# Prospective phase II trial to evaluate the complications and kinetics of chimerism induction following allogeneic hematopoietic stem cell transplantation with fludarabine and busulfan

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**This prospective trial assessed the safety and efficacy of allogeneic hematopoietic stem cell transplantation from a HLA-matched donor with a reduced-intensity regimen (RIST) consisting of iv fludarabine 30 mg/m$^2$ for 6 days and oral busulfan 4 mg/kg/day for 2 days in patients older than 50 years with hematological malignancies. Cyclosporine alone or cyclosporine with short-term methotrexate was randomized for graft-versus-host disease prophylaxis. After 30 patients had been enrolled, an interim analysis was performed, and this report focuses on a precise evaluation of the toxicity profile and chimerism kinetics. Sustained engraftment in all patients, no severe regimen-related toxicity (RRT) within 20 days, and no transplant-related mortality through Day 100 were observed. T-cell (CD3+) full-donor (over 90%) chimerism was observed in 22 of the 30 patients, while the remaining eight had mixed-donor chimerism over 77% on Day 90. Thereafter, five subsequently converted to full-donor chimerism without donor lymphocyte infusion by day 120 ($n$ = 4) or Day 180 ($n$ = 1). Two showed persistent mixed chimerism without relapse through Day 180. Grade III–IV acute graft-versus-host disease and extensive chronic graft-versus-host disease occurred in 10% and 73%, respectively. With a median follow-up of 1.5 years, overall survival and disease-free survival at 1 year was 83% and 62%, respectively. Seven patients hematologically relapsed overall, and five of them had myelodysplastic syndrome with poor prognostic factors. In older patients, RIST with fludarabine and busulfan was associated with acceptable toxicities and a satisfactory antileukemia effect, regardless of the early chimerism status. Am. J. Hematol. 82:873–880, 2007.** © 2007 Wiley-Liss, Inc.

## Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is a potentially curative treatment of choice for hematological malignancies. However, many centers limit HSCT to younger patients because of the threat of a higher risk of treatment-related toxicities including graft-versus-host disease (GvHD), nonrelapse mortality, and lower disease-free survival (DFS) in the older population, although the median age of onset of chronic myeloid leukemia (CML) is in the sixth decade of life, and the peak incidence of acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) is in the seventh decade. To overcome this obstacle, allogeneic HSCT with a reduced-intensity (RIST) or nonmyeloablative conditioning regimen has recently been explored for patients who are ineligible to receive conventional myeloablative HSCT (CIST) due to age limits or comorbidities. Many studies suggested that RIST is a reasonable option for older patients or patients with comobidities with acceptable treatment-related complications or morbidity, while preserving adequate antitumor effects [1–12]. However, these studies mostly pursued different variables including disease types, stages [1,4–6, 8,12], donor type [1,2,5,10], graft source [1,2], conditioning

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**TABLE I. Patient and Donor Characteristics**

