 (3%)	
Sex matching					<.001
Female donor to male recipient	1030 (75%)	73 (14%)	67 (23%)	153 (27%)	
Others	293 (21%)	443 (86%)	227 (77%)	360 (64%)	
Unknown	54 (4%)	0 (0%)	1 (<1%)	53 (9%)	
Body weight, median (range), kg	56 (32.0-102.4)	58.5 (32.0-102.4)	58.9 (35.1-92.0)	54.0 (32.0-86.0)	<.001
Disease					<.001
AML	902 (65%)	314 (61%)	180 (61%)	408 (72%)	
ALL	244 (18%)	96 (19%)	47 (16%)	101 (18%)	
MDS	231 (17%)	106 (20%)	68 (23%)	57 (10%)	
Disease status at transplantation					<.001
Early phase	471 (34%)	223 (43%)	94 (32%)	154 (27%)	
Intermediate phase	221 (16%)	82 (16%)	58 (20%)	81 (14%)	
Advanced phase	685 (50%)	211 (41%)	143 (48%)	331 (59%)	
Year of transplantation					
2000-2004	343 (25%)	128 (25%)	74 (25%)	141 (25%)	1
2005-2009	1034 (75%)	388 (75%)	221 (75%)	425 (75%)	
Conditioning regimen					
Myeloablative	653 (47%)	291 (56%)	147 (50%)	215 (38%)	<.001
CY + TBI (≥8 Gy)	174 (12%)	79 (15%)	43 (15%)	52 (9%)	
CY + TBI (≥8 Gy) + other	135 (10%)	46 (9%)	19 (6%)	70 (13%)	
BU + CY	110 (8%)	64 (12%)	33 (12%)	13 (2%)	
FLU + BU (>8 mg/kg)	44 (3%)	34 (7%)	5 (2%)	5 (1%)	
FLU + BU (>8 mg/kg) + TBI (<8 Gy)	40 (3%)	14 (3%)	7 (2%)	19 (3%)	
FLU + MEL (>140 mg/m^2)	57 (4%)	28 (5%)	20 (7%)	9 (2%)	
Other TBI-based regimen	66 (5%)	19 (4%)	13 (4%)	34 (6%)	
Other BU-based regimen	27 (2%)	7 (1%)	7 (2%)	13 (2%)	
RIC/NMA	712 (52%)	217 (42%)	145 (49%)	350 (62%)	
FLU + BU (≤8 mg/kg)	25 (2%)	5 (1%)	5 (2%)	15 (3%)	
FLU + BU (≤8 mg/kg) + TBI (<8 Gy)	206 (15%)	91 (17%)	58 (20%)	57 (10%)	
FLU + BU (≤8 mg/kg) + MEL (≤140 mg/m^2)	26 (2%)	13 (3%)	5 (2%)	8 (1%)	
FLU + BU (≤8 mg/kg) + other	33 (2%)	12 (2%)	16 (5%)	5 (1%)	
FLU + MEL (≤140 mg/m^2)	64 (5%)	33 (6%)	16 (5%)	15 (3%)	
FLU + MEL (≤140 mg/m^2) + TBI (<8 Gy)	219 (16%)	33 (6%)	26 (9%)	160 (28%)	
FLU + MEL (≤140 mg/m^2) + TBI (<8 Gy) + other	20 (2%)	3 (1%)	1 (<1%)	16 (3%)	
FLU + CY + TBI (<8 Gy)	56 (4%)	3 (1%)	2 (1%)	51 (9%)	
Other regimen including TBI (<8 Gy)	33 (2%)	13 (3%)	10 (3%)	10 (2%)	
Other regimen not including TBI (<8 Gy)	30 (2%)	11 (2%)	6 (2%)	13 (2%)	
Unknown	12 (1%)	8 (2%)	3 (1%)	1 (<1%)	
TBI-containing conditioning regimen	962 (70%)	306 (59%)	184 (62%)	472 (83%)	<.001
Addition of ATG to conditioning regimen	46 (3%)	17 (3%)	19 (6%)	10 (2%)	.001
GVHD prophylaxis					
CyA + other	370 (27%)	129 (25%)	52 (18%)	189 (33%)	<.001
CyA alone	68 (5%)	5 (1%)	3 (1%)	60 (11%)	
TAC + other	775 (56%)	359 (70%)	226 (76%)	190 (33%)	
TAC alone	138 (10%)	15 (3%)	11 (4%)	112 (20%)	
Others	13 (1%)	7 (1%)	3 (1%)	3 (1%)	
None	13 (1%)	1 (<1%)	0 (0%)	12 (2%)	
Total cell dose (range, ×10^7/kg)				2.56 (2.00-5.62)	
CD34$^+$ cell dose (range, ×10^5/kg)				.83 (.01-14.02)	
HLA-A, B, DR antigen level					
Matched (6/6)		516 (100%)	295 (100%)	46 (8%)	
One-antigen mismatched (5/6)		0	0	159 (28%)	
Two-antigen mismatched (4/6)		0	0	361 (64%)	

HLA indicates human leukocyte antigen; TBI, total body irradiation; GVHD, graft-versus-host disease; CY, cyclophosphamide; BU, busulfan; FLU, fludarabine; MEL, melphalan; NMA, nonmyeloablative; ATG, antithymocyte globulin; CyA, cyclosporine A; TAC, tacrolimus.

probability of neutrophil recovery by day 50 was significantly lower in recipients of 4/6 to 6/6 HLA–matched UCBT (72% [95% confidence interval (CI), 68% to 75%]) than in those of 8/8 HLA–matched UBMT (95% [95% CI, 92% to 96%]) or 7/8 HLA–matched UBMT (90% [95% CI, 85% to 93%]). On multivariate analysis, the 4/6 to 6/6 HLA–matched UCBT was an independent negative predictor for neutrophil engraftment when compared with the 8/8 HLA–matched UBMT (hazard ratio [HR], .43 [95% CI, .38 to .50]; $P < .001$) and the 7/8 HLA–matched UBMT (HR, .47 [95% CI, .40 to .56]; $P < .001$) (Table 2).

