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Unrelated Umbilical Cord Blood Transplantation Using a TBI/FLAG Conditioning Regimen for Adults with Hematologic Malignancies

Masaya Okada,[1,2] Yoshihiro Fujimori,[2] Mahito Misawa,[1,2] Shunro Kai,[2,3] Toshiyuki Nakajima,[1] Yoshiko Okikawa,[1] Atsushi Satake,[1] Hisayuki Itoi,[1] Hiroyuki Takatsuka,[1,2] Takeyoshi Itsukuma,[2] Keisuke Nishioka,[1] Hiroya Tamaki,[1] Kazuhiro Ikegame,[1] Hiroshi Hara,[2,4] Hiroyasu Ogawa[1,2]

[1] Division of Hematology, Department of Internal Medicine, [2] Laboratory of Cell Transplantation, Institute for Advanced Medical Sciences, and [3] Division of Blood Transfusion, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan; and [4] Department of Internal Medicine, Uegahara Hospital, Nishinomiya, Hyogo, Japan

Correspondence and reprint requests: Yoshihiro Fujimori, MD, PhD, Laboratory of Cell Transplantation, Institute for Advanced Medical Sciences, Hyogo College of Medicine, 1-1 Mukogawa-cho, Nishinomiya, Hyogo 663-8501, Japan (e-mail: fuji-y@hyo-med.ac.jp).

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ABSTRACT

A combined chemotherapy regimen comprising fludarabine, cytosine arabinoside, and granulocyte colony-stimulating factor (FLAG) has been used in the treatment of relapsed or refractory leukemias. We here report 38 patients with hematologic malignancies who underwent single-unit cord blood transplantation (CBT) with a conditioning regimen comprising 12-Gy total-body irradiation (TBI) and FLAG therapy (TBI/FLAG). Graft-versus-host disease (GVHD) prophylaxis consisted of tacrolimus or cyclosporin A and/or methotrexate. The median nucleated cell dose was 2.43 × 10^7/kg (range: 1.96-3.55 × 10^7/kg). Of 34 evaluable recipients, the cumulative incidence of donor engraftment was 97%. The median time to reach an absolute neutrophil count of 500/μL was 23 days (range: 18-35 days). The median time to an untransfused platelet count of 50,000/μL was 45.5 days (range: 28-208 days). Sixteen patients developed grades II-IV of acute GVHD. Fourteen patients were alive at a median follow-up of 46 months (range: 4-77 months). The estimated event-free survival at 3 years for all patients was 33.5%, with 72.7% in the standard-risk group (n = 11) and 17.7% in the high-risk group (n = 27) (P = .0075). These results showed that this novel regimen was well tolerated by patients and able to establish sustained donor cell engraftment, indicating the feasibility of TBI/FLAG as a conditioning regimen for CBT in adults with hematologic malignancies.

KEY WORDS

Cord blood transplantation (CBT) • Adult • Conditioning regimen • FLAG • Total body irradiation (TBI)

INTRODUCTION

Umbilical cord blood transplantation (CBT) has increasingly been performed as an alternative to human leukocyte antigen (HLA)-matched sibling or unrelated bone marrow transplantation (BMT) [1-3]. The advantages of CBT in comparison to BMT include prompt availability of cryopreserved cells, a less stringent requirement for HLA-type matches between donors and recipients, and a low risk of inducing severe graft-versus-host disease (GVHD). The major drawbacks of CBT are slow hematopoietic recovery and a high incidence of graft failure, mainly because of a small number of progenitors being infused, which is more pronounced in adults with greater body weight [4]. Generally, the overall outcome in adult CBT needs to be improved in comparison to that in adult allogeneic BMT [2]. A standard conditioning regimen with cyclophosphamide and total-body irradiation (TBI) produces favorable results for BMT [5], but a standard conditioning regimen for CBT has not yet been firmly established.

Intensive combination chemotherapy has significantly improved the prognosis of patients with hematologic malignancy [6]. FLAG therapy using fludarabine (Flu), cytosine arabinoside (Ara-C), and

granulocyte colony-stimulating factor (G-CSF) has been shown to be effective against a variety of hematologic malignancies, including high-risk acute myeloid leukemias [7,8] and acute lymphoblastic leukemia [9]. The use of FLAG therapy for the treatment of leukemias is based on the following arguments: (1) infusion of fludarabine before Ara-C increases the accumulation of the active metabolite ara-C triphosphate in leukemic cells [10], (2) G-CSF shortens the duration of neutropenia and reduces infection rates in leukemia patients [11], and (3) G-CSF may sensitize leukemic blasts to S-phase-specific Ara-C by recruiting quiescent cells into the cell cycle and increasing Ara-C phosphorylation [12]. Thus, FLAG therapy was pharmacokinetically designed to increase antileukemic metabolites, and was intended to exert an efficient antileukemic effect in the treatment of relapsed or refractory leukemias.

Fludarabine is highly immunosuppressive and shown to be especially effective in a nonmyeloablative preparative regimen for allogeneic stem cell transplantation (SCT) [13]. Pawson et al. [14] used FLAG with or without idarubicin as a reduced-intensity conditioning (RIC) regimen for second allogeneic peripheral blood SCT in the treatment of relapsed leukemia patients. Thus, FLAG therapy may act not only as an effective antileukemic chemotherapy regimen, but also as an efficient preparative regimen for SCT.

In the present study, we developed a new conditioning regimen consisting of FLAG therapy combined with 12-Gy TBI (TBI/FLAG). We performed CBT using this regimen in 38 adult patients with hematologic malignancies in our single institution. Our results demonstrated the feasibility of this TBI/FLAG as a novel myeloablative preparative regimen for CBT.

PATIENTS AND METHODS

Eligibility

Patients were eligible if they were in a condition requiring SCT but had no 6/6 or 5/6 allele HLA-matched related donor or 6/6 HLA-matched unrelated donor available, or needed urgent SCT within 3 months. Patients receiving a transplant during the first or second complete remission of leukemia or non-Hodgkin's lymphoma, or those who had refractory anemia of myelodysplastic syndrome (MDS) were placed in the standard-risk group. Patients in their third or subsequent remission, relapse, or partial remission with refractory leukemia and those with chronic myelogenous leukemia (CML) beyond the first chronic phase at the time of CBT were considered to be in the advanced phase of disease and were placed in the high-risk group. Patients with diseases with high-risk cytogenetics, such as acute lymphoblastic leukemia (ALL) with t(9;22) and acute myelogenous leukemia (AML) with −5, del(5q), −7, del(7) or del(11), were also included in the high-risk group [15]. This study was approved by the institutional review board of Hyogo College of Medicine. All patients provided written informed consent.

CB grafts

Appropriate cord blood (CB) was identified through the Japan Cord Blood Bank Network (JCBBN), which maintains information on the holdings of 11 local CB banks in Japan [16]. In the first 19 patients, CB grafts were selected on the basis of serologic matching at 4-6 of 6 HLA loci (class I HLA-A and -B, and class II HLA-DR alleles) as determined by a standard complement-dependent microlymphocytotoxicity test [17]. In the subsequent 19 patients, high-resolution DNA typing of class II DRB1 alleles was used for selection of class II alleles according to the availability of the high-resolution class II data. CB grafts selected had a cryopreserved cell dose of at least 2×10^7 nucleated cells (NC) per kilogram of recipient body weight (NC/kg). Confirmatory high-resolution DNA typing of class I HLA-A and -B and class II DRB1 alleles was also performed [18-20]. All CB used were single units and were not depleted of T lymphocytes.

Preparative Regimen

The TBI/FLAG regimen comprised TBI (12 Gy), Flu (150 mg/m^2), Ara-C (10 g/m^2), and G-CSF. TBI was administered daily at 3 Gy for 4 days (day −10 to day −7). Flu, Ara-C, and G-CSF were administered daily for 5 days (day −6 to −2). Flu (30 mg/m^2) was administered intravenously over 2 hours. Four hours after the completion of Flu infusion, Ara-C (2 g/m^2) was administered intravenously over 2 hours. The TBI/FLAG regimen was performed irrespective of prior Ara-C treatment. G-CSF (300 μg/m^2) was administered subcutaneously. In the first 24 consecutive patients, G-CSF was administered to all patients, but in the subsequent 14 patients, the G-CSF administration was omitted in patients with lymphoid leukemias and lymphomas (n = 7), because efficacy of G-CSF on lymphoid malignancy is not firmly established.

GVHD Prophylaxis and Treatment

GVHD prophylaxis was tacrolimus (n = 11) or cyclosporin A (CsA) (n = 1) alone during the years 2000 to 2002. Tacrolimus plus short-term methotrexate (MTX) (n = 9) or CsA plus short-term MTX (n = 17) was used since August 2002. Administration of tacrolimus (0.02 mg/kg/day) or CsA (3 mg/kg/day) in a continuous infusion was started on day −1 and continued until the patient became tolerate to oral administration. Short-term MTX was administered at 10 mg/m^2 on day 1 and 7 mg/m^2 on days 3 and 6 [21]. After neutrophil engraftment, and in the absence of acute

GVHD (aGVHD), tacrolimus or CyA was tapered 10% per week starting at approximately day 35. Acute GVHD was clinically diagnosed using the criteria of Glucksberg et al. [22]. Grade II to IV aGVHD was treated with methylprednisolone at 1-2 mg/kg/day. Patients who survived for >100 days were analyzed for chronic GVHD (cGVHD).

Supportive Care

Each patient was isolated in a laminar air-flow room. Ciprofloxacin at 400 mg/day and fluconazole at 300 mg/day were administered from day −14 until neutrophil recovery. G-CSF at 300 $\mu g/m^2$ was again administered to all patients from day 5 until neutrophil recovery. Acyclovir was administered at 750 mg/day for 5 weeks after transplantation to prevent herpes simplex virus infection. Ganciclovir 10 mg/kg was administered in 2 divided doses from day −10 to day −3 as prophylaxis for cytomegalovirus (CMV) infection. Detection of CMV antigenemia was performed using an immunoperoxidase-conjugated antibody, HRP-C7, which binds to an immediate-early antigen of CMV, pp65 antigen. After grafting, ganciclovir administration was reinstituted in patients demonstrating positive CMV antigenemia.

Donor Chimerism Analysis

Donor chimerism was analyzed using marrow and/or blood samples. Chimerism was determined by quantitative PCR analysis of informative short tandem repeat regions in the recipients and donors (STR-PCR) [21,23]. DNA was extracted from marrow or blood cells using a SepaGene isolation kit (Sankyo Pure Chemical, Tokyo, Japan), and amplified with fluorescent PCR primers (AmpFlSTR profiler PCR amplification kit; Applied Biosystems, San Jose, CA). The fluorescent PCR products were separated by capillary electrophoresis using a 310 Genetic Analyzer (Applied Biosystems). GeneScan software and GeneMapper software (Applied Biosystems) were used to calculate the percentage of donor and recipient DNA.

