

ORIGINAL ARTICLE

New myeloablative conditioning regimen with fludarabine and busulfan for allogeneic stem cell transplantation: comparison with BuCy2

YS Chae¹, SK Sohn¹, JG Kim¹, YY Cho¹, JH Moon¹, HJ Shin², JS Chung², GJ Cho², DH Yang³, J-J Lee³, Y-K Kim³ and H-J Kim³

¹Department of Hematology/Oncology, Kyungpook National University Hospital, Daegu, Korea; ²Department of Hematology/Oncology, Chonnam National University Hospital, Gwangju, Korea and ³Department of Hematology/Oncology, Pusan National University Hospital, Pusan, Korea

A regimen of busulfan and cyclophosphamide (BuCy2) is regarded as the standard myeloablative regimen for SCT. This study evaluated the hypothesis that fludarabine can replace cyclophosphamide for myeloablative allogeneic SCT. Ninety-five patients underwent allogeneic SCT from HLA-identical donors, following BuCy2 ($n=55$) or busulfan + fludarabine (BF, $n=40$). The efficacy of fludarabine compared to cyclophosphamide was retrospectively evaluated. The BF group exhibited a shorter duration until engraftment ($P=0.001$), lower incidence of acute and chronic GVHD ($P<0.001$ and $P=0.003$, respectively), and non-relapse mortality (NRM) ($P=0.039$). Furthermore, the event-free survival and overall survival were significantly higher for the BF group compared to the BuCy2 group ($P=0.004$ and 0.002 , respectively). After adjusting for age, the risk status of disease, GVHD prophylaxis and donor type, the BF regimen was found to be an independent favorable risk factor for event-free survival (hazard ratio (HR), 0.181; 95% confidence interval, 0.045–0.720; $P=0.016$) and overall survival (HR, 0.168; 0.035–0.807; $P=0.026$). The replacement of cyclophosphamide with fludarabine for myeloablative conditioning seems to be more effective in terms of short-term NRM, and GVHD compared to BuCy2 regimen in allogeneic transplantation.

Bone Marrow Transplantation (2007) **40**, 541–547; doi:10.1038/sj.bmt.1705770; published online 16 July 2007

Keywords: myeloablative conditioning; HLA identical; allogeneic stem cell transplantation; fludarabine

Introduction

Busulfan and cyclophosphamide (BuCy2) has been regarded as a standard myeloablative regimen for allogeneic

stem cell transplantation (SCT). However, combination of these two alkylating agents caused a few unwished adverse effects like severe veno-occlusive disease (VOD), mucositis or enteritis, and enhanced GVHD, which could lead to high morbidity and mortality of recipients. Especially, the specific metabolite of cyclophosphamide, *o*-carboxyethylphosphoramide mustard, could cause major toxicity and an increased treatment-related mortality (TRM).¹

Fludarabine is a purine analog that inhibits lymphocyte proliferation, thus causing potent immunosuppression to enhance engraftment and hematologic recovery, and may reduce GVHD. As fludarabine also prevents alkylator-induced DNA repair, thus inducing apoptosis of hematologic malignant cells, it is known to have a synergistic tumor-killing effect with busulfan.^{2,3} Furthermore, as it has shown a safe toxicity profile, lower TRM would be anticipated in the allogeneic transplantation setting when replacing cyclophosphamide for conditioning. Due to these properties, fludarabine has been successfully utilized in reduced intensity conditioning regimens especially with busulfan,^{4–7} and more recently it has been used in combination with myeloablative doses of busulfan.^{8–11}

This study retrospectively evaluated the role of a myeloablative busulfan and fludarabine (BF) regimen compared to a historical control using standard BuCy2 for allogeneic transplantation.

Patients and methods

Patients and donors

From December 2004 to November 2006, 40 patients who underwent an HLA-identical allogeneic SCT with myeloablative BF regimen in three SCT centers were enrolled in this study. The HLA genotyping was performed in the same way at each center, where HLA matching was determined by DNA genotyping in sibling transplants, and serologic typing for an unrelated setting.

All patients received allogeneic SCT consecutively in each center. For comparison, a review was conducted on the medical records of 55 patients who consecutively received allogeneic SCTs at Kyungpook National

Correspondence: Dr SK Sohn, Department of Hematology/Oncology, Kyungpook National University Hospital, 50 Samduk 2-ga, Jung-Gu, Daegu 700-721, Korea.

E-mail: sksohn@knu.ac.kr

Received 8 March 2007; revised 3 May 2007; accepted 4 June 2007; published online 16 July 2007

University Hospital with a BuCy2 conditioning regimen from September 1998 to September 2004.