The probability of platelet recovery by day 180 was also significantly lower in the 4/6 to 6/6 HLA–matched UCB recipients (54% [95% CI, 50% to 58%]) than in those who received the 8/8 HLA–matched UBMT (83% [95% CI, 79% to 86%]) or the 7/8 HLA–matched UBMT (75% [95% CI, 70% to 80%]). The median times from transplantation to platelet recovery in the recipients of 8/8 HLA–matched

Table 2
Multivariate Analysis of Transplantation Outcomes

Outcome	HR (95% CI)	*P* Value
Overall survival*	Overall	<.001
4/6-6/6–Matched UCB versus 8/8 HLA–matched UBM	1.47 (1.24-1.74)	<.001
4/6-6/6–Matched UCB versus 7/8 HLA–matched UBM	1.03 (.86-1.24)	.75
Relapse†	Overall	.02
4/6-6/6–Matched UCB versus 8/8 HLA–matched UBM	1.35 (1.05-1.74)	.02
4/6-6/6–Matched UCB versus 7/8 HLA–matched UBM	1.18 (.89-1.56)	.26
NRM‡	Overall	.013
4/6-6/6–Matched UCB versus 8/8 HLA–matched UBM	1.32 (1.06-1.64)	.013
4/6-6/6–Matched UCB versus 7/8 HLA–matched UBM	.98 (.77-1.25)	.88
Neutrophil recovery§	Overall	<.001
4/6-6/6–Matched UCB versus 8/8 HLA–matched UBM	.42 (.37-.48)	<.001
4/6-6/6–Matched UCB versus 7/8 HLA–matched UBM	.47 (.40-.55)	<.001
Platelet recovery‖	Overall	<.001
4/6-6/6–Matched UCB versus 8/8 HLA–matched UBM	.36 (.30-.42)	<.001
4/6-6/6–Matched UCB versus 7/8 HLA–matched UBM	.44 (.37-.53)	<.001
Grade II-IV acute GVHD¶	Overall	.36
4/6-6/6–Matched UCB versus 8/8 HLA–matched UBM	1.10 (.89-1.36)	.38
4/6-6/6–Matched UCB versus 7/8 HLA–matched UBM	.69 (.56-.87)	.001
Extensive chronic GVHD#	Overall	.022
4/6-6/6–Matched UCB versus 8/8 HLA–matched UBM	.65 (.46-.92)	.015
4/6-6/6–Matched UCB versus 7/8 HLA–matched UBM	.56 (.38-.82)	.003

UCB indicates umbilical cord blood; UBM, unrelated bone marrow.

* For overall survival, hazard ratio is adjusted with recipient age, sex, primary disease, disease status at transplantation, and year of transplantation.

† For relapse, hazard ratio is adjusted with primary disease, the use of TBI, the use of antithymocyte globulin, and disease status at transplantation.

‡ For NRM, hazard ratio is adjusted with recipient sex, the use of TBI, and year of transplantation.

§ For neutrophil recovery, hazard ratio is adjusted with disease status at transplantation, conditioning regimen, the use of TBI, and GVHD prophylaxis.

‖ For platelet recovery, hazard ratio is adjusted with recipient sex, disease status at transplantation, the use of TBI, year of transplantation, and GVHD prophylaxis.

¶ For grade II to IV acute GVHD, hazard ratio is adjusted with age, disease status at transplantation, and the use of TBI.

\# For extensive chronic GVHD, hazard ratio is adjusted with recipient sex.

UBMT, 7/8 HLA–matched UBMT, and 4/6 to 6/6 HLA–matched UCBT were 29 days (range, 1 to 228 days), 32 days (range, 1 to 323 days), and 66 days (range, 8 to 230 days), respectively. Platelet recovery was also faster in recipients with early phase disease or intermediate phase disease than in those with advanced phase disease in ($P < .001$). A 4/6 to 6/6 HLA–matched UCBT was a strong independent negative predictor for platelet engraftment within the multivariate analysis (versus 8/8 HLA–matched UBMT, HR, .36 [95% CI, .30 to .42]; $P < .001$, versus 7/8 HLA–matched UBMT, HR, .44 [95% CI, .37 to .53]; $P < .001$, respectively) (Table 2). MAC was not a negative predictor for platelet engraftment.

GVHD

The cumulative incidence of grade II to IV acute GVHD by 100 days after transplantation was lower in recipients of an 8/8 HLA–matched UBMT (34% [95% CI, 30% to 39%]) than in recipients of a 7/8 HLA–matched UBMT (50% [95% CI, 44% to 56%]) or a 4/6 to 6/6 HLA–matched UCBT (41% [95% CI, 36% to 45%]). More recipients who received a TBI-containing regimen experienced grade II to IV acute GVHD by day 100 than did those who received a non-TBI regimen (43% [95% CI, 40% to 46%] versus 34% [95% CI, 29% to 39%], $P = .001$). The 4/6 to 6/6 HLA–matched UCBT recipients had a similar risk of grade II to IV acute GVHD to the 8/8 HLA–matched UBMT recipients within the multivariate analysis (HR, 1.10 [95% CI, .89 to 1.36]; $P = .38$) (Table 2). However, the 4/6 to 6/6 HLA–matched UCBT recipients had a significantly lower risk of grade II to IV acute GVHD than did the 7/8 HLA–matched UBMT recipients (HR, .69 [95% CI, .56 to .87]; $P = .001$) (Table 2).

The cumulative incidence of the extensive type of chronic GVHD by 2 years after transplantation was lower in recipients of the 4/6 to 6/6 HLA–matched UCB (15% [95% CI, 11% to 19%]) than in those who received the 8/8 HLA–matched UBMT or 7/8 HLA–matched UBMT (23% [95% CI, 19% to 27%] and 25% [95% CI, 20% to 32%], respectively). The same relationship was observed when performing the multivariate analysis (versus 8/8 HLA–matched UBMT, HR, .65 [95% CI, .46 to .92]; $P = .015$, versus 7/8 HLA–matched UBMT, HR, .56 [95% CI, .38 to .82]; $P = .003$, respectively) (Table 2).