Engraftment

Engraftment was considered to have occurred when whole blood cell counts of absolute neutrophil counts of >500/μL were obtained for 3 consecutive days after transplantation, accompanied by the detection of donor chimerism. Graft failure was considered to have occurred when peripheral and marrow hypoplasia were noted after transplantation, and donor markers could not be detected by using cytogenetic and/or molecular techniques.

Regimen-Related Toxicity (RRT) and Transplantation-Related Mortality (TRM)

RRT, the nonhematologic toxicities directly caused by a given preparative regimen by day 28, were analyzed using Bearman's criteria [24]. TRM was defined as death without primary disease progression.

Statistical Analysis

The probability of event-free survival (EFS) was estimated using the Kaplan-Meier method with Mantel-Cox log rank test. In this analysis, graft failure, relapse, disease progression, and death were defined as events. We used Cox proportional hazards models to determine which independent patient-, disease- and transplant-related variables predict EFS. We first fitted univariable models, then all variables with $P < .10$ were included in a multivariable model. Hazard ratios were estimated with 95% confidence intervals. Categoric variables were compared using the χ^2 test. Values of $P < .05$ were considered to be significant. All statistical analyses were carried out with StatView version 5.0 software (SAS Institute, Cary, NC).

RESULTS

Patient Characteristics

Thirty-eight patients underwent CBT with a TBI/FLAG conditioning regimen between December 2000 and February 2007 at our institution. The median age of the patients was 38.5 years (range: 16-52 years) and the median weight was 58 kg (range: 39-81 kg). Details of patients' characteristics are listed in Table 1. Eleven patients (29%) who were in first or second remission were placed in the standard-risk group. The remaining 27 patients (71%), who were placed in the high-risk group, include 14 in relapse or partial remission with

Table 1. *Patient Characteristics*

Number of patients		**38**	
Sex (male/female)		**20/18**	
Age (year); median (range)		**38.5 (16-52)**	
Disease			
	Standard-Risk CR1/CR2 RA, CP	**High-Risk > CR3/>AP HRC**	**High-Risk Rel/Ref**
AML	5	4	5
ALL	4	5	2
MLL	2	0	0
NHL	0	1	1
ATL	0	0	2
MDS	0	0	2
CML	0	3	0
CLL	0	0	2
Total	11	13	14

AML indicates acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; MLL, mixed-lineage leukemia; NHL, non-Hodgkin lymphoma; ATL, adult T cell leukemia; MDS, myelodysplastic syndrome; CML, chronic myelogenous leukemia; CLL, chronic lymphocytic leukemia; CR1, CR2, CR3 first, second, and third complete remission; RA, refractory anemia; CP, chronic phase of CML, Rel, relapse; Ref, refactory disease; AP, accelerated phase of CML; HRC, high-risk cytogenetics.

refractory disease, 13 in their third or subsequent remission or with high-risk cytogenetics (Table 1).

Graft Characteristics

The median number of nucleated cells infused was 2.43 × 10^7/kg body weight (range: 1.96-3.55 × 10^7/kg) and that of $CD34^+$ cells was 0.87 × 10^5/kg body weight (range: 0.24-3.98 × 10^5/kg) (Table 2). CB grafts were primarily selected on the basis of serologic matching at HLA-A, -B, and -DR alleles (n = 19) or serologic matching at HLA-A and -B and high-resolution DNA typing of DRB1 alleles (n = 19). Only 1 graft (2%) in primary selection was 3 HLA mismatches. However, confirmatory high-resolution DNA typing of both class I and class II alleles revealed that 12 (31%) of the CB grafts had 3 or 4 mismatched antigens (Table 2).

Recovery of Peripheral Blood Cell Counts and Engraftment

Four of 38 patients were not evaluated for donor engraftment because of early death from sepsis (n = 2) (day 7, day 21) or bleeding (n = 2) (days 17, 22). Of 34 evaluable recipients, the cumulative incidence of primary donor engraftment was 97% (33 patients) as 1 patient experienced graft rejection with autologous marrow recovery. The median time for neutrophil recovery (>500/μL) was 23 days (range: 18-35 days; n = 33) (Figure 1A). All of these patients accompanied by donor chimerism by 86% to 100% using STR-PCR analysis of bone marrow cells at approximately day 21. After neutrophil recovery, 6 patients did not achieve subsequent reticulocyte recovery; 5 patients died between day 25 and day 100, and 1 patient experienced relapse. The median time for reticulocyte recovery (>1%) was 29 days (range: 25-57 days; n = 27) (Figure 1B). Thereafter, 1 patient, who experienced relapse, failed to achieve platelet recovery. The median time for platelet recovery (>50,000/μL) was 45.5 days (range: 28-208 days; n = 26) (Figure 1C).

Table 2. *Graft Characteristics and GVHD Prophylaxis*

Cord blood		
Total cells (×10^7/kg)	**2.43 (1.96-3.55)**	
$CD34^+$ cells (×10^5/kg)	**0.87 (0.24-3.98)**	
HLA mismatch	**Primary***	**Confirmatory†**
0/6	**1**	**0**
1/6	**9**	**6**
2/6	**27**	**20**
3/6	**1**	**10**
4/6	**0**	**2**
GVHD prophylaxis		
CsA/CsA + sMTX	**1/17**	
Tacrolimus/tacrolimus + sMTX	**11/9**	

GVHD indicates graft-versus-host disease; CsA, cyclosporin A; sMTX, short-term methotrexate.

*Primary HLA mismatches were detected on the basis of serological HLA-A, -B, and -DR alleles (n = 19) or serologic HLA-A and -B and high-resolution DRB1 alleles (n = 19).

†Confirmatory HLA mismatches were detected on the basis of high-resolution HLA -A, -B, and -DRB1 alleles.

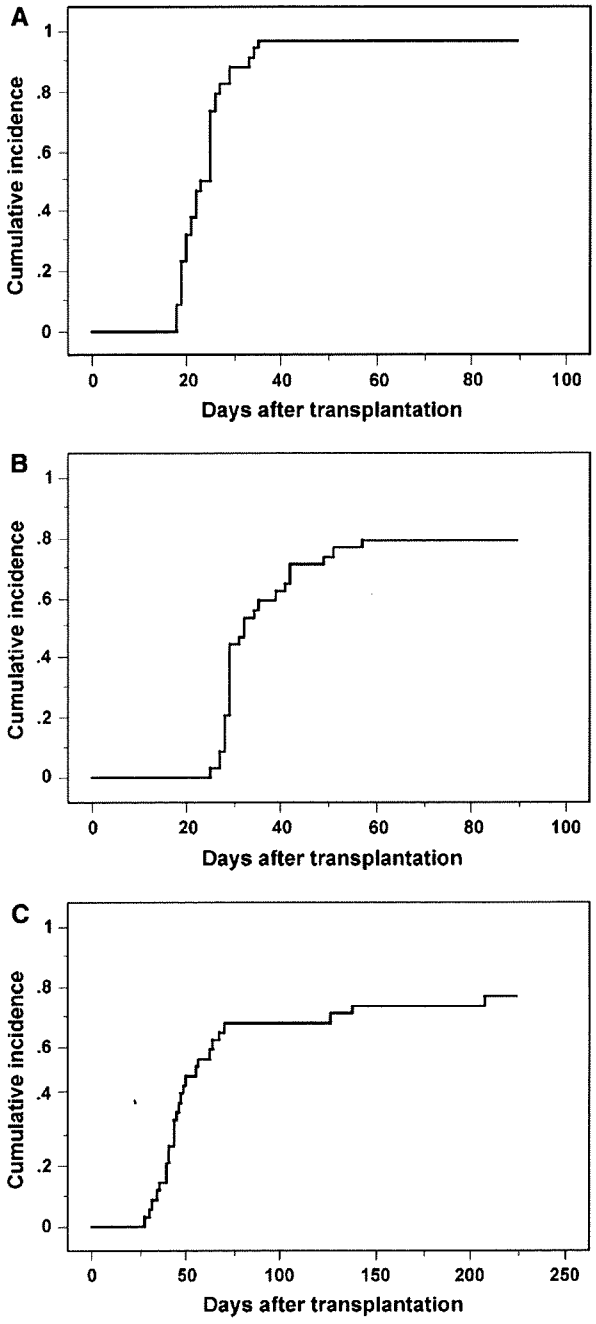

Figure 1. Neutrophil, reticulocyte, and platelet engraftment. Kaplan-Meier estimates of the probability of achieving a neutrophil count of >500/μL (A), a reticulocyte count of >1% (B), and an untransfused platelet count of >50,000/μL (C).

Early Organ Toxicity

Early organ toxicity caused by the TBI/FLAG preparative regimen by day 28 was graded by the regimen-related toxicity (RRT) grading system [24]. Toxicities because of infection, bleeding, GVHD, and drugs administered posttransplant were excluded from this

analysis. Grade I stomatitis was observed in 13 patients, grade I hepatic toxicity in 7 patients, and Grade I gastrointestinal toxicity (diarrhea) in 11 patients. No patient developed cardiac toxicity (electrocardiograph abnormality), pulmonary toxicity (dyspnea), renal toxicity (increase in creatinine), or bladder toxicity (haematuria).

Infection

Five patients developed sepsis, 6 pneumonia, 1 human herpesvirus-6 (HHV-6) encephalitis and 1 interstitial pneumonitis. Reactivation of cytomegalovirus was documented in 16 patients and gancyclovir was administered. One of them developed fatal interstitial pneumonitis because of CMV. No obvious fungemia and invasive aspergillosis were observed.

GVHD

Twenty-eight patients who attained engraftment and survived >40 days were evaluated for aGVHD. The cumulative incidence of grade II-IV aGVHD was 57% (16/28), with grades II, III, and IV occurring in 7, 8, and 1 patients, respectively. Appearance of aGVHD varied depending on GVHD prophylaxis used. The incidence of grade II-IV aGVHD was 88% (n = 8) when single agent (tacrolimus or CsA alone) was used in 2000 to 2002, and it was significant reduced (45%, n = 20) when short-term MTX was used in combination with tacrolimus or CsA after August 2002 ($P = .04$ by the χ^2 test). Chronic GVHD developed in 11 (41%) of 27 evaluable patients who survived >100 days. Of the 11 patients, 8 patients developed limited cGVHD and 3 extended cGVHD.