Conditioning regimen and GVHD prophylaxis

In the BF group, fludarabine was given as 30 mg/m² i.v. for 6 days (total 180 mg/m², days -8 to -3), whereas busulfan (Busulfex, Orphan Medical Inc., Minnetonka, MN, USA) was infused 3.2 mg/kg/day i.v. for 4 days (total 12.8 mg/kg, days -7 to -4). In the BuCy2 group, busulfan was given orally (p.o.) (16 mg/kg) or i.v. (12.8 mg/kg) every 6 h for 4 days followed by i.v. cyclophosphamide (120 mg/m²) for 2 days according to the protocol used in previous studies. The chemotherapy doses were based on the ideal body weight (IBW), except for patients whose real body weight (RBW) exceeded their IBW by more than 20%, for whom the doses were based on an adjusted IBW, consisting of IBW + (0.25 (RBW-IBW)).

Acute GVHD (aGVHD) prophylaxis consisted of methotrexate and cyclosporin A or FK506. Cyclosporin A was started at a dose of 5 mg/kg/day (continuous i.v.) on day -1, and reduced to a dose of 2.5 mg/kg/day i.v. on day +7, then changed to a dose of 3 mg/kg/day (twice daily p.o.) when tolerable. FK506 was used in case of unrelated SCT setting with a starting dose of 0.05 mg/kg. Methotrexate was treated 15 mg/m² on day 1, and 10 mg/m² on days 3, 6 and 11. The last dose of methotrexate was omitted when mucositis over grade 4 or renal impairment was observed. Allogeneic donor hematopoietic stem cells were infused on day 0 using standard infusion technique.

Prophylactic antibiotics and other medications as protocol

Infection prophylaxis consisted of ciprofloxacin (250 mg twice daily p.o.)/metronidazole (500 mg thrice daily p.o.)/fluconazole (100 mg/day p.o.) beginning with the initiation of conditioning and acyclovir (600 mg twice daily p.o.) from day -1. Co-trimoxazole was started after engraftment. Ursodeoxycholic acid was used for VOD prophylaxis beginning with the initiation of conditioning.

Monitoring and management of CMV antigenemia

CMV antigenemia assay was performed every week till day +100, every 2 weeks till 6 months and every 2 or 4 weeks till 12 months after engraftment. CMV antigenemia surveillance was extended when late-CMV reactivation or a recurrent episode of CMV reactivation was documented or when a clinical sign of GVHD was observed. CMV antigenemia assay was performed as described elsewhere,¹² and determined as positive when any polymorphonuclear leukocytes (PMNLs) were stained in 200 000 PMNLs per slide.

When CMV pp65 antigenemia was detected, pre-emptive ganciclovir (GCV) therapy (5 mg/kg i.v. twice daily) was administered daily for at least 2 weeks until antigenemia resolved, followed by 5 mg/kg/day i.v. every other day for another week. CMV disease was treated with GCV 5 mg/kg i.v. twice daily for at least 3 weeks followed by 5 mg/kg i.v. for 5 days a week until clinical signs of CMV disease improved and CMV antigenemia was undetectable. If CMV antigenemia persisted, the treatment was continued with a weekly antigenemia assay.

Definition

Engraftment was confirmed by peripheral blood counts (myeloid: peripheral absolute neutrophil count more than $0.5 \times 10^9/l$; megakaryocyte: peripheral platelet count more than $20 \times 10^9/l$ for 3 consecutive days without requiring transfusion). The diagnosis of aGVHD was carried out as described previously by consensus criteria.¹³⁻¹⁵ The day of onset of aGVHD was defined as the date of initiation of clinical symptoms or signs of aGVHD. Primary treatment failure was defined as the necessity for secondary treatment for aGVHD due to a lack of response or progression following initial treatment or patient death due to aGVHD while receiving initial treatment. The diagnosis of chronic GVHD (cGVHD) was prepared by the revised Seattle criteria.¹⁶ The day of the onset of overall and extensive cGVHD was defined as the date of initiation of clinical sign(s) of cGVHD or the date of histological confirmation. High-risk disease status was defined as acute leukemia beyond the first remission, chronic myeloid leukemia beyond the first chronic phase, and multiple relapsing or chemo-resistant malignancies. The overall survival (OS) was defined as the time from transplantation until death from any cause, while event-free survival (EFS) time was counted from day 0 to relapse or death. Non-relapse mortality (NRM) was defined as death from any cause other than refractory disease or relapse, while treatment-related mortality (TRM) was confined within day 90. The cumulative incidence of relapse was defined as the time from transplantation until disease progression.