Relapse

The cumulative incidence of relapse by 2 years was significantly higher in patients receiving the 4/6 to 6/6 HLA–matched UCBT (26% [95% CI, 22% to 30%]) than in those who received the 8/8 HLA–matched UBMT (18% [95% CI, 15% to 22%]) or those who received the 7/8 HLA–matched UBMT (21% [95% CI, 16% to 26%]). However, according to disease status at transplantation, the relapse rate by 2 years after the 8/8 HLA–matched UBMT, 7/8 HLA–matched UBMT, and 4/6 to 6/6 HLA–matched UCBT were not statistically different regardless of disease status at transplantation (8/8 HLA–matched UBMT, 7/8 HLA–matched UBMT, and 4/6 to 6/6 HLA–matched UCBT; early phase disease, 11% [95% CI, 7% to 16%], 15% [95% CI, 8% to 23%], and 19% [95% CI, 12% to 26%]; intermediate phase disease, 22% [95% CI, 13% to 32%], 26% [95% CI, 15% to 39%], and 17% [95% CI, 10% to 27%]; advanced phase disease, 35% [95% CI, 28% to 42%], 36% [95% CI, 28% to 44%], and 43% [95% CI, 38% to 49%], respectively) (Figure 1A–C). On multivariate analysis, the 4/6 to 6/6 HLA–matched UCBT recipients had a significantly higher risk of relapse than did the recipients of the 8/8 HLA–matched UCBT (HR, 1.35 [95% CI, 1.05 to 1.74]; $P = .02$) and had a similar risk to that of the 7/8 HLA–matched UBMT recipients (HR, 1.18 [95% CI, .89 to 1.56]; $P = .26$) (Table 2).

According to primary disease, the cumulative incidence of relapse after the 4/6 to 6/6 HLA–matched UCBT was higher than that after the 8/8 HLA–matched UBMT only in MDS patients and was similar both in AML patients and in ALL patients (Supplemental Table 4).

According to conditioning regimen, the cumulative incidence of relapse after the 4/6 to 6/6 HLA–matched UCBT was higher than that after the 8/8 HLA–matched UBMT only in recipients of MAC (Supplemental Table 5). Among the patients who received RIC, the cumulative incidence of relapse after the 4/6 to 6/6 HLA–matched UCBT was significantly higher than that after the UBMT in recipients without extensive chronic GVHD. However, the cumulative incidence of relapse after the 4/6 to 6/6

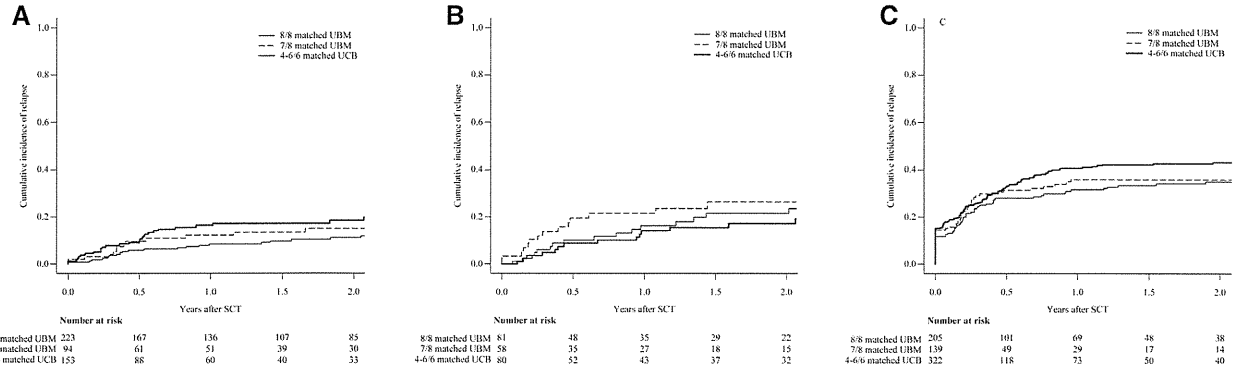

Figure 1. Cumulative incidence of relapse in patients with early phase disease, in those with intermediate phase disease, and in those with high-risk disease according to hematopoietic stem cell source and donor-recipient HLA match. (A) The cumulative incidences of relapse in patients with early phase disease by 2 years after an 8/8 HLA–matched unrelated bone marrow transplantation (UBMT), a 7/8 HLA–matched UBMT, and a 4/6 to 6/6 HLA–matched umbilical cord blood transplantation (UCBT) were 11% (95% CI, 7% to 16%), 15% (95% CI, 8% to 23%), and 19% (95% CI, 12% to 26%), respectively. (B) The cumulative incidences of relapse in patients with intermediate phase disease by 2 years after an 8/8 HLA–matched UBMT, a 7/8 HLA–matched UBMT, and a 4/6 to 6/6 HLA–matched UCBT were 22% (95% CI, 13% to 32%), 26% (95% CI, 15% to 39%), and 17% (95% CI, 10% to 27%), respectively. (C) The cumulative incidences of relapse in patients with intermediate phase disease by 2 years after an 8/8 HLA–matched UBMT, a 7/8 HLA–matched UBMT, and a 4/6 to 6/6 HLA–matched UCBT were 35% (95% CI, 28% to 42%), 36% (95% CI, 28% to 44%), and 43% (95% CI, 38% to 49%), respectively.

HLA–matched UCBT was not statistically different from that after UBMT among the recipients of MAC (Supplemental Figure 1).