Relapse

Overall, 11 patients (28.9%) relapsed after CBT. Cumulative incidence of relapse is shown in Figure 2. Of these patients, 10 were in the high-risk group (3 with AML, 1 with MDS, 3 with ALL, 2 with non-Hodgkin lymphoma), and 1 was in the standard risk group (1 with ALL).

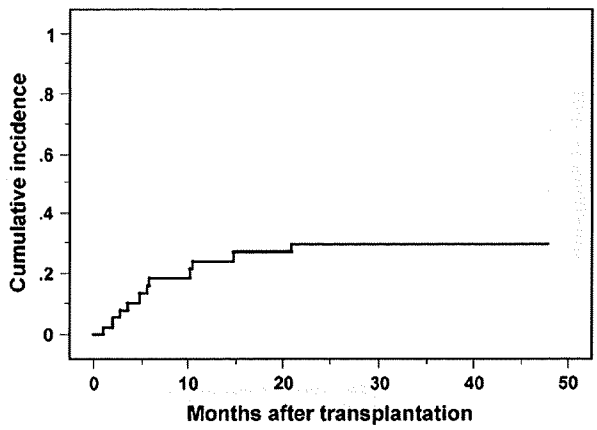

Figure 2. Cumulative incidence of relapse estimated by Kaplan-Meier method.

Causes of Death

TRM within 100 days was 23.6% (9 of 38 patients). The main cause of death was bleeding (pulmonary and cerebral) in 2 cases, sepsis in 2, multiple organ failure in 2, herpes simplex virus-6 encephalitis in 1, and pneumonia in 2. One patient died of relapse within 100 days. The cause of death after 100 days was relapse in 10 cases, sepsis in 1, pneumonia in 1, interstitial pneumonitis in 1, and cGVHD in 1. TRM at day 365, which excluded primary disease progression, was 34.2% (13 out of 38 patients).

Survival and Prognostic Factors

Fourteen out of 38 patients were alive at a median follow-up of 46 months (range: 4-77 months). Three-year EFS was 33.5% (Figure 3). Using Cox proportional hazards models, sex, weight, HLA match (primary and confirmatory), cell dose, GVHD prophylaxis, and the presence of grade II-IV GVHD had no apparent effect on EFS (Table 3). In contrast, age and disease status at transplantation had significant impacts on EFS in both univariable and multivariable analysis (Table 3). Kaplan-Meier estimates indicated that patients who were 42 years old or younger (n = 26) showed significantly better survival (39.8%) than those who were older than 42 years (n = 12) (19.4%) ($P = .0422$) (Figure 4). Regarding the disease status at transplantation, EFS was 72.7% in the standard risk group (n = 11) and 17.7% in the high-risk group (n = 27) ($P = .0075$) (Figure 5). As the number of patients included in this study is small, the results shown above should be interpreted with caution.

DISCUSSION

Intensified chemotherapy can be effective in the treatment of chemotherapy-sensitive malignant

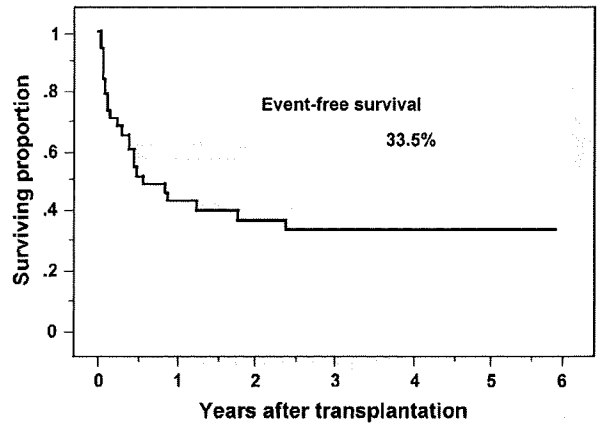

Figure 3. Kaplan-Meier estimates of EFS in CBT (n = 38).

Table 3. *Risk Factors for Event-Free Survival (EFS) after CBT*

Factors	Hazard Ratio	95% Confidential Interval	P-Value
Univariate analysis			
Sex	0.881	0.394-1.966	.7568
Age >42	2.325	1.007-5.493	.0481
Weight	1.016	0.969-1.064	.5168
Disease status: high risk	4.604	1.356-15.634	.0143
Cell dose: total cells	0.696	0.239-2.064	.5140
HLA match (primary)*	0.885	0.458-1.708	.7152
HLA match (confirmatory)†	1.022	0.666-1.567	.9218
GVHD prophylaxis			
CsA vs tacrolimus:	0.701	0.301-1.585	0.3939
CsA			
MTX + vs −: +	0.738	0.322-1.691	0.4730
Grade II-IV acuteGVHD	0.688	0.215-2.200	0.5282
Multivariate analysis			
Age >42	2.828	1.140-7.091	0.0250
Disease status: high-risk	5.245	1.505-18.281	0.0093

GVHD indicates graft-versus-host disease; CsA, cyclosporin A; MTX, methotrexate.

*Primary HLA matches were detected on the basis of serological HLA-A, -B, and -DR alleles (n = 19) or serologic HLA-A and -B and high-resolution DRB1 alleles (n = 19).

†Confirmatory HLA matches were detected on the basis of high-resolution HLA -A, -B, and -DRB1 alleles.

diseases [6]. The curative effect of allogeneic SCT is derived partly from the antileukemic effect of myeloablative therapy and partly from a graft-versus-leukemia effect of donor immune cells on the residual leukemia. Transplantation using CB cells as alternative to the bone marrow cells or peripheral blood cells has increasingly been performed for the treatment of hematologic malignancies [1-3]. However, a standard preparative conditioning regimen has not been firmly established. We here report the results of CBT using a new myeloablative regimen, TBI/FLAG.

In the present study, we used 12-Gy TBI (in 4 fractions) and FLAG comprising 10 g/m^2 Ara-C, 150 mg/m^2 Flu, and G-CSF. High-dose Ara-C has been found to be effective in the treatment of myeloid and lymphoid leukaemia patients with poor prognoses [25,26]. A conditioning regimen using TBI and high cumulative doses of Ara-C (24 or 36 g/m^2) achieves a lower relapse rate [27]. However, a significant proportion of allogeneic or autologous BMT patients who received high cumulative doses of Ara-C (36 g/m^2) has been reported to die early as a result of toxicity [28]. The incidence of pulmonary complications, including interstitial pneumonia and obvious infection, and the risk of pulmonary toxicity, increases with age. These results suggest that the use of high cumulative doses of Ara-C (36 g/m^2) for conditioning should be avoided. Tomonari et al. [29] carried out a preliminary trial in which 5 patients who received CBT were conditioned with 24 g/m^2 Ara-C, 90 mg/m^2 Flu, and 12-Gy TBI. All patients showed favorable prognosis. Furthermore, Takahashi et al. [30] reported that a conditioning regimen comprising 12-Gy TBI, Ara-C (12 g/m^2), cyclophosphamide (120 mg/kg), and G-CSF produced very good outcomes. Thus, appropriate doses of Ara-C may be effective as part of a preparative regimen. In the TBI/FLAG regimen used in this study, the total dose of Ara-C was limited to 10 g/m^2, and its activity was pharmacokinetically augmented by concomitant use of fludarabine [10]. This preparative regimen was found to be associated with minimal early RRT within 28 days and without any enhancement of later pulmonary or other life-threatening toxicities.

In CBT, the incidence of grade II-IV aGVHD has been reported to be 40% to 70% [31,32]. It was 58% in this study. When single-agent (tacrolimus or CsA alone) was used in 2000 to 2002, the incidence of grade

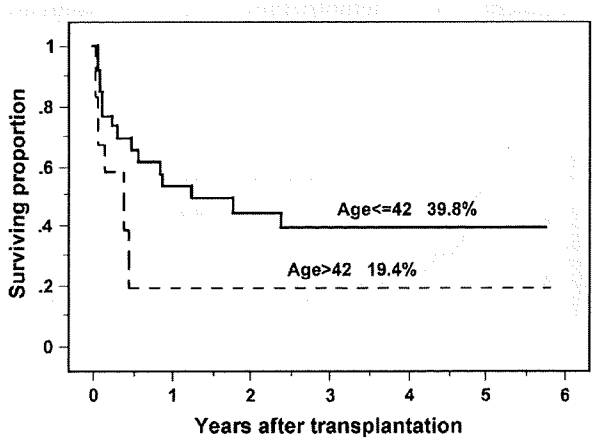

Figure 4. EFS in relation to age. Kaplan-Meier estimates of EFS in patients 42 years old or younger (n = 26) and older than 42 (n = 12) (P = .0422).

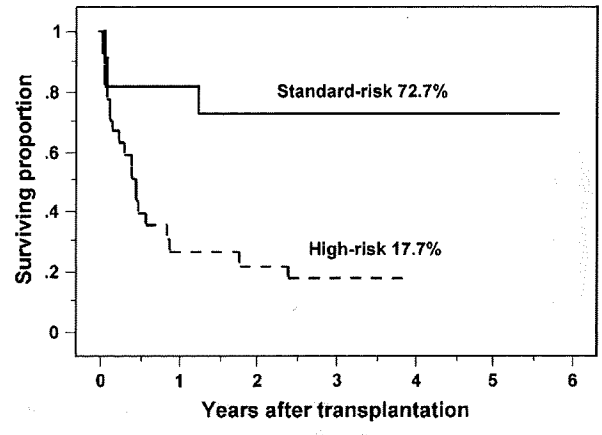

Figure 5. EFS in relation to disease status. Kaplan-Meier estimates of EFS in standard-risk patients (n = 11) and high-risk patients (n = 27) (P = .0075).

II-IV aGVHD was high (88%, n = 8), but it was significantly reduced to 45% (n = 20) when short-term MTX was used together with tacrolimus or CsA after August 2002. However, the combined use of MTX for GVHD prophylaxis did not result in the improvement of the survival rate in our analysis (Table 3). Rather, the disease status had the strongest impact on the survival rate (Table 3). Our patients' response to steroid therapy was generally good for those with grade II and III aGVHD (data not shown).