Statistics

The results were analyzed according to the information available on November 2006. The day of the stem cell infusion was defined as day 0. We evaluated the difference of baseline characteristics of the patients and transplantation procedures, and the transplant outcomes between different conditioning regimens using χ^2 -test or the Student's *t*-test. The cumulative incidence or survival was plotted according to the Kaplan-Meier method, whereas log-rank test was used to analyze the difference between the two groups. Univariate and multivariate analyses for hazard ratio (HR) or 95% confidence interval (CI) were conducted using Cox's regression analysis. The basic statistical data were obtained using the SPSS software package (SPSS 11.5 Inc., Chicago, IL, USA). Statistical significance is represented by two-tailed *P*-values. A cutoff value of 0.05 was adopted for all statistical analyses.

Results

Patient characteristics and transplantation procedures

Ninety-five patients were enrolled in this study (BuCy2, *n* = 55; BF, *n* = 40), and the patient characteristics are summarized in Table 1. The median age was 36 years (range, 17-54), and 40 patients (42.1%) were at high risk. All donors were HLA identical with recipients, wherein 15 donors (15.8%) were unrelated. Seventy-three patients underwent peripheral blood stem cell transplantation

Table 1 Patient characteristics and transplantation procedure

	BuCy2	BF	P-value
Number	55	40	
Median follow-up (days)	311 (29–2821)	321 (13–685)	
Age (median, range)	36.0 (17–49)	38.5 (21–54)	0.010
<i>Sex (%)</i>			
Male	33 (60.0)	21 (52.5)	0.466
Female	22 (40.0)	19 (47.5)	
<i>Disease</i>			
AML (1 CR)	24	16	
AML (> 1 CR/relapse)	12	10	
ALL (1 CR)	3	4	
ALL (> 1 CR/relapsed)	3	2	
CML (chronic phase)	5	0	
CML (> chronic phase)	5	0	
MDS	2	4	
NHL	1	4	
HLH	0	2	
<i>Risk (%)</i>			
Standard	33 (60.0)	22 (55.0)	0.626
High	22 (40.0)	18 (45.0)	
<i>Donor type (%)</i>			
Sibling	49 (89.1)	31 (77.5)	0.126
Unrelated	6 (10.9)	9 (22.5)	
<i>Stem cell source (%)</i>			
BM	14 (25.5)	5 (12.5)	0.081
PB	38 (69.1)	35 (87.5)	
BM + PB	3 (5.5)	0	
<i>Infused cell</i>			
MNC	6.57 ± 4.22	7.40 ± 3.94	0.347
CD34	6.10 ± 3.38	5.99 ± 3.76	0.876
CD3	2.12 ± 1.83	2.79 ± 1.47	0.084

Abbreviations: BuCy2 = busulfan and cyclophosphamide; BF = busulfan and fludarabine; CR = complete remission; HLH = hemophagocytic lymphohistiocytosis; MDS = myelodysplastic syndrome; NHL = non-Hodgkin's lymphoma.

(PBSCT), and 19 BMT, whereas BM harvest was conducted after PBSC in three patients.

The infused MNCs and CD34+ stem cells were 6.92 ± 4.10 and 6.06 ± 3.52, respectively, and the median follow-up duration was 311 days (range, 13–2821 days).

Between the two groups, there were no differences in the sex of the recipients, stem cell source, donor type, infused stem cell dose or use of immunosuppressants, including the administration of methotrexate at day + 11; however, the median age was higher in the BF group (38.5 vs 36.0, $P=0.010$).

Comparison of transplantation outcomes

Hematopoietic recovery. Among 95 patients, the failure of engraftment was observed in one patient, who had been treated with BuCy2 (Table 2). Engraftment after transplantation was faster in the BF group for neutrophil (11.0 vs 14.0 days, $P<0.001$) and platelet (11.0 vs 16.0, $P=0.001$) than the BuCy2 group.