| UPN | Patient age/sex | Donor age/sex | Stage and diagnosis | IPSS/cytogenetic risk in MDS patients | CD34+ cells ($10^6$) | Blood type patient/donor |
|---|---|---|---|---|---|---|
| 1 | 60/M | 46/F | MDS (RA) | Intermediate-2/Poor | 3.92 | A/A |
| 2 | 61/M | 54/F | MDS (RAEB) | Intermediate-2/Poor | 5.84 | O/O |
| 3 | 67/M | 60/F | AML (M4) in 2CR | | 2.74 | B/O |
| 4 | 60/M | 55/F | AML (M2) in 2CR | | 4.58 | B/B |
| 5 | 63/M | 60/M | CML in 2CP | | 12.59 | O/O |
| 6 | 54/M | 59/F | MDS (RAEB) | High/Intermediate | 5.4 | O/A |
| 7 | 52/M | 55/F | AML (M2) in 1CR | | 6.77 | A/A |
| 8 | 61/M | 54/M | AML (M1) in 1CR | | 3.29 | B/A |
| 9 | 58/F | 64/F | CML in 1CP | | 2.9 | A/AB |
| 10 | 64/F | 59/F | ALL (L2) in 1CR | | 5.54 | A/A |
| 11 | 55/M | 44/M | AML (M1) in 1CR | | 3.13 | A/A |
| 12 | 55/F | 51/F | CML in 1CP | | 4.94 | A/O |
| 13 | 52/F | 42/M | AML (M4) in 1CR | | 3.59 | A/A |
| 14 | 59/M | 64/M | MDS (RAEB) | Intermediate-2/intermediate | 3.58 | A/AB |
| 15 | 59/M | 56/M | MDS (RA) | Intermediate-1/Good | 3.58 | AB/A |
| 16 | 53/F | 55/F | MDS (RA) | Intermediate-2/Poor | 2.2 | O/O |
| 17 | 55/F | 68/M | AML (M3) in 2CR | | 2.63 | A/A |
| 18 | 54/M | 50/M | MDS (RA) | Intermediate-1/Poor | 3.74 | O/B |
| 19 | 51/M | 44/F | AML (M1) in 1CR | | 4.86 | AB/A |
| 20 | 64/F | 66/M | CML in 2CP | | 3.59 | O/A |
| 21 | 68/F | 64/M | MDS (RAEB) | Intermediate-1/Good | 3.56 | B/B |
| 22 | 53/M | 44/M | MDS (RAEB) | High/Intermediate | 7.2 | B/B |
| 23 | 60/F | 53/M | AML (M2) in 1CR | | 2.83 | A/B |
| 24 | 59/M | 62/M | AML (M4) in 2CR | | 5.47 | A/O |
| 25 | 51/F | 47/F | MDS (RAEB) | Intermediate-2/Poor | 5.93 | A/A |
| 26 | 59/M | 62/F | MDS (RA) | Intermediate-2/Poor | 4.02 | B/O |
| 27 | 59/M | 48/M | AML (M2) in 2CR | | 4.94 | B/A |
| 28 | 56/M | 62/F | MDS (RAEB-t) | High/Good | 4.38 | AB/A |
| 29 | 53/F | 62/F | AML (M2) in 1CR | | 3.06 | O/O |
| 30 | 54/F | 63/M | AML (M2) in 1CR | | 6.47 | A/O |

M, male; F, female; MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; ALL, acute lymphoblastic leukemia; RA, refractory anemia; RAEB, refractory anemia with excess blasts; RAEB-t, refractory anemia with excess blasts in transformation; CR, complete remission; CP, chronic phase. All donors were HLA-matched siblings.

regimens [4,5,7,9], and/or GvHD prophylaxis [1,4,9]. This makes overall interpretation of studies difficult. Additionally, there has been no study to prospectively assess whether RIST consisting of 180 mg fludarabine plus 8 mg/kg busulfan without antithymocyte globulin actually produces less significant organ toxicities and treatment-related toxicities in an older patient population. Information regarding the impact of the speed and degree of lineage-specific donor chimerism on clinical outcomes after RIST in older patients has been limited [3,8,13–17]. Moreover, even studies evaluated with more homogeneous patient population, type of GvHD prophylaxis and/or tempo of withdrawal of immunosuppressive agents varied depending on transplant centers and a feasible prophylaxis regimen for acute GvHD has not been well evaluated in RIST, which is considered to require a sophisticated balance between GvHD and a graft-versus-leukemia (GvL) effect.

To address these points, we conducted a prospective randomized clinical trial to evaluate the safety and efficacy of RIST with fludarabine and oral busulfan in patients aged over 50 years and with appropriate GvHD prophylaxis. In this report, the results of an interim analysis, including clinical outcomes, complications, and chimerism kinetics, were compared with those previously published in the literature.