Rocha et al. [33] reported the results of CBT on 98 patients in multicenter analysis, which showed 36% of 2-year survival. Laughlin et al. [2] also reported multicentric analysis of CBT including 150 patients that showed 26% of 3-year survival. In single-institution studies of adult CBT, Long et al. [34] reported 3-year survival of 19% of 57 patients, whereas Takahashi et al. [30] showed 2-year survival of 74% of 113 patients. In our single-institution study using a single-conditioning regimen, 3-year EFS was 33.5% (Figure 3). Cell dose of CB graft is known to be one of the critical factors that affect EFS in CBT [31], but we did not find this is to be the case in our analysis (Table 3). This is probably because we used CB with relatively large number of cells, with the median cell number of 2.43×10^7 cells/kg. We found that patients older than 42 showed poor EFS (Figure 4), and this FLAG/TBI conditioned CBT is favorable to those who are 42 or younger. Regarding disease status and survival, 3-year EFS was 72.7% in the standard risk group (n = 11) and 17.7% in the high-risk group (n = 27) ($P = .0075$) (Figure 5). The EFS of 17.7% in the high-risk group in our study is comparable to previously reported rates of 15% to 20% [2]. The results of the present study are encouraging because standard-risk patients had 72.7% survival, which is comparable to that seen in standard-risk patients receiving allogeneic BMT or peripheral blood stem cell transplantation from HLA-matched donors [35]. This may indicate that CBT has almost the same efficacy as BMT in standard-risk patients. Although the finding must be confirmed in a larger scale study, our study suggests that CBT following conditioning with the TBI/FLAG regimen may be a reasonable option for adults with hematologic malignancies.

The results presented above show that CBT with a TBI/FLAG preparative regimen was well tolerated without significant RRT, and offered sustained donor cell engraftment. Patients who are 42 years old or younger and in standard risk may obtain a favorable outcome in this TBI/FLAG regimen. Further studies are needed to optimize this procedure to establish an effective treatment modality for hematologic malignancies.

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poorly phrased. There is no standard by which one can compare an odds ratio for CR to a hazard ratio for OS. Given how difficult it has been historically to improve upon the results with MP, and the conflicting results of the various MPT vs MP trials, the clear survival advantage seen with MP-Bortezomib in the VISTA trial is truly remarkable, and by far the most important take-home message. It is not appropriate to directly contrast the results of the E4A03 and VISTA studies (which I agree are both excellent). The contrast I tried to make in the editorial was in the reaction to these trials of stock analysts (whose obtrusive presence at the meeting has recently been noted[16]), who appear to place an excessively high value on improvements in CR. In my opinion this is not always appropriate, and a more balanced approach is warranted, with better surrogates (for example, molecular CR, suppression of cytogenetic abnormalities) for OS needed.

PL Bergsagel
Professor of Medicine, Mayo Clinic, Mayo Clinic Cancer Center, Scottsdale, AZ, USA
E-mail: bergsagel.leif@mayo.edu

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Molecular detection of *AML1-MTG8*-positive cells in peripheral blood from a patient with isolated extramedullary relapse of t(8;21) acute myeloid leukemia

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A few studies have reported that *AML1-MTG8* expression levels in bone marrow (BM) are 1- to 3-log higher than those in peripheral blood (PB) when detected by quantitative PCR methods in acute myeloid leukemia (AML) with the t(8;21) translocation.[1–3] However, the relationship between BM and PB is retained at any time during the clinical course is unknown. Here we present a patient with t(8;21) AML who demonstrated isolated ovarian relapse after allogeneic BM transplantation (BMT). *AML1-MTG8* chimeric transcripts could be repeatedly detected in both BM and PB during the clinical course. Moreover, the *AML1-MTG8* expression levels detected by real-time quantitative (RQ)-PCR methods in PB were higher than those in BM before and at the time of the extramedullary relapse (EMR). Thus, we propose that the presence of EMR is responsible for repeated detection of minimal residual disease (MRD) and discuss the clinical significance of different *AML1-MTG8* expression levels between BM and PB for the diagnosis of isolated EMR.

A 22-year-old woman was diagnosed with AML (French-American-British (FAB) subtype M2) with thoracic vertebrae involvement in March 1998. Cytogenetic evaluation revealed the t(8;21)(q22;q22) chromosomal translocation. She achieved complete remission (CR) with induction chemotherapy and

radiotherapy; however, in December 1998 she had a BM relapse, involving the thoracic vertebrae and the spine. Salvage chemotherapy reduced the total number of leukemic blasts in BM to below 5%. In March 1999, she underwent BMT from her HLA-2-antigen-mismatched/haploidentical sister at Osaka University Hospital. A total of 3.0×10^8 per kg unmanipulated nucleated cells were infused. The transplant protocol consisted of a high dose of cytarabine and cyclophosphamide and total body irradiation (12 Gy), followed by a short course of methotrexate, tacrolimus and methylprednisolone (2 mg/kg) for graft-versus-host disease (GVHD) prophylaxis. The patient achieved an absolute neutrophil count above 0.5×10^9 per liter on day 20. The last platelet transfusion was performed on day 125. CR and complete donor chimerism was confirmed by a BM examination on day 24. No acute GVHD developed.

We sequentially measured *AML1-MTG8* expression levels using RQ-PCR methods during the clinical course as previously described.[4] *AML1-MTG8* levels in BM stayed below 1.0×10^{-5} after BMT, but increased to 3.8×10^{-5} on day 96. Thereafter, *AML1-MTG8* levels in BM and PB were monitored biweekly and weekly, respectively. Whereas *AML1-MTG8* levels in BM and PB showed parallel movement, those in PB were constantly over 2.0×10^{-4} and were higher than those in BM. Because a BM examination still revealed CR, we suspected a regrowth of leukemia cells in the patient and performed a systemic examination. Computed tomography scans of the pelvis revealed little ascites in the patient on day 109, but an enlargement of the left ovary with a diameter of 5 cm with moderate ascites on day 158 (Figure 1). The ovarian tumor was diagnosed as EMR due to the contamination of t(8;21)-positive leukemia cells in ascites by culdocentesis.

As tacrolimus and prednisolone were tapered rather rapidly for induction of a graft-versus-leukemia effect, skin GVHD developed on day 170. Following increase in tacrolimus and prednisolone, the skin rash disappeared in about a week, followed by shrinkage of the tumor to a diameter of 2.5 cm and

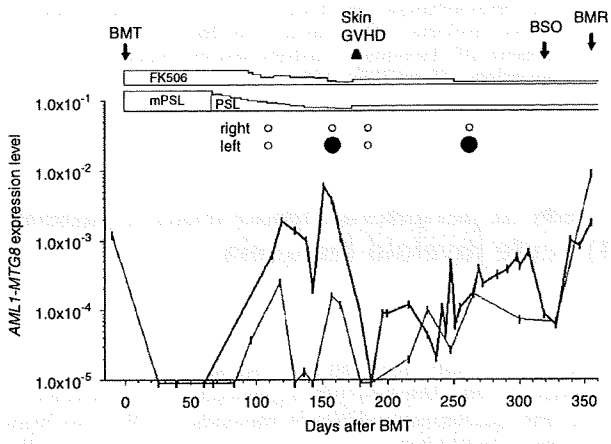

Figure 1 Clinical course and kinetics of the *AML1-MTG8* gene-transcript expression levels in bone marrow (BM) and peripheral blood (PB). Thin and thick lines indicate changes in *AML1-MTG8* expression levels in BM and PB, respectively. In the results of imaging studies of the ovary, open circles indicate the normal size ovary, whereas closed circles indicate the enlarged ovary. Real-time quantitative-PCR was performed with ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). *AML1-MTG8* expression levels in Kasumi-1 cell lines were defined as 1.0. BMR, bone marrow relapse; BMT, bone marrow transplantation; BSO, bilateral salpingo-oophorectomy; FK506, tacrolimus; GVHD, graft-versus-host disease; mPSL, methylprednisolone; PSL, prednisolone.

disappearance of the ascites on day 185. *AML1-MTG8* levels in both BM and PB decreased to below 1.0×10^{-5} on day 187; however, the levels increased again with some fluctuations. Magnetic resonance imaging of the pelvis on day 262 showed enlargement of the left ovarian tumor again. Because BM was still present in CR, bilateral salpingo-oophorectomy was performed on day 319. The bilateral ovaries appeared to be involved, making complete resection impossible due to tight adhesion with surrounding tissues. *AML1-MTG8* levels were highest in the BM greater than those in PB, when morphologic BM relapse occurred on day 355 (Figure 1). Despite chemotherapy and donor lymphocyte infusion, the patient died of renal failure due to obstruction of the bilateral ureters by abdominal mass on day 474.

EMR of leukemia after transplant occurs in diverse sites such as the central nervous system and testis, which makes an early diagnosis difficult. One feasible approach to overcome this problem would be monitoring MRD that is involved in BM.[5,6] In the present case, continuous detection of *AML1-MTG8* chimeric transcripts not only in BM, but also in PB was quite helpful in detecting the presence of EMR. So far nested PCR detection of *AML1-MTG8* chimeric transcripts in BM and PB have not been the indicators of subsequent relapse in t(8;21) AML after BMT.[7] Meanwhile, recent studies using quantitative PCR methods reported that there is a threshold of *AML1-MTG8* expression levels at which subsequent relapse occurs.[1,8,9] Therefore, frequent monitoring of *AML1-MTG8* expression levels or systemic screening for extramedullary disease should be considered, especially as *AML1-MTG8* chimeric transcripts continued to be detected despite CR.

Interestingly, the *AML1-MTG8* levels in BM and PB showed reversal between day 112 and 179, suggesting that t(8;21)-positive leukemia cells originated from extramedullary disease were constantly present in PB, rather than in BM. Given that this unusual relationship returned to the original state at the time of BM relapse, higher *AML1-MTG8* expression levels in PB compared to BM suggest a sign of isolated EMR. However, further studies are required to determine whether screening of PB is superior to BM for early detection of isolated EMR by PCR-based monitoring *AML1-MTG8* expression levels.