VOD. Ten cases of VOD were observed only in the BuCy2 group. Among these cases, eight patients received

Table 2 Transplantation outcomes

	BuCy2	BF	P-value
Number	55	40	
<i>Engraftment, median days (range)</i>			
WBC	14 (9–25)	11 (8–16)	0.000
ANC	15 (10–30)	13 (10–19)	0.000
Platelet	16 (9–56)	11 (9–29)	0.001
<i>VOD (%)</i>	10 (18.2)	0	
Moderate to severe	5 (9.1)	0	
<i>aGVHD (%)</i>	45 (81.8)	16 (40.0)	0.000
Grades 2–4 aGVHD	39 (70.9)	5 (12.5)	0.000
<i>cGVHD^a (%)</i>	39/46 (84.8)	15/34 (44.1)	0.000
Extensive cGVHD	24 (52.2)	8 (23.5)	0.001
<i>CMV antigenemia^b (%)</i>	30 (54.5)	12 (30.0)	0.017
Refractory	1	0	
Relapse (%)	16 (29.1)	7 (17.5)	0.193
Event (%)	34 (61.8)	8 (20.0)	0.000
NRM (%)	17 (30.9)	4 (10.0)	0.023
Death (%)	33 (60.0)	6 (15.0)	0.000

Abbreviations: aGVHD = acute GVHD; cGVHD = chronic GVHD; NRM = non-relapse mortality; VOD = veno-occlusive disease.

^aEvaluated the patients who survived over +100 days after transplantation.

^bUsed CMV pp65 antigen.

oral busulfan, while two received i.v. busulfan. Moderate-to-severe VOD was observed in five patients, and one died from VOD.

Acute and chronic GVHD. Grades 2–4 aGVHD developed in 39 (70.9%) of 55 patients in the BuCy2 group and 5 (12.8%) of 40 in the BF group, resulting in a 100-day cumulative incidence of 71.6 and 15.5%, respectively ($P<0.001$; Figure 1, left).

cGVHD developed within 1 year after transplantation in 39 (84.8%) of 46 evaluable patients in the BuCy2 group and 15 (44.1%) of 34 in the BF group ($P<0.0001$). Among them, extensive cGVHD developed in 24 (52.2%) patients in the BuCy2 group and 8 (25%) in the BF group ($P=0.001$) with a cumulative incidence of extensive cGVHD being 56.3 and 28.7%, respectively ($P=0.003$; Figure 1, right).

CMV infection. CMV antigenemia developed in 42 patients (44.2%) without any evidence of overt disease, wherein the cumulative incidence of CMV antigenemia at day +100 and 1 year were 50.2 and 59.4% in the BuCy2 group, and 31.5 and 31.5% in the BF group ($P=0.049$), respectively. However, the incidence of overt CMV disease was not different between the two groups.

NRM

The causes of death excluding relapsed or refractory disease in the BF group included infection (two cases) and aGVHD (two cases). The NRM was 17 (30.9%) in the BuCy2 group and 4 (10.0%) in the BF group ($P=0.023$), resulting in a 1 year and 2 year cumulative incidence of NRM being 31.0

and 34.2%, and 10.4 and 10.4% ($P=0.039$), respectively (Figure 2, left). The causes of NRM were infection ($n=4$), organ failure ($n=4$), acute and chronic GVHD ($n=3$), hemorrhage ($n=2$) and VOD ($n=1$) in the BuCy2 group, while the causes were infection ($n=2$) and acute GVHD ($n=2$) in the BF group.

Relapse and refractory

Twenty-three (24.5%) patients relapsed and one patient had persistent primary disease even after SCT, where 17 (30.9%) patients were in the BuCy2 group and 7 (17.5%) in the BF group, resulting in a 2-year cumulative incidence of 39.8 and 23.3%, respectively ($P=0.200$; Figure 2, right).

Survival

The OS rate was significantly superior in the BF (mean, 40.64 vs 19.88 months; $P=0.002$) yielding an estimated

2-year OS rate of 83.45 vs 37.69% (Figure 3, left). In a univariate analysis, the variables that decreased the OS probability were (1) BuCy2 regimen ($P=0.003$); (2) high-risk disease status ($P=0.028$); and (3) delayed engraftment of neutrophil or platelet ($P<0.001$; Table 3). In a multivariate analysis, the BF regimen was an independent favorable prognostic index of OS (HR=0.168; 95% CI, 0.035–0.807; $P=0.026$), whereas a high-risk status and delayed engraftment of platelet were marginally associated with a poor OS (HR=2.348; 95% CI, 0.987–5.585; $P=0.054$, and HR=1.052, 95% CI, 1.000–1.106; $P=0.050$, respectively).