## Patients and Methods

### Patient eligibility and accrual

Eligible patients ranged in age from 50 to 69 years (median 58.5, range 51–68 years) and had a hematological malignancy, including AML or acute lymphoblastic leukemia (ALL) in 1st or 2nd complete remission (CR), CML in 1st or 2nd chronic phase (CP), and MDS. They were required to have an HLA-identical related donor. The study protocol was reviewed and approved by the institutional review boards of the participating transplantation centers (Appendix). Eligible patients and their donors gave written informed consent before enrollment. The enrollment criteria included a performance status (PS) of the Eastern Cooperative Oncology Group (ECOG) of less than two, a serum creatinine concentration of less than 2.0 mg/dl, a cardiac ejection fraction of more than 50%, arterial oxygen saturation without supplemental oxygen of more than 93%, liver function tests less than fourfold the upper limit of normal, total bilirubin less than 2.0 mg/dl, no active infection, and no previous allergy for drugs used for conditioning or GvHD prophylaxis. Donors were required to have a normal physical examination, and normal values in the serum chemistry and blood counts, and negative results of serologic testing for human immunodeficiency virus and hepatitis B. The patient and donor characteristics are shown in Table I. Those with AML/ALL in 1st CR, CML in 1st CP, or MDS in refractory anemia were defined as low risk, and the others were defined as high risk. All 12 patients with MDS except one (UPN 22) were transfusion dependent, and all those were grouped according to the International Prognostic Scoring System (IPSS) into intermediate or high risk at the time of transplantation: Intermediate-1, $n = 3$; intermediate-2, $n = 6$; high risk, $n = 3$. By IPSS criteria, 3 patients had good-risk, 3 had intermediate-risk, and 6 had poor-risk cytogenetics.

### Donor selection and blood stem cell harvest

Related donors were selected based on compatibility of HLA-A, B and DRB1 by intermediate- or high-resolution DNA typing. After G-CSF treatment, apheresis procedures were performed daily until at least $2.0 \times 10^6$ CD34+ cells per kilogram of the recipient's body weight, up to three times, and all of the collected cells were cryopreserved until stem cell infusion.

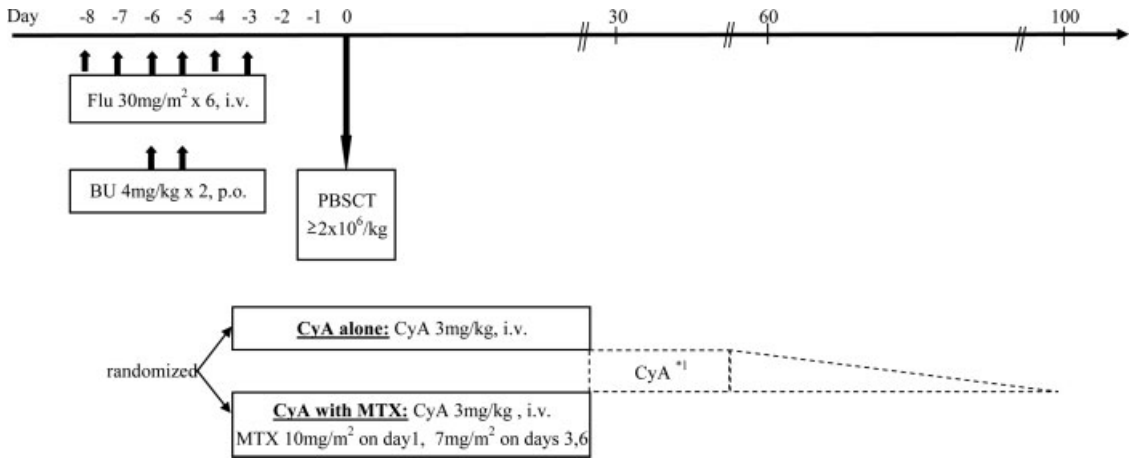


**Figure 1. Treatment schedule. CyA; cyclosporine, MTX; methotrexate. *1: When acute GvHD was not observed, CyA was tapered by 10% a week starting at Day 28, and was eliminated by Day 100. When mixed chimerism was seen without active acute GvHD over Day 60, CyA was tapered and discontinued within 2 weeks. Patients who did not convert to complete chimerism after CyA withdrawal received donor lymphocyte infusion.**