H Tamaki[1], S Yoshihara[1], T Fujioka[1], M Kawakami[2], Y Oka[3] and H Ogawa[1]
[1]Division of Hematology, Department of Internal Medicine, Hyogo College of Medicine, Hyogo, Japan;
[2]Department of Medicine, National Hospital Organization, Osaka Minami Medical Center, Osaka, Japan and
[3]Department of Respiratory Medicine, Allergy and Rheumatic Diseases, Osaka University Graduate School of Medicine, Osaka, Japan
E-mail: tamakhi@hyo-med.ac.jp

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The B-cell calcium sensor predicts progression of chronic lymphocytic leukemia

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Identifying mechanisms responsible for the clinical heterogeneity of chronic lymphocytic leukemia (CLL) is important to develop better treatments for this disease. Variations in responsiveness to immunoreceptor signaling may be responsible for differences in proliferation of CLL cells *in vivo*.[1] Accordingly, we examined the status of the B-cell calcium sensor (Ca_oS) in primary CLL cells, as it responds to extracellular calcium (Ca_o^{2+}) fluctuations by modulating subsequent signal transduction through immunoreceptors.[2]

In contrast to normal B cells, nearly half (23/51) of the CLL samples examined (with approval from the Sunnybrook Health Sciences Center Research Ethics Board) did not release intracellular calcium (Ca_i^{2+}) in response to $CaCl_2$ (labeled Ca_o^{2+} non-responders) (Table 1; Figure 1a). This impaired Ca_oS activity was not due to decreased stores of Ca_i^{2+} in the endoplasmic reticulum, as the Ca^{2+} ATPase inhibitor, thapsigargin, was able to mobilize Ca_i^{2+} in these cells (not shown). While normal B-cells mobilized Ca_i^{2+} in response to as little as 250 μM $CaCl_2$, Ca_o^{2+} non-responder CLL cells remained insensitive to doses as high as 1.5 mM (above which, calcium was toxic) (not shown). These results suggested that the defective responses to Ca_o^{2+} were not due to reduced expression or

Table 1 Summary of clinical properties of CLL patients classified on the basis of release of Ca_i^{2+} stores by their tumor cells in response to Ca_o^{2+}

Variable	*All patients*	*Ca_o^{2+} responders*	*Ca_o^{2+} non-responders*	P-*value*
No. of patients	51	28	23	
Median age, years	61	60.5	63	NS
Sex, no. (%)				
Female	27 (52.9)	14 (50.0)	13 (56.5)	NS
Male	24 (47.1)	14 (50.0)	10 (43.5)	NS
Years after diagnosis, mean ± s.e.	6.1 ± 0.6	6.1 ± 0.9	6.1 ± 0.7	NS
WBC count, × 1000 cells/μl, mean ± s.e.	65.9 ± 11.2	62.7 ± 14.2	69.8 ± 18.3	NS
Rai stage III–IV, no. (%)	30 (58.8)	19 (67.9)	11 (47.8)	0.08
CD38%, mean ± s.e.	16.8 ± 3.4 (*n* = 46)	25.3 ± 5.4 (*n* = 26)	5.9 ± 0.9 (*n* = 20)	<0.02
β2-Microglobulin, mg/l, mean ± s.e.	2.3 ± 0.3 (*n* = 16)	2.6 ± 0.4 (*n* = 11)	1.8 ± 0.3 (*n* = 5)	0.05
Genomic aberrations, no. (%)				
Deletion 11	3 (8.1)	2 (9.1)	1 (6.6)	
Deletion 17	5 (13.5)	4 (18.8)	1 (6.6)	
Trisomy 12	3 (8.1)	2 (9.1)	1 (6.6)	
Deletion 13	21 (56.8)	15 (68.2)	6 (40.0)	
Normal	11 (29.7)	6 (27.3)	5 (33.3)	
Not available	14	6	8	
High-risk cytogenetics[a], no. (%)	10 (27.0)	7 (31.8)	3 (20.0)	NS
LDTs, months, mean ± s.e.	28.3 ± 5.6 (*n* = 44)	10.9 ± 3.3 (*n* = 22)	49.5 ± 9.4 (*n* = 22)	<0.001
Received treatment, no. (%)	25 (49.0)	17 (60.7)	8 (34.8)	0.03
No. of treatments/patient, mean ± s.e.	1.3 ± 0.3	1.9 ± 0.4	0.7 ± 0.3	0.02

Abbreviations: CLL, chronic lymphocytic leukemia; LDTs, lymphocyte doubling times; NS, not significant; WBC, white blood cell.
Assume *n* = 51, unless otherwise indicated.
[a]High-risk cytogenetics include patients with $17p^-$ deletions, $11q^-$ deletions, trisomy 12 or complex multiple abnormalities.

Hematopoietic stem cell transplantation for core binding factor acute myeloid leukemia: t(8;21) and inv(16) represent different clinical outcomes

Yachiyo Kuwatsuka,[1] Koichi Miyamura,[1] Ritsuro Suzuki,[2] Masaharu Kasai,[3] Atsuo Maruta,[4] Hiroyasu Ogawa,[5] Ryuji Tanosaki,[6] Satoshi Takahashi,[7] Kyuhei Koda,[8] Kazuhiro Yago,[9] Yoshiko Atsuta,[2] Takashi Yoshida,[10] Hisashi Sakamaki,[11] and Yoshihisa Kodera[1]

[1]Department of Hematology, Japanese Red Cross Nagoya First Hospital, Nagoya; [2]Department of HSCT Data Management, Nagoya University School of Medicine, Nagoya; [3]Department of Hematology, Sapporo Hokuyu Hospital, Sapporo; [4]Department of Hematology, Kanagawa Cancer Center, Yokohama; [5]Department of Molecular Medicine, Osaka University Graduate School of Medicine, Osaka; [6]Stem Cell Transplantation Unit, National Cancer Center Hospital, Tokyo; [7]Department of Hematology, Institute of Medical Science, The University of Tokyo, Tokyo; [8]Department of Hematology, Asahikawa Red Cross Hospital, Asahikawa; [9]Department of Hematology, Shizuoka General Hospital, Shizuoka; [10]Hematology Department, Toyama Prefectural Hospital, Toyama; and [11]Department of Hematology, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan

We analyzed 338 adult patients with acute myeloid leukemia (AML) with t(8;21) and inv(16) undergoing stem cell transplantation (SCT) who were registered in the Japan Society for Hematopoietic Cell Transplantation database. At 3 years, overall survival (OS) of patients with t(8;21) and inv(16) was 50% and 72%, respectively ($P = .002$). Although no difference was observed when restricted to allogeneic SCT in first complete remission (CR; 84% and 74%), OS of patients with t(8;21) and inv(16) undergoing allogeneic SCT in second or third CR (45% and 86% at 3 years; $P = .008$) was different. OS was not different between patients in first CR who received allogeneic SCT and those who received autologous SCT for both t(8;21) AML (84% vs 77%; $P = .49$) and inv(16) AML (74% vs 59%; $P = .86$). Patients with inv(16) not in CR did better after allogeneic SCT than those with t(8;21) (70% and 18%; $P = .03$). Patients with t(8;21) and inv(16) should be managed differently as to the application of SCT. SCT in first CR is not necessarily recommended for inv(16). For t(8;21) patients in first CR, a prospective trial is needed to clarify the significance of autologous SCT and allogeneic SCT over chemotherapy. (Blood. 2009;113:2096-2103)

Introduction

Core binding factor (CBF) acute myeloid leukemia (AML) including t(8;21)(q22;q22) and inv(16)(p13q22)/t(16;16)(p13;q22) [t(8;21) and inv(16)] is considered to be a favorable cytogenetic subgroup in clinical studies.[1-4] Patients with t(8;21) and inv(16) have shown a markedly improved outcome with repetitive use of high-dose cytarabine.[5-13] However, the major treatment failure is disease recurrence.[14-16] These patients frequently become stem cell transplantation (SCT) candidates.

Both t(8;21) and inv(16) AMLs are associated with disruption of genes encoding subunits of the CBF, a heterodimeric transcriptional factor involved in the regulation of hematopoiesis.[17,18] Although these 2 different cytogenetics also share common clinical characteristics, they are associated with different clinical features such as morphologic presentation and immunophenotypic marker expression.[19]

Several reports demonstrated inferior outcome of t(8;21) compared with inv(16), but the number of patients who underwent transplantation was limited.[14,15,20] A recent study from the Dana-Farber Cancer Institute reported that both patients with t(8;21) and inv(16) de novo AML who underwent allogeneic transplantation performed favorably compared with other karyotypes.[21] To identify the survival data and prognostic factors among the CBF leukemia population who received SCT, we conducted a retrospective analysis using a Japanese multi-institution database with a large number of patients.

Methods

Study population

A total of 2802 adult patients who underwent autologous or allogeneic SCT from 1996 and 2004 for AML were registered in the Japan Society for Hematopoietic Cell Transplantation (JSHCT) database. Patients who underwent SCT from unrelated donors were registered in the different registry in the study period, but not all of the patients undergoing unrelated SCT were registered in the JSHCT database. Demographic, diagnostic, clinical, cytogenetics, induction, and outcome information were collected for each patient, and were sent to a central registration center. Cytogenetic studies were performed in each center, but a central review of cytogenetic analysis was not performed.

Patients with de novo AML aged 16 to 70 years who received hematopoietic SCT as the first transplant were included in the study. No patients with prior history of autologous or allogeneic SCT were included in the study. Of the remaining 2164 patients, 178 patients with t(15;17) or PML/RARα were excluded from the analysis below (Table 1). Finally, of the 1986 patients included in the analysis, 255 were reported to have t(8;21) abnormality, and 83 to have inv(16). A total of 194 patients had no available cytogenetic data. The remaining 1454 patients with normal karyotype and other cytogenetic abnormalities were further coded and analyzed according to published Southwest Oncology Group (SWOG) criteria.[3] The intermediate risk category included patients characterized by +8, −Y, +6, del(12p), or normal karyotype. The unfavorable risk category was defined by the presence of one or more of −5/del(5q), −7/del(7q), abn 3q, 11q, 20q, or

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Table 1. Cytogenetic risk groups of patients with AML who received autologous SCT and allogeneic SCT

Cytogenetic risk groups	No. patients		
	Auto-SCT	Allo-SCT	Total
t(8;21)	61	194	255
inv(16)	17	66	83
t(15;17)*	65	113	178
Intermediate	140	749	889
Unfavorable	35	325	360
Unknown			
Unknown cytogenetic risk	27	178	205
No available cytogenetic data	44	150	194
Total	389	1775	2164

Auto-SCT indicates autologous stem cell transplantation; Allo-SCT, allogeneic stem cell transplantation.

*Patients with t(15;17) were excluded from the analysis.

21q, del(9q), t(6;9), t(9;22), abn 17p, and complex karyotypes defined as 3 or more abnormalities. Patients with other cytogenetic aberrations were considered an unknown risk group, and were analyzed together with 194 patients with no cytogenetic data.

This study was approved by the Committee for Nationwide Survey Data Management of the JSHCT. Informed consent was obtained in accordance with the Declaration of Helsinki.

Transplantation

A total of 1662 patients underwent allogeneic SCT, and 324 underwent autologous SCT. Patients were treated with various conditioning regimens, but most of those who underwent autologous transplantation received non–total body irradiation (TBI) regimens (97%), including busulfan (BU), cytarabine (CA), and etoposide. The most frequently used conditioning regimens before allogeneic SCT were cyclophosphamide (Cy) plus TBI (n = 327 patients), and BU plus Cy (n = 267). Conditioning regimens before allogeneic SCT also included more intensified regimens such as CA plus Cy plus TBI (n = 262) and BU plus Cy plus TBI (n = 146), or reduced-intensity conditioning regimens with fludarabine (n = 241) or cladribine (n = 19).