Although relapse rates were similar in the two groups, EFS was significantly higher in the BF group compared to the BuCy2 group (mean, 37.01 vs 16.65 months; $P=0.004$) yielding an estimated 2-year EFS rate of 77.3 vs 37.19% (Figure 3, right). In a univariate analysis, the variables that decreased the EFS probability were (1) BuCy2 regimen

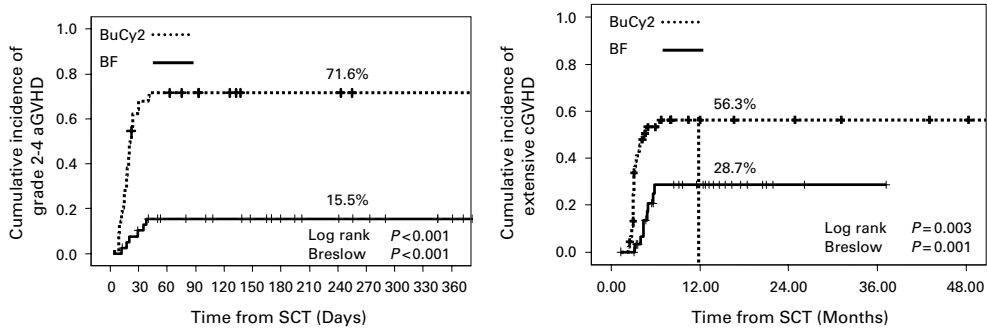


Figure 1 Cumulative incidence of grades 2–4 aGVHD (left) and extensive cGVHD (right). aGVHD = acute GVHD; cGVHD = chronic GVHD.

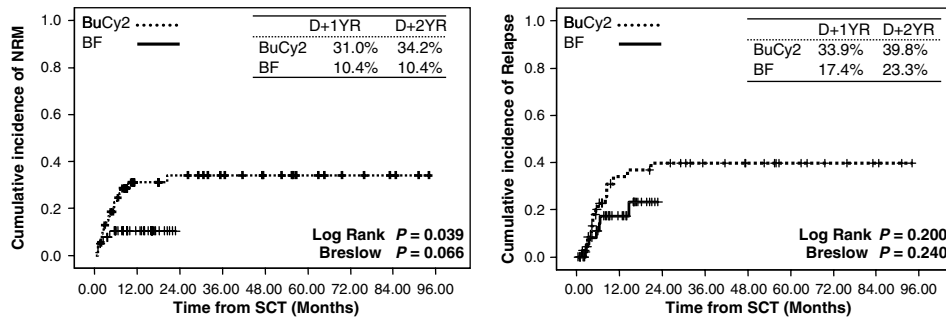


Figure 2 Cumulative incidence of NRM (left) and relapse (right). NRM = non-relapse mortality.

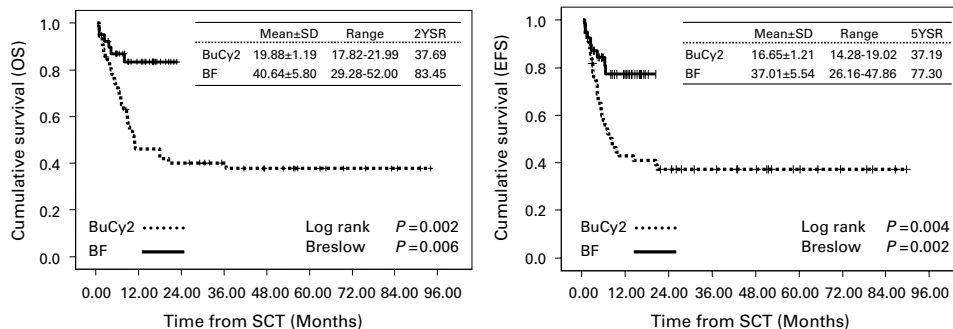


Figure 3 Cumulative OS (left) and EFS (right). OS = overall survival; EFS = event-free survival.