## Treatment schedule

The treatment schedule is shown in Fig. 1. The conditioning regimen consisted of fludarabine (30 mg/m$^2$/day) infused over 30 min once a day on Days 8, 7, 6, 5, 4, and 3, and oral busulfan (4 mg/kg/day) on Days 6 and 5. To prevent seizures, the patients received oral valproate sodium, at a dose of 600 mg divided into 3 doses 2 days before busulfan administration, and this was continued until 24 hr after the last dose of busulfan.

Patients were randomized to receive either cyclosporine (CyA) alone or CyA plus short-term methotrexate (MTX) for GvHD prophylaxis. Randomization was performed by stratifying according to disease (AML, ALL, CML or MDS), transplant center, age (less than 60 years or more than or equal to 60 years), and sex (male or female). All patients received 3 mg/kg/day CyA by continuous iv infusion daily from Day 1 to maintain a therapeutic trough level of 250–400 ng/ml, and thereafter orally in an attempt to maintain a therapeutic trough level of 150–250 ng/ml. The patients who were assigned to CyA plus short-term MTX received a dose of 10 mg/m$^2$ iv MTX on Day +1, and 7 mg/m$^2$ on Days +3 and +6 after stem cell infusion. CyA was tapered starting at Day 28 in the absence of acute GvHD and was discontinued by Day 100 after transplantation. When a patient did not achieve complete donor chimerism by Day 60, CyA was tapered rapidly and discontinued within 2 weeks if clinically feasible, since anti-leukemic effect was presumed to occur after development of complete donor chimerism [14]. Cases of Grade II–IV acute GvHD were treated with 2 mg/kg/day of methylprednisolone in addition to CyA.

## Supportive care

The following infection prophylaxis was recommended: prophylactic antibiotics (fluoroquinolones) were given during cytopenia, fluconazole (200 mg/day) was given at the start of conditioning and continued until the discontinuation of immunosuppressant, and oral acyclovir (1,000 mg/day) or iv acyclovir (750 mg/day) was given for prophylaxis of herpes simplex virus (HSV) and varicella zoster virus (VZV) from Day $-7$ to Day 35. Prophylaxis against *Pneumocystis carinii* was consisted of trimethoprim-sulfamethoxazole after neutrophil engraftment ($\geq 0.5 \times 10^9$ L$^{-1}$) and was continued until the discontinuation of immunosuppressant. During the first 100 days after transplantation, cytomegalovirus antigenemia assay with HRP-C7 or C10/C11 monoclonal antibody was performed weekly after neutrophil engraftment until Day 100 after transplantation. Pre-emptive therapy with ganciclovir was recommended upon the detection of positive antigenemia and was continued until it became negative. Patients were treated with G-CSF from Day +6 to neutrophil engraftment.

## Chimerism analysis

Hematopoietic chimerism was evaluated with regard to peripheral T cell (CD3+) fraction by an analysis of DNA microsatellite polymorphisms by polymerase chain reaction (PCR) with D18S51, D20S471, and D22S684 fluorescence-labeled primers, which identified differences between patient and donor (on the basis of polymorphisms found in pretransplant patient/donor samples) using an BECKMAN COULTER CEQ8000 GENETIC ANALYSIS SYSTEM. T cell (CD3+) chimerism studies post HSCT were performed on Days 30, 60, 90, 120, and thereafter every other month through 1 year.

## Assessment of response

Day 0 was defined as the day of stem cell infusion day. The day of neutrophil engraftment was defined as the first of two consecutive days on which the patient's absolute neutrophil count was above $0.5 \times 10^9$ L$^{-1}$. The day of platelet engraftment was defined as the first of seven consecutive days on which the platelet count was above $20 \times 10^9$ L$^{-1}$ without platelet transfusion.