NRM

The 2-year cumulative incidences of NRM after the 8/8 HLA–matched UBMT, 7/8 HLA–matched UBMT, and 4/6 to 6/6 HLA–matched UCBT were 32% (95% CI, 27% to 36%), 40% (95% CI, 33% to 46%), and 38% (95% CI, 34% to 43%), respectively. Among patients with early phase disease, the cumulative incidence of NRM at 2 years after the 8/8 HLA–matched UBMT was significantly lower than that after the 7/8 HLA–matched UBMT or 4/6 to 6/6 HLA–matched UCBT (25% [95% CI, 19% to 32%], 35% [95% CI, 25% to 45%], and 37% [95% CI, 29% to 46%]) (Figure 2A). Among patients with intermediate phase disease or advanced phase disease, NRM by 2 years was not statistically different among 3 groups (8/8 HLA–matched UBMT, 7/8 HLA–matched UBMT, and 4/6 to 6/6 HLA–matched UCBT; intermediate phase disease; 32% [95% CI, 31% to 43%], 27% [95% CI, 16% to 40%], and 28% [95% CI, 18% to 38%]; advanced phase disease, 34% [295% CI, 7% to 41%], 41% [95% CI, 32% to 50%], and 36% [95% CI, 30% to 41%], respectively) (Figure 2B,C). On multivariate analysis, the 4/6 to 6/6 HLA–matched UCBT recipients had a higher risk of NRM than the 8/8 HLA–matched UBMT recipients (HR, 1.32 [95% CI, 1.06 to 1.64]; $P = .013$); however, they had a similar risk to the 7/8 HLA–matched UBMT recipients (HR, .98 [95% CI, .77 to 1.25]; $P = .88$) (Table 2). According to primary disease, NRM by 2 years after the 4/6 to 6/6 HLA–matched UCBT was likely higher than that after the 8/8 HLA–matched UBMT only among patients with MDS; however, the difference was not significant regardless of primary diseases (Supplemental Table 4). On multivariate analysis of subgroup analysis according to conditioning regimen, NRM after the 8/8 HLA–matched UBMT was significantly lower than that after the 7/8 HLA–matched UBMT and 4/6 to 6/6 HLA–matched UCBT only among recipients of RIC (Supplemental Table 5).

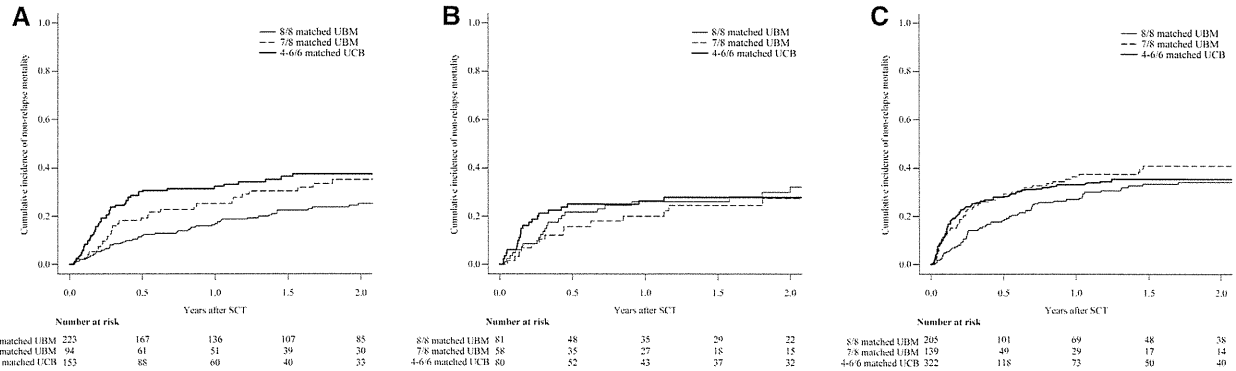

Figure 2. Cumulative incidence of NRM in patients with early phase disease, in those with intermediate phase disease, and in those with advanced phase disease according to hematopoietic stem cell source and donor-recipient HLA match. (A) The cumulative incidences of NRM in patients with early phase disease by 2 years after an 8/8 HLA–matched unrelated bone marrow transplantation (UBMT), a 7/8 HLA–matched UBMT, and a 4/6 to 6/6 HLA–matched umbilical cord blood transplantation (UCBT) were 25% (95% CI, 95% CI, 19% to 32%), 35% (95% CI, 95% CI, 25% to 45%), and 37% (95% CI, 29% to 46%), respectively. (B) The cumulative incidences of NRM in patients with intermediate phase disease by 2 years after an 8/8 HLA–matched UBMT, a 7/8 HLA–matched UBMT, and a 4/6 to 6/6 HLA–matched UCBT were 32% (95% CI, 31% to 43%), 27% (95% CI, 16% to 40%), and 28% (95% CI, 18% to 38%), respectively. (C) The cumulative incidences of NRM in patients with advanced phase disease by 2 years after an 8/8 HLA–matched UBMT, a 7/8 HLA–matched UBMT, and a 4/6 to 6/6 HLA–matched UCBT were 34% (95% CI, 27% to 41%), 41% (95% CI, 32% to 50%), and 36% (95% CI, 30% to 41%), respectively.

Survival

The 2-year unadjusted probabilities of OS after the 8/8 HLA–matched UBMT (51% [95% CI, 46% to 56%]) were significantly higher than those of the 7/8 HLA–matched UBMT (39% [95% CI, 32% to 45%]) and 4/6 to 6/6 HLA–matched UCBT (35% [95% CI, 31% to 39%]) recipients, respectively. The adjusted probabilities of OS at 2 years were also significantly better in recipients of the 8/8 HLA–matched UBMT than in the recipients of the 7/8 HLA–matched UBMT or 4/6 to 6/6 HLA–matched UCBT (49% [95% CI, 44% to 54%], 38% [95% CI, 32% to 45%], 39% [95% CI, 35% to 44%], respectively). This finding was also observed in the subgroup analysis for disease status (at early phase: the adjusted probabilities of OS at 2 years after the 8/8 HLA–matched UBMT, 7/8 HLA–matched UBMT, and 4/6 to 6/6 HLA–matched UCBT were 69% [95% CI, 62% to 76%], 54% [95% CI, 44% to 66%], and 46% [95% CI, 38% to 56%]; at intermediate phase: 53% [95% CI, 42% to 67%], 55% [95% CI, 42% to 72%], and 62% [95% CI, 52% to 74%], respectively; at advanced phase: 31% [95% CI, 24% to 39%], 24% [95% CI, 17% to 33%], and 25% [95% CI, 21% to 31%], respectively) (Figure 3).

According to the multivariate analysis, the 4/6 to 6/6 HLA–matched UCBT recipients had a significantly higher risk of overall mortality than did the 8/8 HLA–matched UBMT recipients (HR, 1.47 [95% CI, 1.24 to 1.74]; $P < .001$) (Table 2). However, the 4/6 to 6/6 HLA–matched UCBT recipients had a similar risk of overall mortality when compared with the 7/8 HLA–matched UBMT recipients (HR, 1.03 [95% CI, .86 to 1.24]; $P = .75$) (Table 2). The adjusted probabilities of OS at 2 years after 8/8 HLA–matched UBMT were superior to those after 4/6 to 6/6 HLA–matched UCBT, regardless of primary disease and conditioning regimen, especially in the patients with MDS (Supplemental Figure 2, Supplemental Tables 4 and 5).