Table 3 Univariate analysis of EFS and OS

	Event-free survival			Overall survival		
	HR	95% CI	P-value	HR	95% CI	P-value
Age (R)	0.996	0.966–1.027	0.811	0.997	0.966–1.030	0.873
Male (R)	0.991	0.537–1.826	0.976	0.883	0.466–1.672	0.703
Age (D)	0.996	0.966–1.027	0.811	1.001	0.969–1.034	0.144
Male (D)	0.991	0.537–1.826	0.976	0.731	0.388–1.377	0.232
Sibling (vs unrelated)	0.632	0.292–1.368	0.224	0.615	0.270–1.402	0.248
BuCy2 (vs BF)	2.985	1.375–6.479	0.006	3.705	1.545–8.886	0.003
High risk (vs standard)	2.217	1.205–4.082	0.011	2.033	1.080–3.831	0.028
FK506 (vs CyA)	1.038	0.370–2.190	0.944	1.281	0.394–4.169	0.681
MNC	0.922	0.847–1.004	0.063	0.917	0.839–1.002	0.056
CD34	0.940	0.856–1.032	0.191	0.927	0.838–1.024	0.137
WBC engraftment	1.120	1.034–1.212	0.005	1.149	1.058–1.248	0.001
ANC engraftment	1.129	1.063–1.199	0.000	1.149	1.079–1.223	0.000
Platelet engraftment	1.079	1.044–1.114	0.000	1.075	1.044–1.108	0.000
Grades 2–4 aGVHD	1.460	0.785–2.217	0.232	1.621	0.845–3.012	0.146
Extensive cGVHD	1.792	0.733–4.386	0.201	2.058	0.962–4.405	0.063
CMV– (vs CMV+)	0.898	0.489–1.547	0.728	0.962	0.512–1.809	0.904

Abbreviations: aGVHD = acute GVHD; cGVHD = chronic GVHD; CI = confidence interval; CyA = cyclosporine A; D = donor; EFS = event-free survival; HR = hazard ratio; OS = overall survival; R = recipient.

Table 4 Multivariate analysis of EFS and OS

	EFS			OS		
	HR	95% CI	P-value	HR	95% CI	P-value
BF regimen (vs BuCy2)	0.181	0.045–0.726	0.016	0.168	0.035–0.807	0.026
High risk (vs standard)	4.205	1.800–9.823	0.001	2.348	0.987–5.585	0.054
Platelet engraftment ^a	1.070	1.009–1.134	0.023	1.052	1.000–1.106	0.050
ANC engraftment ^a	1.053	0.952–1.165	0.315	1.059	0.954–1.175	0.282
Extensive cGVHD	0.613	0.256–1.468	0.272	1.067	0.472–2.412	0.876

Abbreviations: cGVHD = chronic GVHD; CI = confidence interval; EFS = event-free survival; HR = hazard ratio; OS = overall survival.

^aAnalyzed using continuous variables.

($P=0.006$); (2) high-risk disease status ($P=0.011$); and (3) delayed engraftment of neutrophil or platelet ($P<0.001$; Table 3). In the multivariate analysis, the BF regimen was an independent favorable prognostic index of EFS (HR = 0.181; 95% CI, 0.045–0.726; $P=0.016$), whereas a high-risk status and delayed engraftment of platelet were significantly associated with a poor EFS (HR = 4.205; 95% CI, 1.800–9.823; $P=0.001$, and HR = 1.070, 95% CI, 1.009–1.134; $P=0.023$, respectively) (Table 4).

Forty-one of the 55 patients in the BuCy2 group received p.o. busulfan and 14 were treated with i.v. busulfan. The OS and EFS were slightly higher in the i.v. busulfan group; however, no statistical significance was observed ($P=0.063$ in OS and $P=0.118$ in EFS).

Discussion

BuCy regimen has been used most commonly for conditioning in allogeneic SCT. Yet, one major concern was high TRM caused by additive cytotoxicity of these two alkylators, which play an important role in the development of organ damage or hepatic VOD by depleting glutathione in hepatocytes contributing to cellular damage.^{17–19} In particular, specific metabolites of cyclophosphamide are known to be associated with increased transplantation-induced mortality after conditioning, especially combined with busulfan.^{1,20}

Like cyclophosphamide, fludarabine also has a potent immunosuppressive effect without increasing toxicity of busulfan. Thus, combination of fludarabine and busulfan in the preparative regimen for allogeneic SCT has recently been tried for myeloablative allogeneic SCT^{8–11} as well as nonmyeloablative transplantation.^{4–7}

In the present study, the regimen had a positive effect in terms of rapid engraftment, lower incidence of grades 2–4 aGVHD, extensive cGVHD and NRM compared to BuCy2 conditioning regimen. Although the relapse probabilities were not significantly different between the two groups, the lower NRM in the BF group could account for the significantly higher EFS in the BF group. Nonetheless, this study has limitations in interpreting the results regarding cGVHD or survival due to the relatively short follow-up duration in the BF group.