Regimen-related toxicity (RRT) was graded using the Seattle criteria [18] on the day before the initiation of conditioning regimens and at least 3 days a week until Day 20 after transplantation. All other observed adverse events were graded according to the National Cancer Institute Common Toxicity Criteria version 2.0 (NCI-CTC ver. 2.0) until Day 100 after transplantation. Infectious diseases were diagnosed based on any positive blood culture or histologic evidence of tissue invasion.

To evaluate the general condition of patients associated with the toxicity profile, PS, and dietary oral intake were also reported at least three times a week during the initial hospitalization and once a week afterwards up to Day 100 post-transplant.

The diagnosis and grading of acute and chronic GvHD was made based on the date of onset (within or beyond 100 days) and clinical findings in conjunction with biopsy of the skin and digestive tract using the published criteria [19,20]. Patients who survived 100 days or longer were evaluable for the assessment of chronic GvHD.

## Pharmacokinetic studies of fludarabine phosphate and busulfan

Blood sampling for pharmacokinetic studies was done on Day $-5$ to investigate the effect of concomitant busulfan administration on the pharmacokinetics of 2-fluoro-ara A (2F-ara-A), which is the major metabolite of fludarabine phosphate. Blood samples for determining the 2F-ara-A plasma level were collected at 0, 0.5, 1, 2, 5, and 23.5 hr after the 4th infusion of fludarabine. We also obtained blood samples for determining the busulfan plasma level at 0, 0.5, 1, 1.5, 2, 3, and 6 hr after the sixth administration of busulfan (1 mg/kg/dose for 8 times). Blood samples were taken in tubes containing heparin and erythro-9-(2-hydroxy-3-nonyl)adenine. Plasma was obtained by centrifugation, and then transported to the laboratory and were stored at $-20^\circ$C until analysis. Plasma levels of 2F-ara-A and busulfan were determined using high-performance liquid chromatography with fluorescence and UV detection, respectively. The accuracy and precision of the assays for 2F-ara-A and busulfan were confirmed by measuring QC samples of both before this study. The maximum concentration of drug in plasma after drug administration ($C_{max}$, $C_{peak}$) and the time to reach

the maximum concentration following drug administration ($T_{max}$) were observed. The area under the plasma concentration-versus-time curve (AUC) for 2F-ara-A or busulfan was calculated by dividing the administered dose by the final plasma clearance estimate, whereas the plasma clearance was determined by modeling all plasma concentration versus time data. Terminal half-lives ($T_{1/2}$) were calculated from the primary parameters.

### Statistical analysis

The primary endpoint of this study was to determine the percentage of patients who were alive at 100 days after transplantation with complete donor chimerism (over 90%) achieved by Day 90. Secondary endpoints included the time to engraftment of neutrophils and platelets, the incidence and severity of RRT, the incidence and severity of acute and chronic GvHD, the anti-leukemia effect, DFS, and overall survival (OS). A descriptive statistical analysis was performed to assess patient baseline characteristics and disease. Time to engraftment, complete chimerism, acute or chronic GvHD, OS, and DFS were calculated using the Kaplan–Meier method. OS was defined as the time between stem cell infusion to death from any cause. DFS was defined as the time between stem cell infusion to relapse and death from any cause, whichever occurred first. After 30 patients had been enrolled in the study, a data and safety monitoring committee undertook an interim analysis. This analysis, completed in October 2004, included data for the primary endpoint, i.e. survival at Day 100 and chimerism status at Day 90, and data on acute and chronic GvHD, survival, chimerism status, and anti-tumor effect through Day 180. Neither of the predefined criteria for stopping the study was met; however, a review of available safety data including incidence and severity of RRT and Day 100 mortality indicated that this conditioning regimen was adequately safe for older patients. According to the recommendation of the committee, we decided to continue the study and published an interim report when 30 patients were enrolled and evaluated without comparing the two different GvHD prophylaxis procedures. This report includes data on these 30 patients with all available follow-up data through December 2005, and does not include the results of a comparison of the two different GvHD prophylaxis procedures.