To identify the population of UCBT recipients who had a similar OS to those of 8/8 HLA–matched UBMT, we evaluated the impact of cell dose, HLA matching, and GVHD prophylaxis on the OS of UCBT recipients. The 2-year unadjusted OS of UCBT recipients who received $\geq .84 \times 10^5$ CD34$^+$ cells/kg, which was median cell dose, was significantly higher than those who received $< .84 \times 10^5$ CD34$^+$ cells/kg (Supplemental Figure 3A). HLA matching did not have an effect on OS (Supplemental Figure 3B). GVHD prophylaxis with calcineurin inhibitor (CNI) and other agents improved OS compared with that with CNI alone (Supplemental Figure 3C). Therefore, we compared the OS of 4/6 to 6/6 HLA–matched UCBT recipients who received umbilical cord blood units containing $\geq .84 \times 10^5$ CD34$^+$ cells/kg with 8/8 HLA–matched UBMT recipients, among those with AML and those with ALL who received GVHD prophylaxis with CNI and other agent. The unadjusted 2-year OS after 8/8 HLA–matched UBMT was higher than 4/6 to 6/6 HLA–matched UCBT in patients with early phase disease. Among those with intermediate phase disease, the unadjusted 2-year OS after 4/6 to 6/6 HLA–matched UCBT was likely higher than 8/8 HLA–matched UBMT. Among those with advanced phase disease, the 2-year OS were similar between 2 groups (8/8 HLA–matched UBMT versus 4/6 to 6/6 HLA–matched UCBT; the unadjusted OS of early phase disease, 67% [95% CI, 59% to 74%] versus 55% [95% CI, 40% to 67%], $P = .044$; the unadjusted OS of intermediate disease, 52% [95% CI, 39% to 64%] versus 77% [95% CI, 56% to 89%], $P = .08$; the unadjusted OS of advanced phase disease, 25% [95% CI, 17% to 33%] versus 26% [95% CI, 16% to 36%], $P = .82$) (Figure 4A,C). The adjusted probability of OS were similar between 2 groups (8/8 HLA–matched UBMT versus 4/6 to 6/6 HLA–matched UCBT; the adjusted OS, 49% [95% CI, 43% to 55%] versus 49% [95% CI, 41% to 58%], $P = .74$, respectively) (Figure 4D).

DISCUSSION

The primary objectives of this study were to compare OS after 4/6 to 6/6 HLA–matched UCBT with those after 8/8 and 7/8 HLA–matched UBMT in patients with hematologic malignancies ages 50 years or older and to provide useful data for the selection of an appropriate unrelated stem cell source for those patients who do not have an available HLA-identical sibling. Our findings suggested that an 8/8 HLA allele–matched unrelated donor is the best alternative to a HLA-identical sibling donor. Four of 6 to 6/6 HLA–matched UCBT had a similar OS to 8/8 HLA–matched UBMT for patients with AML and for those with ALL when the umbilical cord blood unit containing $\geq .84 \times 10^5$ CD34$^+$ cells/kg is available.

Neutrophil and platelet recovery were significantly slower after the 4/6 to 6/6 HLA–matched UCBT than after the

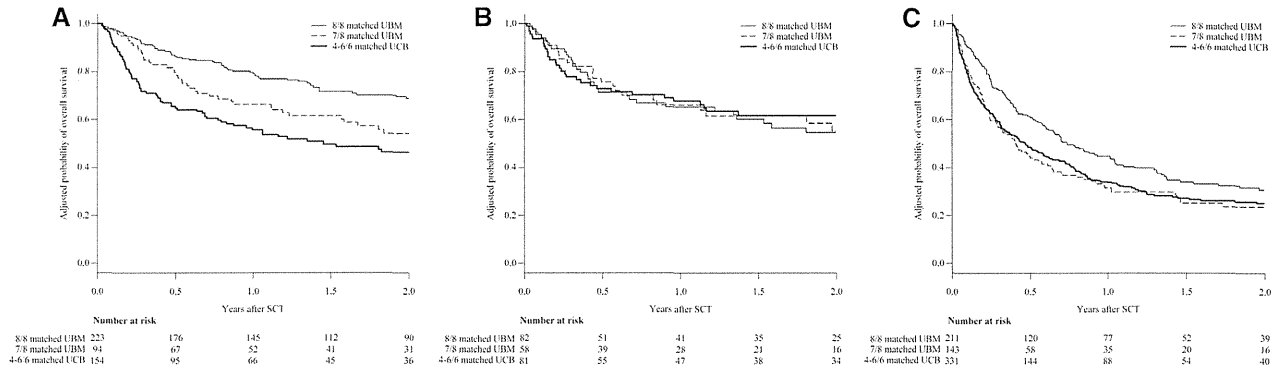

Figure 3. Adjusted probabilities of OS in patients with early phase disease, in those with intermediate phase disease, and in those with advanced phase disease according to hematopoietic stem cell source and donor-recipient HLA match. (A) The adjusted probabilities of the 2-year OS after transplantation in patients with early phase disease who received an 8/8 HLA–matched unrelated bone marrow transplantation (UBMT), a 7/8 HLA–matched UBMT, and a 4/6 to 6/6 HLA–matched umbilical cord blood transplantation (UCBT) were 69% (95% CI, 62% to 76%), 54% (95% CI, 44% to 66%), and 46% (95% CI, 38% to 56%), respectively. (B) The adjusted probabilities of the 2-year OS after transplantation in patients with intermediate phase disease who received an 8/8 HLA–matched UBMT, a 7/8 HLA–matched UBMT, and a 4/6 to 6/6 HLA–matched UCBT were 53% (95% CI, 42% to 67%), 55% (95% CI, 42% to 72%), and 62% (95% CI, 52% to 74%), respectively. (C) The adjusted probabilities of the 2-year OS after transplantation in patients with intermediate phase disease who received an 8/8 HLA–matched UBMT, a 7/8 HLA–matched UBMT, and a 4/6 to 6/6 HLA–matched UCBT were 31% (95% CI, 24% to 39%), 24% (95% CI, 17% to 33%), and 25% (95% CI, 21% to 31%), respectively.