Stem cell sources for allogeneic SCT were bone marrow in 871 patients, peripheral blood stem cell in 570 patients, bone marrow plus peripheral blood stem cell in 23 patients, and cord blood in 190 patients. A total of 1242 patients underwent allogeneic SCT from a related donor, and 404 patients underwent SCT from an unrelated donor.

Of the 1637 patients who had available data, 74% received transplants from human leukocyte antigen (HLA)–matched donors. Among patients who received unrelated bone marrow transplants, 156 patients were HLA genotypically matched and 51 were HLA mismatched. HLA data for 39 mismatched unrelated bone marrow transplantation patients were available. A total of 32 patients were one locus mismatched, and 7 patients were 2 loci mismatched. Among patients receiving unrelated cord blood transplants, 19 patients were serologically HLA matched and 170 patients were mismatched. HLA incompatibility was 5 of 6 HLA matched in 57 patients, 4 of 6 HLA matched in 99 patients, 3 of 6 HLA matched in 7 patients, and 1 of 6 HLA matched in 1 patient.

Graft-versus-host disease (GVHD) prophylaxis mostly consisted of methotrexate and a calcineurin inhibitor, either cyclosporin A or tacrolimus. Several other prophylaxes include mycophenolate mofetil, antithymocyte globulin, and $CD34^+$ selection. The incidence of acute GVHD was evaluated in 1488 patients who survived more than 28 days, and chronic GVHD was evaluated in 1302 patients who survived more than 100 days after allogeneic SCT. GVHD was evaluated in each center.

Statistical analysis

Correlation between the 2 groups was examined with the chi-square test, Fisher exact test, and the Mann-Whitney *U* test. Disease-free survival (DFS) was calculated from the date of transplantation until the date of relapse or the date of death in CR. Patient survival data were analyzed with the method of Kaplan and Meier and compared by the log-rank test.

Univariate and multivariate analyses for OS were performed with the aid of the Cox proportional hazard regression model, and variables were selected with the stepwise method. The following variables were evaluated: age, sex, and disease status at transplantation; CR versus not in CR; the number of induction courses to achieve CR; one course versus more than one course and failure; type of transplantation (allogeneic SCT vs autologous SCT); conditioning regimen (reduced intensity vs myeloablative); TBI regimen or not; and the existence of additional karyotype abnormalities or not. For those who received allogeneic SCT, in addition to these variables, the following were also evaluated: type of GVHD prophylaxis; short-course methotrexate plus cyclosporin A or short methotrexate plus FK506; acute GVHD, grade II to IV or grade III to IV; chronic GVHD; HLA mismatch; donor; and donor source. The doses of methotrexate were not surveyed. Each factor was considered to be prognostic if the *P* value was less than .05. Data were analyzed with the Stata 9.2 statistical software (College Station, TX).

Results

Initial characteristics of patients

The median age of all patients with AML in total was 41 years old (range, 16-70 years old). Median follow-up period of living patients was 37.3 months (range, 0.4-108 months). Patients were categorized into 5 cytogenetic subgroups: with t(8;21), with inv(16), intermediate risk cytogenetics, unfavorable cytogenetics, and an unknown risk group. Table 1 shows the number of patients in each cytogenetic subgroup and patients with t(15;17), who were excluded from the analysis.

Characteristics of the patients with CBF who underwent allogeneic SCT or autologous SCT are shown in Table 2. No significant difference was observed between characteristic of 2 groups of patients with CBF who received autologous SCT, except for the initial white blood cell count.

Of the 259 patients with CBF who received allogeneic SCT, significantly more patients with t(8;21) had failed to achieve CR with a single course of induction chemotherapy at diagnosis ($P = .002$), and were not in CR at the time of transplantation ($P < .001$). Among patients in CR at transplantation, the ratio of those in first, second, or third CR was not different between t(8;21) and inv(16) subgroups. Significantly more patients with inv(16) received transplants from an unrelated donor ($P = .004$). Table 3 and Table S1 (available on the *Blood* website; see the Supplemental Materials link at the top of the online article) summarize the transplantation data of those undergoing allogeneic SCT. More patients with inv(16) received unrelated transplants compared with t(8;21) patients ($P = .004$).

Overall survival

The OS of 1986 patients with AML at 3 years was 48%, and those with t(8;21), inv(16), intermediate, unfavorable, and unknown cytogenetic risks showed OS of 50%, 72%, 52%, 35%, and 45%, respectively ($P < .001$). Figure 1 shows survival curves of patients with AML patients who underwent allogeneic SCT in first CR (Figure 1A), in second or third CR (Figure 1B), or not in CR (Figure 1C), categorized by the cytogenetic abnormalities. Survival data are listed in Table 4. The OS of patients with t(8;21), inv(16), and intermediate, unfavorable, and unknown risk undergoing allogeneic SCT in first CR was 84%, 74%, 69%, 53%, and 52%, respectively ($P < .001$), and that of patients undergoing allogeneic-SCT

Table 2. Characteristics of patients with CBF AML

	Auto-SCT			Allo-SCT		
	t(8;21) (n = 61), no.	inv(16) (n = 17), no.	P	t(8;21) (n = 194), no.	inv(16) (n = 66), no.	P
Median age, y (range)	44 (17-68)	37 (19-61)	.59	39 (16-70)	34 (16-64)	.054
Median WBC, g/L (range)	8.8 (0.2-94)	33 (2.1-199)	.02	11 (.6-366)	53 (1.8-284)	< .001
Sex						
Male	41	12	.79	117	40	.93
Female	20	5		74	26	
No. of induction chemotherapy at diagnosis of AML						
1 course	48	15	.72	125	55	.002
> 1 or failure*	11	2		56	7	
Additional cytogenetic abnormalities						
None	53	15	> .999	153	54	.61
Positive	8	2		41	12	
Disease status at SCT						
CR	55	16	> .999	108	52	< .001
Not in CR	6	1		85	11	
CR1	43	13	.98	49	21	.29
CR2	7	1		45	26	
CR3	0	1		5	4	
Conditioning regimen						
TBI	0	1	.22	118	47	.078
Not TBI	61	16		71	16	

Correlation between the two groups was examined.
WBC indicates white blood cell count; g/L, 10^9/L; CR1, first complete remission; and CR2 or 3, second or third CR.
*More than 1 or failure includes patients who did not achieve complete remission after first course of induction chemotherapy, and those who were resistant to induction chemotherapy.

in second or third CR was 45%, 86%, 57%, 44%, and 64%, respectively ($P = .09$). OS of patients undergoing allogeneic SCT not in CR was 18%, 70%, 25%, 15%, and 18%, respectively ($P = .003$).

Table 3. Summary of allogeneic SCT

	t(8;21) (n = 194), no.	inv(16), (n = 66), no.	P
Conditioning regimen			
RIST	31	9	.66
Myeloablative	161	56	
GVHD prophylaxis*			
sMTX+CyA	136	48	.78
sMTX+FK	20	8	
HLA			
Match	146	47	.5
Mismatch	45	18	
Donor			
Related	161	44	.004
Unrelated	32	22	
Stem cell source			
BM	101	40	.27
PB	72	17	
CB	18	7	
aGVHD grade			
0-I	117	37	.54
II-IV	60	22	
cGVHD type			
None	64	28	.28
Lmt/Ext	67	20	

Correlation between the two groups was examined. Some of the missing data was not available, and total numbers do not add up to the number of the patients in each group.
RIST indicates reduced intensity stem cell transplantation; sMTX, short-course methotrexate; CyA, cyclosporin A; FK, tacrolimus; BM, bone marrow; PB, peripheral blood; CB, cord blood; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; Lmt, limited; and Ext, extensive.
*Dose of methotrexate was not surveyed in the study. Detail of other GVHD prophylaxis regimens are in Table S1.

When patients undergoing allogeneic SCT in first CR were analyzed, 3-year OS was not significantly different between patients with t(8;21) and inv(16) (84% and 74%, respectively; $P = .28$), between inv(16) and intermediate risk groups (74% and 69%, respectively; $P = .84$), or between t(8;21) and intermediate risk groups (84% and 69%, respectively; $P = .06$). However, when patients undergoing allogeneic SCT in second or third CR were analyzed, the 3-year OS of patients with inv(16) was significantly better than patients with t(8;21) (86% and 45%, respectively; $P = .008$), and better than intermediate risk patients (86% and 57%, respectively; $P = .03$). Difference was not significant between patients in the intermediate risk group and t(8;21) undergoing allogeneic SCT in second or third CR ($P = .36$). The OS of inv(16) patients undergoing allogeneic SCT not in CR was 70% at 3 years, which was also significantly better than that of t(8;21) (18%; $P = .03$) and the intermediate risk group (25%; $P = .045$).

In addition, the OS of t(8;21) undergoing allogeneic SCT in first CR was significantly better than that of the unfavorable risk group (84% and 53%, respectively; $P < .001$), but the difference between the 2 groups was not significant among patients undergoing allogeneic SCT in second or third CR. In contrast, OS was not different between inv(16) and unfavorable groups undergoing allogeneic SCT in first CR, but it was significantly different when they underwent allogeneic SCT in second or third CR (86% and 44%, for inv(16) and unfavorable groups, respectively; $P = .01$) or allogeneic SCT in non-CR (70% and 15%, respectively; $P = .006$).