## Results

### Engraftment and chimerism analysis

The results are summarized in Table II. One and four patients were not evaluated for neutrophil and platelet engraftment, respectively, because they did not show a nadir. The remaining patients achieved sustained engraftment and none experienced graft failure. The median number of days to achieve a neutrophil count $\geq 0.5 \times 10^9\ L^{-1}$ was 13 (range, 10–25 days), and this was 18 (range, 11–24 days) for a platelet count $\geq 20 \times 10^9\ L^{-1}$ without transfusion. Full-donor (over 90%) T-cell (CD3+) chimerism was observed in 2 and 9 of the 30 patients on Day 30 and Day 60, respectively (median [range], Day 30:71 [40 to $\geq$90] %, day 60:81 [41 to $\geq$90] %). Twenty-two patients achieved full-donor chimerism, while the remaining eight patients had mixed chimerism ranging from 78% to 88% on Day 90. Among those with mixed chimerism on Day 90, five subsequently converted to full-donor chimerism without early CyA withdrawal because of the severe acute GvHD ($n = 2$: UPN 1 and 15) and/or donor lymphocyte infusion (DLI) by day 120 ($n = 4$) or day 180 ($n = 1$). One achieved full-donor chimerism on Day 120 after DLI since the patient did not respond to the discontinuation of immunosuppressive drugs, and two had persistent mixed chimerism without relapse through 180 days after transplantation (71% and 75% donor-type chimerism on Day 180). The diagnoses of two patients with persistent mixed chimerism through Day 180 were CML and MDS, and they had not received proceeding cytotoxic chemotherapy; the patient with CML (UPN 12) received immunomodulators, imatinib mesylate and hydroxyurea, and the patient with MDS (UPN 21) received low-dose cytarabine and aclarubicin in combination with granulocyte colony stimulating factor before RIST.

### Regimen-related toxicities, complications, and general condition

The frequencies of Grade I–IV organ toxicities within 20 days after transplantation are listed in Table III. Although non-fatal toxicities including Grade I/II were seen in all 30 patients, all of the observed episodes were reversible and in no case required suspension of fludarabine. Stomatitis was the most frequently observed organ toxicity (57%, 17/30), with 47% of them (8/17) had Grade II events. None of the patients experienced veno-occlusive disease of the liver (VOD). Twenty patients had at least one episode of infectious complications within the first 100 days, with a total of 44 documented episodes (median, 2; range, 1–7 episodes) within the first 100 days after transplantation. These included proven bacterial infection (1 episode), suspected bacterial infection (1), suspected fungal infection (2), cytomegalovirus antigenemia (6), HSV infection (1), suspected viral infection (1), and uncertain causes (33). All infectious complications were recovered with or without appropriate antibiotic therapy.

The median PS for the first 28 days was 0 (range, 0–3). The worst PS of 2 ($n = 5$) or 3 ($n = 2$) within the first 28 days was experienced temporarily due to infection ($n = 2$), Grade III GvHD ($n = 1$), and nausea/vomiting ($n = 4$). Those ($n = 6$) observed from Day 29 to Day 100 were all caused by Grade II or III acute GvHD. A one-thirds reduction in dietary oral intake was temporarily seen in 20 and 11 patients within the first 28 days and from 29 days to 100 days post HSCT, respectively, which resulted from nausea/vomiting ($n = 18$) and treatment-related mucositis ($n = 2$) within Day 28, and Grade II–III acute GvHD ($n = 9$), prolonged infection with Grade II acute GvHD ($n = 1$) and gastroesophageal reflux disease ($n = 1$) between Day 29 and Day 100.