Russell *et al.*¹⁰ also demonstrated the efficacy and safety of fludarabine (total, 250 mg/m²) with i.v. busulfan (3.2 mg/kg for 4 days), showing a low incidence of aGVHD (grades 2–4, 25%) and TRM. As previous studies demonstrated that organ damage by conditioning was one main cause of the development of GVHD,²¹ less host tissue damage by

fludarabine seemed to lead to a lower incidence or severity of GVHD in the BF group, which would also be associated with lower late morbidity and mortality. Also, in the present study, the incidence of aGVHD was much lower in the BF group, which included even more allogeneic PBSCT recipients compared with BuCy2 group. As PBSCT is known to be associated with a higher GVHD rate by relatively higher transfused cell dose, myeloablative BF regimen may be more useful in allogeneic PBSCT rather than BMT in terms of prevention of GVHD.

Although the results showed less CMV reactivation in the BF group compared to the BuCy2 group, the regimen was not entirely responsible for this impact. As CMV reactivation is known to be related with GVHD and the use of immunosuppressants, the high incidence of acute and chronic GVHD followed by the increased dose or duration of immunosuppressants may have caused the higher incidence of CMV reactivation in the BuCy2 group.

The earlier engraftment in the BF group may have been due to the lower cytotoxicity induced by fludarabine, causing a better marrow environment for engraftment compared to cyclophosphamide, as well as the immunosuppressive effect of fludarabine. In addition, the high proportion of PBSC in the BF group may also have played a role in the faster engraftment.

However, less organ damage by BF regimen might cause a concern of a lower anti-leukemic effect or a higher recurrence of primary diseases compared to BuCy2 regimen. Previous studies demonstrated that nucleoside analogues combined with alkylating agents exert synergistic tumor-killing effect by inhibiting the mechanisms of repair from alkylator-induced DNA damage.^{2,3} In the present study, no difference in the relapse rate was observed between two regimens, and other studies using BF regimen also reported similar relapse rates.^{8,10} Furthermore, we experienced encouraging results even in a setting of autologous transplantation for six patients with AML showing no TRM and one relapse (follow-up duration, 83–748 days). Therefore, fludarabine combined with busulfan may have sufficient tumor-killing effect with limited toxicity.

In the present study, busulfan was infused for 4 days in accordance with original BuCy2, and fludarabine was infused from 24 h before busulfan infusion to 24 h after busulfan infusion (total 6 days) instead of cyclophosphamide. In a few recent studies using BF regimen, variable infusion schedules and doses from 120 to 250 mg/m² were used, wherein a high dose of fludarabine (total 250 mg/m²) was associated with a lower incidence rate of GVHD but delayed hematologic recovery.^{8–11} Yet, the sequence and timing to reach maximum synergy between these two agents has not yet been settled. As fludarabine inhibits recovery from alkylator-induced DNA damage, infusion of fludarabine before busulfan or sequential use of two drugs may be recommended in the BF regimen. However, de Lima *et al.*⁸ demonstrated similar transplantation outcomes using concomitant infusion regimen with fludarabine and busulfan. Although the persistent effect of fludarabine might allow the concomitant peak intracellular concentration of busulfan and fludarabine, the optimal dose and infusion timing of fludarabine needs to be decided in the BF regimen.

In summary, the replacement of cyclophosphamide with fludarabine for myeloablative conditioning seems to be more effective in terms of short-term NRM, and GVHD compared to BuCy2 regimen in allogeneic transplantation; thus, the BF regimen could be considered as a standard regimen in allogeneic SCT. However, the present study has certain limitations in drawing conclusions, due to the retrospective comparison and small number of patients. Furthermore, the follow-up period for the patients treated with BF has not been long, as only 20 of the 40 patients who received the BF regimen have been followed for over 1 year. As fludarabine is known to be a potent immune suppressant, the possibility of late recurrence or secondary malignancy may be a possible concern in the late period after transplantation. Furthermore, as fludarabine may have different efficacy on diverse diseases, subgroup analysis according to different hematologic malignancies needs to be carried out in a larger scale, and a randomized study is also warranted.