Survival curves of patients who underwent autologous SCT in first CR, second or third CR, and not in CR are shown in Figure 2A, 2B, and 2C, respectively. The overall survival of patients with t(8;21), inv(16), and intermediate, unfavorable, and unknown cytogenetic risks in first CR was 77%, 59%, 74%, 38%, and 71%, respectively ($P = .049$), while that of patients undergoing autologous SCT in second or third CR was 43%, 50%, 59%, 44%, and 42%, respectively ($P = .8$). The OS of patients undergoing autologous SCT not in CR with t(8;21), inv(16), intermediate, and

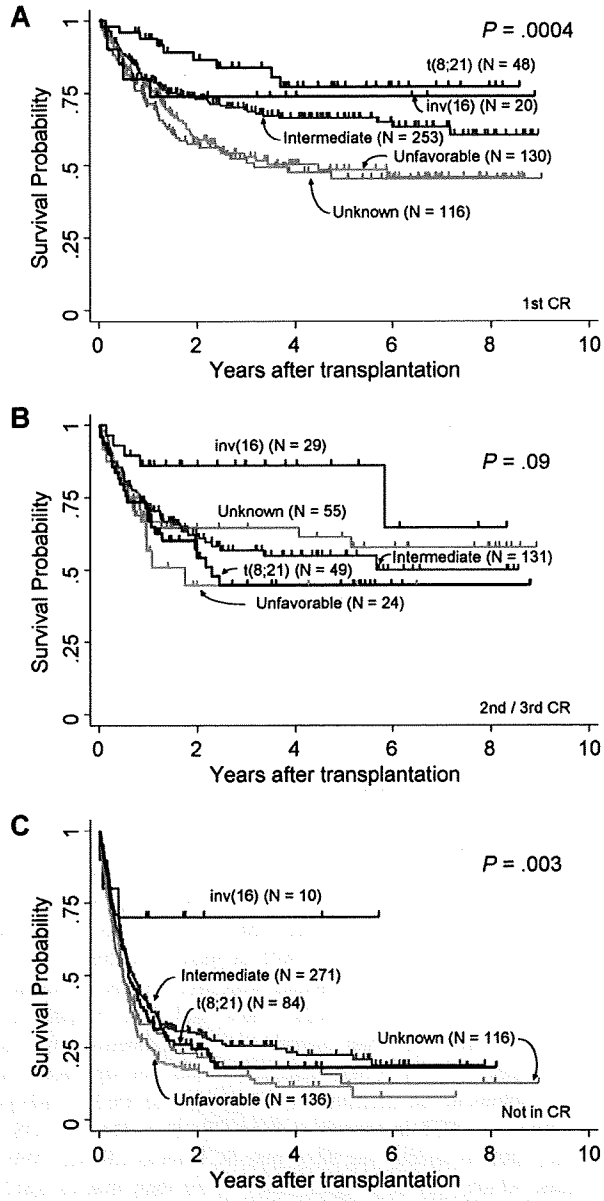

Figure 1. OS difference of patients undergoing allogeneic SCT between cytogenetic subgroups. (A) Survival curves of patients undergoing allogeneic SCT in first CR. (B) Survival curve of patients undergoing allogeneic SCT in second or third CR. (C) Survival curves of patients undergoing allogeneic SCT not in CR. Each are categorized by cytogenetic risk groups, respectively.

unknown risks was 17%, 100%, 25%, and 13%, respectively, and the survival curve of patients in the unfavorable risk group did not reach 3 years ($P = .35$).

Figure 3A and B focus on t(8;21) and inv(16) patients, stratified according to the type of (allogeneic or autologous) and disease status at the time of transplantation (first CR, second or third CR, and not in CR). The 3-year overall survival of t(8;21) patients in first CR was not different between allogeneic and autologous transplantation (84% and 77%, respectively), as well as that of patients in second or third CR (45% and 43%, respectively) and patients not in CR (18% and 17%, respectively). Similarly, the 3-year OS of inv(16) patients was not different between allogeneic and autologous transplantation when they underwent transplantation in first CR (74% and 59%). A significant difference was observed among the 3 disease status groups of t(8;21) patients ($P < .001$; Figure 3A), but not inv(16) patients ($P = .75$; Figure 3B).

The OS of allogeneic SCT, excluding cord blood transplantation, was not different from the analysis presented here, including bone marrow, peripheral blood, and cord blood transplantation (Table S2; Figures S1,S2).

DFS after SCT was also different among cytogenetic risk groups ($P < .001$). DFS of patients with inv(16) (69% at 3 years) was better compared with t(8;21) (49%), intermediate (46%), unfavorable (31%), and unknown (41%) risk groups. Among patients undergoing allogeneic SCT in first CR, DFS was also different among cytogenetic subgroups ($P < .001$). When t(8;21), inv(16), and intermediate cytogenetic subgroups undergoing allogeneic SCT in first CR were compared, the difference was not statistically significant between t(8;21) and inv(16) (78% and 73% at 3 years; $P = .58$), between t(8;21) and intermediate risk group (78% and 63%; $P = .1$), nor between inv(16) and intermediate risk group (73% and 63%; $P = .65$). DFS of patients with t(8;21) undergoing allogeneic SCT in first CR was better than that of the unfavorable risk group (78% and 47%, respectively; $P < .001$), but the difference was not significant between inv(16) and unfavorable risk groups (73% and 47%, respectively; $P = .16$).

DFS was not significantly different when 5 cytogenetic subgroups among patients undergoing allogeneic SCT in second or third CR were compared ($P = .32$). The DFS of patients undergoing allogeneic SCT in second or third CR was not significantly different between t(8;21) and inv(16) (43% and 71% at 3 years; $P = .053$), t(8;21) and the intermediate group (43% and 47%; $P = .76$), or inv(16) and the intermediate group (71% and 47%; $P = .06$). The difference was also not significant between t(8;21) and unfavorable risk groups (43% and 42%; $P = .7$), nor between inv(16) and unfavorable risk groups (71% and 42%; $P = .06$). The DFS of patients undergoing allogeneic SCT who were not in CR was significantly different among the 5 cytogenetic subgroups ($P = .005$), and that of inv(16) (75% at 3 years) was significantly better than t(8;21) (18%; $P = .02$), the intermediate risk group (22%; $P = .03$) and the unfavorable risk group (10%; $P = .003$).

Relapse and TRM

The relapse rate (RR) after SCT also differed among cytogenetic subgroups ($P < .001$). The RR of patients with inv(16) (18% at 3 years) was lower than t(8;21) (38%), intermediate (38%), and unfavorable (56%) risk groups. The RR of t(8;21) and inv(16) after allogeneic SCT was not statistically different in either first CR (16% and 6%; $P = .45$) or second or third CR (34% and 16%, respectively; $P = .09$).

Transplantation-related mortality (TRM) of all patients with AML was 22% at 3 years. The TRM of t(8;21) (18%), inv(16) (11%), and intermediate (21%), unfavorable (24%), and unknown risk groups (27%) was significantly different among cytogenetic risk groups ($P = .02$).

Evaluation of prognostic variables in CBF

Univariate analyses of t(8;21) showed that age ($P = .004$), not in CR at transplantation ($P < .001$), allogeneic SCT ($P = .01$), and TBI regimen ($P = .006$) were significant prognostic factors indicating poor OS (Table 5). Multivariate analysis for OS revealed older age ($P = .01$) and not in CR at transplantation ($P < .001$) as the independent prognostic variables. Univariate analyses of t(8;21) patients who received allogeneic SCT in CR showed that age ($P = .02$), TBI regimen ($P = .01$), and second and third CR at

Table 4. Outcome of the AML patient population by cytogenetic risk groups

	t(8;21)		inv(16)		Intermediate		Unfavorable		Unknown		
	%	N	%	N	%	N	%	N	%	N	*P*
OS											
Allogeneic SCT											
CR1	84	48	74	20	69	253	53	130	52	116	< .001
CR2/CR3	45	49	86	29	57	131	44	24	64	55	.09
Non-CR	18	84	70	10	25	271	15	136	18	116	.003
Autologous SCT											
CR1	77	42	59	13	74	89	38	15	71	39	.05
CR2/CR3	43	7	50	2	59	15	44	6	42	18	.8
Non-CR	17	6	100	1	25	16	0	10	13	8	.35
DFS											
Allogeneic SCT											
CR1	78	48	73	19	63	249	47	129	48	113	< .001
CR2/CR3	43	48	71	27	47	129	42	22	57	54	.32
Non-CR	18	81	75	8	22	255	10	128	16	107	.005
Autologous SCT											
CR1	73	41	62	13	64	81	33	15	61	36	.09
CR2/CR3	43	7	50	2	36	14	50	6	39	18	.89
Non-CR	17	6	100	1	25	16	0	10	17	6	.45

transplantation ($P < .001$) were also significantly prognostic for poor OS. These variables remained significant after multivariate analysis. Univariate analyses for inv(16) patients showed only age ($P = .009$) to be a significant prognostic factor (Table 5). The univariate analysis of inv(16) patients who underwent allogeneic SCT in CR showed only additional karyotype abnormalities to be an unfavorable prognostic variable ($P = .009$).

Additional cytogenetic abnormalities to CBF

A total of 49 patients with t(8;21) and 14 with inv(16) had additional cytogenetic abnormalities. Data for additional cytogenetic abnormalities were obtained in 42 patients with t(8;21) and 13 patients with inv(16) (Table 6). Additional abnormalities were selected that have been reported to be prognostic by others, including loss of sex chromosome (X or Y), trisomy 8, trisomy 4, del(7q), and del(9q) for the t(8;21) group, and trisomy 22, trisomy 8, trisomy 21, del(7q), and del(9q) for the inv(16) group.[14,15,20,22,23] There were no patients with trisomy 21 in the data of patients with CBF. Patients with t(8;21) and patients with inv(16) were analyzed separately. Among t(8;21) patients undergoing allogeneic SCT, survival was not different between patients with and without additional karyotype abnormalities. When patients with inv(16) were analyzed, the survival was not different between patients with (n = 13) and without (n = 67) additional abnormalities (61% and 74%, respectively; $P = .07$). The survival of patients undergoing allogeneic SCT without additional abnormality (n = 52) was significantly better than that with additional abnormality (n = 11), (85% and 53%, respectively; $P = .004$). When analysis was restricted to patients in CR with inv(16) undergoing allogeneic SCT, a similar difference was observed (86% without additional abnormality [n = 42], and 60% with additional abnormality [n = 8], respectively; $P = .03$). Difference in OS was observed among non-CR patients with (n = 9) and without (n = 1) additional abnormality, but this difference may not be relevant with too few patients in the analysis. We further analyzed subgroups of additional abnormalities of the patients with inv(16). Although the number of patients were limited, significant difference was found among 3 groups of patients; trisomy 8 or trisomy 22 as a sole abnormality (n = 4), without additional abnormality (n = 69), and other additional abnormality to inv(16) (n = 10). The OS at 3 years were 100%, 74%, and 42%, respectively ($P = .002$). The OS of patients undergoing allogeneic SCT was also different among these 3 groups (100%, n = 3; 85%, n = 52; and 33%, respectively; $P < .001$).

Discussion

We analyzed the outcome of a large group of patients with adult CBF AML in Japan who were treated with SCT. The current study focused on the different outcome of the 2 different cytogenetic subgroups of patients with CBF AML undergoing SCT. Our study demonstrated a comparable outcome between patients with t(8;21) and inv(16) undergoing SCT in first CR, but the prognosis between these 2 cytogenetic subgroups was different beyond first CR.

In the literature, there have been several reports showing inferior survival of patients with t(8;21) compared with inv(16) patients undergoing induction chemotherapy and SCT.[14,15,20] Other studies categorized both patients with t(8;21) and inv(16) undergoing allogeneic SCT together as good-risk CBF AML,[1,21] with a relatively comparable prognosis. In our study, OS of patients with t(8;21) undergoing allogeneic SCT in first CR was not statistically different from intermediate cytogenetic subgroup (84% and 79% at 3 years, respectively; $P = .058$). Moreover, the survival of inv(16) (74% at 3 years) and intermediate cytogenetic subgroups showed no statistically significant difference.