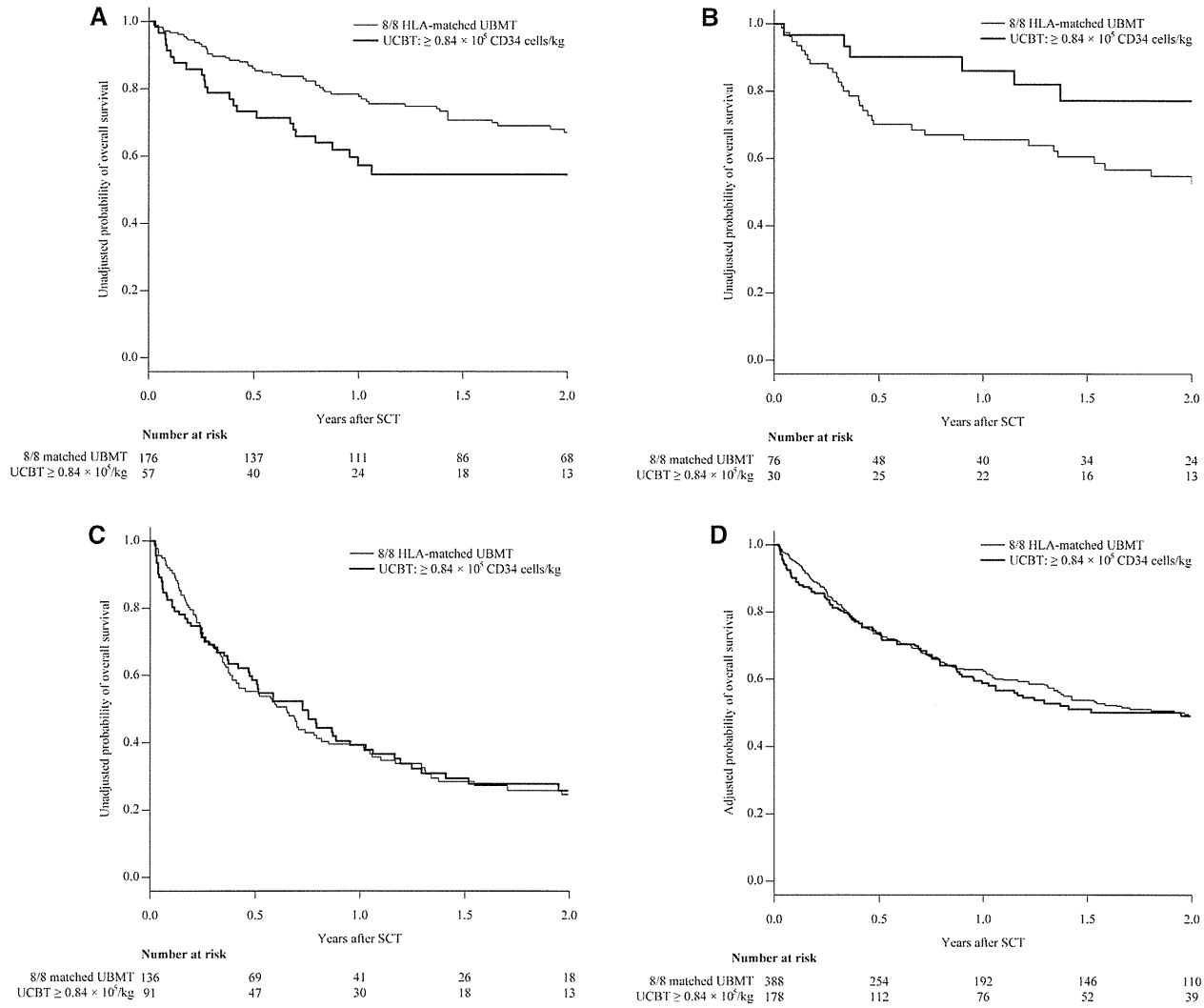

Figure 4. OS in UCBT recipient who received ≥ .84 × 10^5/kg CD34 cells compared with 8/8 HLA–matched UBMT recipients, among those with AML and ALL who prevented graft-versus-host disease with CNI and other agents. (A) The unadjusted probabilities of the 2-year OS after transplantation in patients with acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) at early phase disease and prevented GVHD with CNI and other agent who received an 8/8 HLA–matched unrelated bone marrow transplantation (UBMT) and a 4/6 to 6/6 HLA–matched umbilical cord blood transplantation (UCBT) receiving ≥.84 × 10^5/kg CD34 cells were 67% (95% CI, 59% to 74%) and 55% (95% CI, 40% to 67%), respectively, $P = .044$. (B) The unadjusted probabilities of the 2-year OS after transplantation in patients with AML and ALL at intermediate phase disease and prevented GVHD with CNI and other agent who received an 8/8 HLA–matched UBMT and a 4/6 to 6/6 HLA–matched UCBT receiving ≥.84 × 10^5/kg CD34 cells were 52% (95% CI, 39% to 64%) and 77% (95% CI, 56% to 89%), respectively, $P = .08$. (C) The unadjusted probabilities of the 2-year OS after transplantation in patients with AML and ALL at advanced phase disease and prevented GVHD with CNI and other agent who received an 8/8 HLA–matched UBMT and a 4/6 to 6/6 HLA–matched UCBT receiving ≥.84 × 105/kg CD34 cells were 25% (95% CI, 17% to 33%) and 26% (95% CI, 16% to 36%), respectively, $P = .82$. (D) The adjusted probabilities of the 2-year OS after transplantation in patients with AML and ALL prevented GVHD with CNI and other agent who received an 8/8 HLA–matched UBMT and a 4/6 to 6/6 HLA–matched UCBT receiving ≥.84 × 10^5/kg CD34 cells were 49% (95% CI, 43% to 55%) and 49% (95% CI, 41% to 58%), respectively, $P = .74$.