References

- 1 McDonald GB, Slattery JT, Bouvier ME, Ren S, Batchelder AL, Kalthorn TF *et al.* Cyclophosphamide metabolism, liver toxicity, and mortality following hematopoietic stem cell transplantation. *Blood* 2003; **101**: 2043–2048.
- 2 Gandhi V, Plunkett W. Cellular and clinical pharmacology of fludarabine. *Clin Pharmacokinet* 2002; **41**: 93–103.
- 3 Li L, Liu X, Glassman AB, Keating MJ, Stros M, Plunkett W *et al.* Fludarabine triphosphate inhibits nucleotide excision repair of cisplatin-induced DNA adducts *in vitro*. *Cancer Res* 1997; **57**: 1487–1494.
- 4 Slavin S, Nagler A, Naparstek E, Kapelushnik Y, Aker M, Cividalli G *et al.* Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. *Blood* 1998; **91**: 756–763.
- 5 Bornhauser M, Thiede C, Platzbecker U, Jenke A, Helwig A, Plettig R *et al.* Dose-reduced conditioning and allogeneic hematopoietic stem cell transplantation from unrelated donors in 42 patients. *Clin Cancer Res* 2001; **7**: 2254–2262.
- 6 Murotani Y, Kuroda J, Kimura S, Terao K, Fukiya E, Ozawa M *et al.* Non-myeloablative haematopoietic stem cell transplantation for severe aplastic anaemia with various complications. *Clin Lab Haematol* 2002; **24**: 303–306.
- 7 Or R, Shapira MY, Resnick I, Amar A, Ackerstein A, Samuel S *et al.* Nonmyeloablative allogeneic stem cell transplantation for the treatment of chronic myeloid leukemia in first chronic phase. *Blood* 2003; **101**: 441–445.
- 8 de Lima M, Couriel D, Thall PF, Wang X, Madden T, Jones R *et al.* Once-daily intravenous busulfan and fludarabine: clinical and pharmacokinetic results of a myeloablative, reduced-toxicity conditioning regimen for allogeneic stem cell transplantation in AML and MDS. *Blood* 2004; **104**: 857–864.
- 9 Bornhauser M, Storer B, Slattery JT, Appelbaum FR, Deeg HJ, Hansen J *et al.* Conditioning with fludarabine and targeted busulfan for transplantation of allogeneic hematopoietic stem cells. *Blood* 2003; **102**: 820–826.
- 10 Russell JA, Tran HT, Quinlan D, Chaudhry A, Duggan P, Brown C *et al.* Once-daily intravenous busulfan given with fludarabine as conditioning for allogeneic stem cell transplantation: study of pharmacokinetics and early clinical outcomes. *Biol Blood Marrow Transplant* 2002; **8**: 468–476.

- 11 Chunduri S, Dobogai LC, Peace D, Sauntharajah Y, Chen HY, Mahmud N *et al*. Comparable kinetics of myeloablation between fludarabine/full-dose busulfan and fludarabine/melphalan conditioning regimens in allogeneic peripheral blood stem cell transplantation. *Bone Marrow Transplant* 2006; **38**: 477–482.
- 12 Boeckh M, Bowden RA, Goodrich JM, Pettinger M, Meyers JD. Cytomegalovirus antigen detection in peripheral blood leukocytes after allogeneic marrow transplantation. *Blood* 1992; **80**: 1358–1364.
- 13 Sullivan KM, Shulman HM, Storb R, Weiden PL, Witherspoon RP, McDonald GB *et al*. Chronic graft-versus-host disease in 52 patients: adverse natural course and successful treatment with combination immunosuppression. *Blood* 1981; **57**: 267–276.
- 14 Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J *et al*. 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant* 1995; **15**: 825–828.
- 15 Lee KH, Choi SJ, Lee JH, Lee JS, Kim WK, Lee KB *et al*. Prognostic factors identifiable at the time of onset of acute graft-versus-host disease after allogeneic hematopoietic cell transplantation. *Haematologica* 2005; **90**: 939–948.
- 16 Sullivan KM, Agura E, Anasetti C, Appelbaum F, Badger C, Bearman S *et al*. Chronic graft-versus-host disease and other late complications of bone marrow transplantation. *Semin Hematol* 1991; **28**: 250–259.
- 17 DeLeve LD. Hepatic venoocclusive disease: a major complication of hematopoietic stem cell transplantation in cancer patients. *Tumori* 2001; **87**: S27–S29.
- 18 DeLeve LD. Cellular target of cyclophosphamide toxicity in the murine liver: role of glutathione and site of metabolic activation. *Hepatology* 1996; **24**: 830–837.
- 19 Shulman HM, Luk K, Deeg HJ, Shuman WB, Storb R. Induction of hepatic veno-occlusive disease in dogs. *Am J Pathol* 1987; **126**: 114–125.
- 20 Slattery JT, Kalthorn TF, McDonald GB, Lambert K, Buckner CD, Bensinger WI *et al*. Conditioning regimen-dependent disposition of cyclophosphamide and hydroxycyclophosphamide in human marrow transplantation patients. *J Clin Oncol* 1996; **14**: 1484–1494.
- 21 Ferrara JL. Pathogenesis of acute graft-versus-host disease: cytokines and cellular effectors. *J Hematother Stem Cell Res* 2000; **9**: 299–306.