In contrast, we have here demonstrated that the prognosis of patients with t(8;21) undergoing allogeneic SCT with second or third CR disease was significantly poor compared with those with inv(16). This finding is consistent with those of other studies reporting differences between the 2 types of CBF AML.[14,15] In the present study, non-CR disease with t(8;21) was also significantly poor compared with patients with inv(16). The Acute Leukemia French Association reported that allogeneic donor availability among patients with CBF AML who were in second CR was a prognostic factor for better survival.[16] We believe that different treatment strategies should be applied for patients with t(8;21) and those with inv(16) other than first CR.

Patients with t(8;21) undergoing allogeneic SCT and autologous SCT had a similar survival rate when they underwent transplantation in first CR, and in further CR. No survival difference between allogeneic SCT and autologous SCT was also

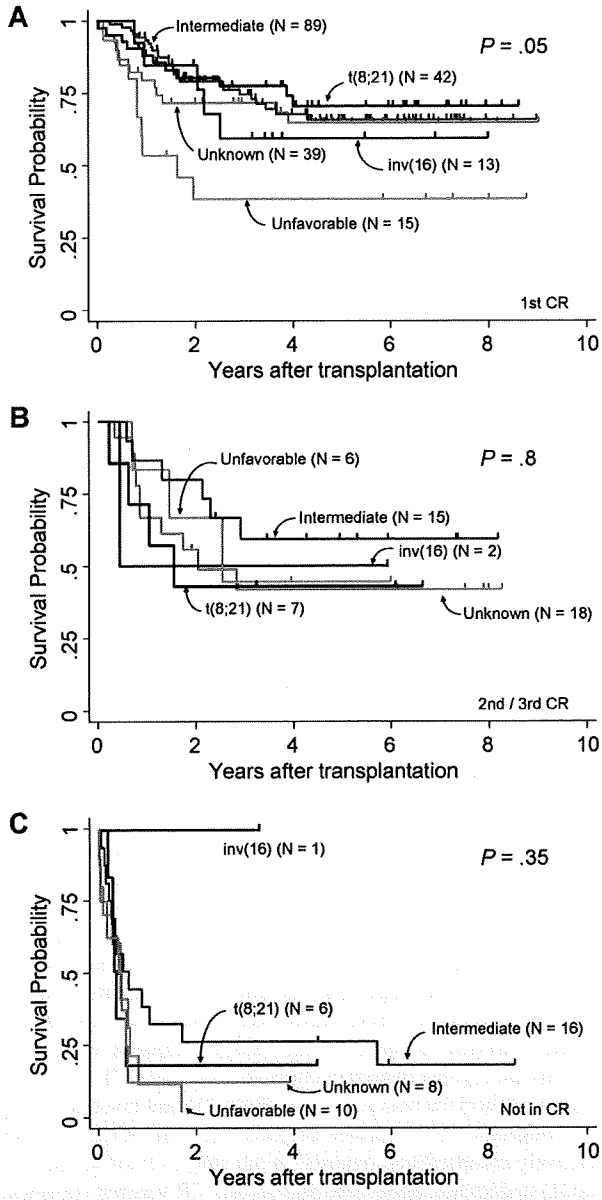

Figure 2. OS difference of patients undergoing autologous SCT between cytogenetic subgroups. (A) Survival curves of patients undergoing autologous SCT in first CR. (B) Survival curves of patients undergoing autologous SCT in second or third CR. (C) Survival curves of patients undergoing autologous SCT not in CR. Each are categorized by cytogenetic risk groups, respectively.

observed among inv(16) patients receiving SCT in first CR (74% and 59%, respectively). The University of California, San Francisco (UCSF) group described the good results of patients with advanced AML undergoing autologous SCT in second or third remission, including patients with CBF.[24] As in our study, the European Group for Blood and Marrow Transplantation (EBMT) reported that the survival rate of t(8;21) patients who received allogeneic bone marrow transplantation was not significantly different from that of patients who received autologous SCT.[1] Results by others showed that allogeneic SCT in first CR did not benefit good-risk cytogenetic subgroups.[3,25,26] Schlenk et al also demonstrated that t(8;21) patients receiving allogeneic SCT or chemotherapy showed no difference in outcome.[23] These results suggest that autologous SCT can be considered as postremission therapy for patients with CBF AML, but it remains unclear whether

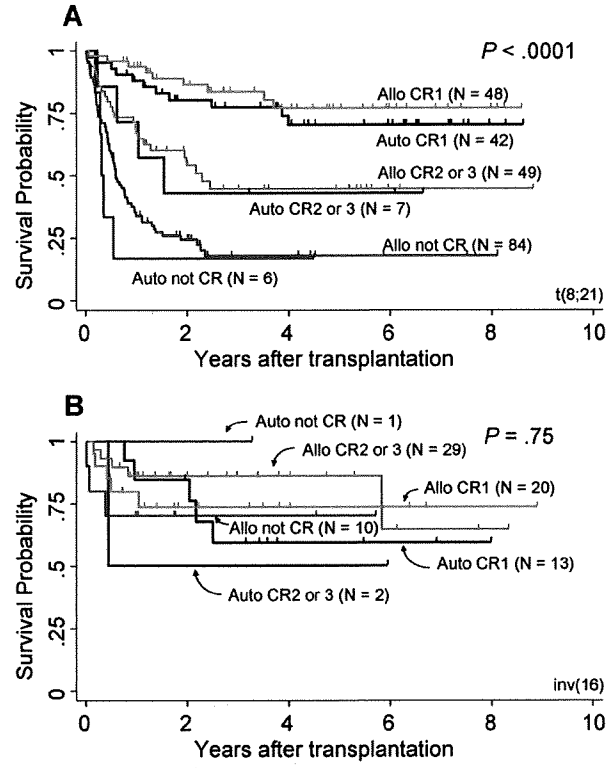

Figure 3. OS of patients with CBF. Survival curves of patients with t(8;21) (A) and with inv(16) (B). Both are stratified according to the type of transplantation (allogeneic or autologous) and disease status at the time of transplantation (first CR, second or third CR, and not in CR).

SCT is more beneficial for patients with CBF than high-dose cytarabine. Survival of patients with inv(16) was favorable beyond first CR. Patients with inv(16) in second or third CR, or even non-CR patients, are good candidates for allogeneic SCT. There are long-term survivors after allogeneic SCT in non-CR disease, so t(8;21) patients with no other choice of treatment, such as those in further CR or non-CR, can proceed to allogeneic SCT. In order to confirm the appropriate treatment for t(8;21) patients in first CR, a prospective trial is needed to compare the results of autologous SCT for t(8;21) in first CR with standard chemotherapy. t(8,21) patients with suitable related or well-matched donors should be recommended to participate in a risk-adopted prospective trial when they receive allogeneic SCT in first CR.

There were differences between the 2 types of CBF AML with respect to prognostic valuables. Age was a significant and independent prognostic variable in both t(8;21) and inv(16) patients, a finding in agreement with reports from some,[14,27] but not all,

Table 5. Prognostic factors affecting overall survival of patients with t(8;21)

Variables	Unfavorable factors	Hazard ratio	95% CI	P
t(8;21)				
Age		1.02	1.01-1.04	.004
Disease status at SCT	Not in CR	4.4	3.1-6.5	< .001
Transplantation	Allo-SCT	1.9	1.2-3.0	.01
Conditioning regimen	TBI	1.7	1.2-2.5	.005
inv(16)				
Age		1.1	1.0-1.1	.009

CI indicates confidence interval.

Table 6. Additional cytogenetic abnormalities among patients with CBF

Additional cytogenetic abnormalities	t(8;21), no.	inv(16), no.
None	206	69
With additional abnormalities	49	14*
−Y	10	0
−X	5	0
Trisomy 22	0	3†
Trisomy 8	0	2†
Trisomy 4	2*	0
Complex	7	4
del(7q)	1†	2
del(9q)	6	0
Other abnormalities	27	9‡
Unknown	7	1

*Patients with additional change to inv(16) and trisomy 4 with t(8;21) tended to show poor survival tendency, with $P < .1$.

†All patients with trisomy 22, trisomy 8 with inv(16), and del(7q) with t(8;21) were alive and censored at survival analysis.

‡Other abnormalities with inv(16) was poorly prognostic, with $P < .001$.

investigators.[28] Transplantation in CR was a significant and independent prognostic factor for patients with t(8;21), but not for those with inv(16). The Cancer and Leukemia Group B (CALGB) also reported differences between t(8;21) and inv(16) in prognostic factors, in terms of race, sex, and secondary cytogenetic abnormalities.[14] Among patients with CBF AML, t(8;21) and inv(16) patients undergoing SCT should be considered 2 separate clinical entities in future clinical studies.

Several specific additional karyotype abnormalities have been reported to be prognostic in patients with CBF AML. Among t(8;21) patients, no specific additional karyotype abnormality was prognostic for overall survival. The poor prognosis of t(8;21) patients with trisomy 4 has been reported by others,[22] but the survival difference was not statistically significant ($P = .085$) in our case series. Since there were limited numbers of patients with additional abnormalities, the real significance of each additional abnormality should be investigated in large numbers of patients.

The reason for the different survival results between patients with t(8;21) and inv(16) undergoing allogeneic SCT in our study remains unclear. The impact of additional mutational events such as c-Kit, FLT3, RAS, and gene-expression profiles was reported to be associated with the clinical outcome of patients with CBF AML.[29-34] The effects of these additional mutational events and gene-expression profiles on the clinical outcome of autologous and allogeneic SCT have not yet been studied. Which proportion of the patients with CBF AML benefited from earlier SCT remains to be identified in future clinical studies. Recent studies by others also suggested that prognosis of CBF AML could differ among different ethnic groups or races.[14,35-37] The background molecular basis among the Japanese population must also be taken into account in future studies.

In conclusion, the survival outcome of patients with CBF AML was similar when they received allogeneic or autologous SCT in first CR. However, the outcomes were significantly different between t(8;21) and inv(16) when they received allogeneic SCT beyond first CR. Therefore, these 2 kinds of CBF AML should be managed differently when applying SCT.

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Authorship

Contribution: Y. Kuwatsuka, K.M., and R.S. contributed to data collection, designed and performed the study, analyzed the data, and wrote the manuscript; M.K., A.M., H.O., R.T., S.T., K.K., K.Y., Y.A., T.Y., and H.S. contributed to data collection and analysis and writing of the paper; and Y. Kodera contributed to data collection and writing of the paper, conceived the study, and provided intellectual input.

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Correspondence: Yachiyo Kuwatsuka, Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya, Aichi 466-8550, Japan; e-mail: kuwatsuka-ngy@umin.ac.jp.

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