8/8 and 7/8 HLA–matched UBMT, which was consistent with findings from previous studies [6-9,11,15]. Neutrophil recovery in patients with early phase disease and intermediate phase disease at transplantation was significantly faster than in those with advanced phase disease, which was consistent with the findings in allogeneic peripheral blood stem cell transplantation that had been previously reported [26]. This may be associated with the fact that patients with advanced phase disease were likely pretreated more heavily than those with early phase disease and intermediate phase disease and that they had damage in the microenvironment of the bone marrow.

UCBT recipients had a lower risk of extensive chronic GVHD and a higher risk of relapse compared with 8/8 HLA–matched UBMT recipients. These findings suggested that the graft-versus-leukemia effect in the UCBT recipients was lower than that in the recipients of 8/8 HLA–matched UBMT.

Several studies comparing transplantation outcomes after UBMT versus after UCBT have been reported [6-9]. In some studies, serological HLA class I typing was used for UBMT [6-8]. In another study, UCBT recipients were significantly younger than UBMT recipients, and all patients received a MAC regimen. As a result, only a small number of patients aged 50 years or older were included [9], so direct comparisons of our findings with previous studies are difficult. We had previously demonstrated that HR of overall mortality after a 4/6 to 6/6 HLA–matched UCBT was significantly

higher than that after an 8/8 HLA–matched UBMT among AML patients but not among ALL patients [15]. By contrast, this study showed that the overall survival after an 8/8 HLA–matched UBMT was superior to that after a 4/6 to 6/6 HLA–matched UCBT for patients with AML and for patients with ALL. The present study included patients 50 years or older who received HSCT between 2000 and 2009 regardless of intensity of the conditioning regimen, whereas our previous study had included the recipients of MAC between 2000 and 2005 ages 16 years or older. Therefore, 20% of the 8/8 HLA–matched UBMT recipients and 10% of the 4/6 to 6/6 HLA–matched UCBT recipients in the present study were also included in our previous study. The discrepancy of the results for ALL may be partly due to differences in conditioning regimens (only recipients of MAC regimens were described in our previous report, whereas more than one half of the patients in this study received RIC regimen). Older patients with ALL had a higher risk of relapse and tended to receive RIC when compared with younger patients [27]; therefore, these patients would need a strong graft-versus-leukemia effect. In addition, short-term methotrexate improved OS in the UCBT recipients [28]. In our cohort, approximately 30% of UCBT recipients received GVHD prophylaxis with cyclosporine or tacrolimus alone, and this reduced OS in UCBT recipients. As previously described [29], UCBT recipients receiving higher $CD34^+$ cells had a higher OS than those receiving lower $CD34^+$ cells. For patients with AML and for patients with ALL, UCBT recipients receiving $\geq .84 \times 10^5$ $CD34^+$ cells/kg had a similar adjusted and unadjusted OS to 8/8 HLA–matched UBMT recipients. These findings suggest that the outcomes of UCBT may improve with graft selection based on $CD34^+$ cell dose. The HR of overall mortality after a 4/6 to 6/6 HLA–matched UCBT was similar to that after a 7/8 HLA–matched UBMT, regardless of disease status at transplantation. To the best of our knowledge, this is the first report to compare transplantation outcomes in patients 50 years or older who received a 4/6 to 6/6 HLA–matched UCBT with those who received a 7-8/8 HLA–matched UBMT in a large cohort.

This study had several limitations. Although we adjusted for known risk factors using multivariate analysis, we could not exclude selection bias because this was a retrospective study based on registry data. Further, donor selection was influenced by several factors that were not statistically adjustable. Some patients with urgent disease who could not wait for the preparation of UBMT received UCBT; in other cases, a suitable UCB unit with enough cell doses was not available, and these patients therefore received UBMT. Patients who planned to receive UBMT and could not receive transplantation because of disease progression during the donor coordination were not included in this analysis. In addition, only 5% of recipients of UBMT received GVHD prophylaxis using only a CNI; on the other hand, approximately 30% of UCBT recipients employed the same protocol, which may have influenced the occurrence of GVHD and overall survival. A randomized controlled trial comparing UCBT with UBMT is needed to validate the findings from the present study; however, a study of that design is very difficult to conduct. Clinical decision analysis may help to address any selection bias caused by the donor search process. From 2000 onwards, UPBSCT was more common than UBMT [5]; however, we could not compare the transplantation outcomes of the 4/6 to 6/6 HLA–matched UCBT with the UPBSCT because more than 99% of the unrelated donors from Japan Marrow Donor Program were harvested bone marrow. A randomized controlled trial comparing UPBSCT with UBMT had shown similar outcomes for OS, NRM, and relapse rate [30]. Taken together, UCBT may also be an alternative stem cell source when a HLA-matched peripheral blood stem cell donor is not available.

In conclusion, UCB is a reasonable alternative donor/stem cell source for elderly patients with AML and for those with ALL with similar outcomes compared with UBM from a 8/8 HLA–matched unrelated donor when UCB unit containing $\geq .84 \times 10^5$ $CD34^+$ cells/kg is available. If urgently needed or if there is no 8/8 HLA–matched unrelated donor, a 4/6 to 6/6 HLA–matched UCBT is an acceptable treatment.

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Supplementary information is available at Leukemia's website.

SUPPLEMENTARY DATA

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bjh research paper

Clinical characteristics of 15 children with juvenile myelomonocytic leukaemia who developed blast crisis: MDS Committee of Japanese Society of Paediatric Haematology/Oncology

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Summary

Juvenile myelomonocytic leukaemia (JMML) is a rare haematopoietic stem cell disease of early childhood, which can progress to blast crisis in some children. A total of 153 children diagnosed with JMML were reported to the Myelodysplastic Syndrome Committee in Japan between 1989 and 2007; 15 of them (9·8%) had 20% or more blasts in the bone marrow (blast crisis) during the disease course. Blast crisis occurred during observation without therapy ($n = 3$) or with oral 6-mercaptopurine treatment ($n = 9$) and in relapse after haematopoietic stem cell transplantation (HSCT; $n = 3$). Six patients had a complex karyotype (5 including monosomy 7) and an additional three patients had isolated monosomy 7 at blast crisis. Seven patients received HSCT after blast crisis and four of them achieved remission. Eleven out of the 15 patients died; the cause of death was disease progression in 10 patients and transplant-related complication in one patient. In summary, patients with blast crisis have poor prognosis and can be cured only by HSCT. The emergence of monosomy 7 and complex karyotype may be characteristic of blast crisis in a substantial subset of children.

Keywords: juvenile myelomonocytic leukaemia, blast crisis, monosomy 7, haematopoietic stem cell transplantation.

Juvenile myelomonocytic leukaemia (JMML) is a rare haematopoietic stem cell disease in infants with features characteristic of both myeloproliferative and myelodysplastic disorders (Aricò *et al*, 1997; Niemeyer *et al*, 1997; Emanuel, 1999; Koike & Matsuda, 2008). Its clinical course is diverse. Sometimes the disease can even spontaneously resolve without any treatment (Matsuda *et al*, 2007). However, most patients have a rapidly progressive disease and early death because of the organ enlargement or bone marrow failure. Many patients are resistant to essentially all chemotherapy regimens and it is thought that haematopoietic stem cell transplantation (HSCT) is the only curative treatment for children with JMML (Locatelli *et al*, 2005; Yabe *et al*, 2008).

According to a previous report (Aricò *et al*, 1997), approximately one-third of the patients present with a rapidly progressive disease and another third of the patients show a more indolent disease course. The latter patients are usually stable, even if they have persistent splenomegaly,

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moderate leucocytosis or monocytosis. However, they may occasionally suddenly experience blast crisis. The remaining patients have an intermediate prognosis.

Luna-Fineman *et al* (1999) reported that 8 (13%) of 60 of patients with JMML developed acute leukaemia. Thus, it is known that JMML can progress to blast crisis in a subset of children. However there are only a few reports accounting for this transformation and the molecular mechanism of acute transformation is unknown.

In order to clarify the characteristics of blast crisis of JMML, we retrospectively analysed in detail those JMML patients who had progressed to blast crisis.

Patients and methods

Between 1989 and 2007, 153 JMML children had been reported to the myelodysplastic syndrome (MDS) committee of the Japanese Society of Paediatric Haematology/Oncology (JSPHO). The diagnosis was made according to the criteria of the International JMML Working Group (Niemeyer *et al*, 1998; Chan *et al*, 2009). Patients registered before 1999 were diagnosed with JMML at individual institutions. Since 1999, bone marrow (BM) and peripheral blood (PB) smears were reviewed by two reference investigators of the MDS Committee at diagnosis and patients were prospectively registered to the MDS Committee (Sasaki *et al*, 2001). Based on clinical information reported to the MDS Committee, 15 JMML patients with blast crisis were identified. Blast crisis was defined as having 20% or more blasts in the BM during the course of illness. Questionnaires inquiring about the detailed clinical and laboratory findings, treatment and clinical outcome were sent to the physicians who treated these patients. These retrospectively collected data were analysed in this study. The study was approved by the MDS Committee of the JSPHO. Informed consent was obtained from the guardians of patients after 1999; however, this was not possible for cases registered before 1999 because an appropriate guideline was not provided for retrospective observational studies in that period.

Complete remission (CR) of JMML after therapy was defined if all of the following criteria were fulfilled: (i) no evidence of circulating blasts in PB, BM with less than 5% blasts and trilineage haematological recovery and (ii) absence of chromosome abnormalities; and iii) disappearance of clinical symptoms of JMML, such as organomegaly.

Results

Fifteen (9·8%) patients of the 153 JMML cases had progressed to blast crisis. The characteristics of these patients at diagnosis are shown in Table I.

There was a male predominance with a male: female rate of 2·8: 1. There was no congenital anomaly, such as Noonan

Table I. Characteristics of the patients at diagnosis.

Patient	Year of Dx	Sex/age (years)	WBC ($\times 10^9$/l)	PB mono ($\times 10^9$/l)	PB blasts (%)	Hb (g/l)	Plt ($\times 10^9$/l)	HbF (%)	BM blasts (%)	Karyotype	Initial treatment	CR before BC
1	1991	M/0·3	38·0	1·9	1·0	104	85	15·4	0·2	46, XY	6MP	No
2	1992	M/1·4	115·9	90·4	0	106	65	13·0	6·2	46, XY	None	No
3	1992	M/2·6	45·2	3·2	1·5	97	17	31·9	3·7	46, XY	6MP + AML type	No
4	1993	M/0·3	89·6	17·0	3·0	90	43	14·7	3·6	46, XY	6MP	No
5	1994	M/0·3	29·0	4·2	0	117	77	53·8	5·8	46, XY	6MP	No
6*	1994	M/3·9	8·9	0·4	6·0	120	43	64·2	16·8	46, XY	6MP	No
7	1995	F/1·5	35·1	5·3	7·0	110	13	24·0	ND	46, XX	6MP + PSL + Etoposide	No
8	1997	M/7·6	29·3	5·3	7·0	69	24	35·9	0	46, XY	None	No
9	1997	F/2·8	15·6	1·4	1·0	113	66	47·0	0	46, XX	None	No
10	1999	M/3·7	72·0	33·5	0	97	94	1·1	14·9	46, XY, −2, +mar	6MP	No
11	1999	M/4·7	98·5	28·1	2·0	70	16	69·5	5·1	46, XY	6MP	No
12	2000	M/1·7	17·8	1·4	0	74	20	55·0	ND	46, XY, t(3;18) (q25;q21)	HSCT	Yes
13	2001	M/4·7	58·5	14·9	0·5	120	102	9·0	0	45, XY, −7	6MP	No
14	2002	F/0·4	376·2	26·3	0	ND	ND	19·5	0	46, XX	6MP + HSCT	Yes
15	2003	F/3·2	27·6	3·0	0	104	32	41·8	18·3	46, XX	6MP + AML type + HSCT	Yes

Dx, diagnosis; WBC, white blood cell count; PB, peripheral blood; Hb, haemoglobulin concentration; Plt, platelet count; ND, data not available; BM, bone marrow; 6MP, 6-mercaptopurine; PSL, prednisolone; AML, acute myeloid leukaemia; HSCT, haematopoietic stem cell transplantation; CR, complete remission; BC, blast crisis.

*This patient did not have monocyte count exceeding 1 × 10^9/l at diagnosis, however, he had splenomegaly and bone marrow mononuclear cells showed hypersensitivity to GM-CS.

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