### GvHD

Grade I–IV acute GvHD at 100 days was documented, respectively, in 5 (17%), 15 (50%), 3 (10%), and 0 (0%) patients. The median time to the occurrence of Grade II–IV acute GvHD was 74 days (range, 18–100 days). All 30 patients survived beyond Day 100 and were evaluated for chronic GvHD. Twenty-six of the 30 patients (87%) developed chronic GvHD (limited type in four cases and extensive type in 22 cases) with the onset at a median of 123 days after transplantation (range, 116–217 days).

### Disease response, survival, and cause of death

No patient died within the first 100 days, and the median follow-up period was 555 days (149–1114 days) after transplantation. Twenty-nine of the 30 patients achieved CR within 100 days after transplantation, but two of them with MDS, who had poor-risk cytogenetics and were classified into intermediate-2, subsequently relapsed on Day 141 (UPN 26) and Day 156 (UPN 25). One was treated with DLI (UPN 25) and showed a temporary response, but died because of the disease progression on Day 401. The other patient (UPN 26) did not respond to DLI and died of progressive disease on Day 412. One patient (UPN 22) with MDS with high risk IPSS achieved full-donor chimerism on Day 90, but could not achieve CR on Day 98 and died with progressive disease on Day 306. This patient showed full-donor chimerism through Day 180. Five other patients died between 100 days and 1 year after transplantation (149, 151, 169, 187, and 354 days). In six patients who died within the first year, two patients were over 60 years and four patients were classified into high risk disease group. Causes of death included progressive disease of MDS with poor IPSS in 1, GvHD and/or its complications in 4, and recurrence of interstitial pneumonia in 1. In four patients, who died of GvHD and/or its complications, all had experienced

**TABLE II. Summary of Clinical Outcomes**

| UPN | Chimerism analysis | | | Post transplant DLI (reason) | GvHD | | Infection until day 100 (etiological agent) | Relapse | Outcome (Cause of death) | Follow up |
|---|---|---|---|---|---|---|---|---|---|---|
| | Day 90(%) | Day 120(%) | Day 180(%) | | Acute | Chronic | | | | |
| 1 | 88.40 | ≥90 | ≥90 | | Gr II (S, G) | Extensive | – | – | Alive | 1,114 |
| 2 | 85 | ≥90 | ≥90 | Yes (d662, relapse) | Gr II (S) | Extensive | Yes (unknown) | Yes (d402) | Dead (recurrent disease and its complication) | 652 |
| 3 | ≥90 | ≥90 | ≥90 | | – | Extensive | Yes (S. maltophilia, unknown) | – | Alive | 735 |
| 4 | ≥90 | ≥90 | D | | Gr II (S) | – | – | – | Dead (IP) | 169 |
| 5 | ≥90 | ≥90 | ≥90 | | – | Extensive | – | – | Alive | 731 |
| 6 | ≥90 | ≥90 | ≥90 | | Gr II (L) | Extensive | – | – | Alive | 716 |
| 7 | ≥90 | ≥90 | ≥90 | | Gr II (S, G) | Extensive | Yes (CMV antigenemia) | – | Dead (GvHD) | 354 |
| 8 | ≥90 | ≥90 | ≥90 | | Gr III (S, G, L) | Extensive | Yes (bacteremia susp., fungal susp., CMV antigenemia, unknown) | – | Alive | 431 |
| 9 | ≥90 | ≥90 | ≥90 | | – | Extensive | Yes (unknown) | – | Alive | 592 |
| 10 | ≥90 | ≥90 | ≥90 | | Gr II (S, G) | Extensive | Yes (unknown) | – | Dead (GvHD) | 757 |
| 11 | ≥90 | ≥90 | ≥90 | | Gr II (S) | Extensive | – | – | Alive | 360 |
| 12 | 88 | 79 | 71 | | Gr III (S, L) | Extensive | Yes (unknown) | – | Alive | 720 |
| 13 | ≥90 | ≥90 | ≥90 | | Gr I (S) | Extensive | Yes (HSV, unknown) | – | Dead (GvHD) | 517 |
| 14 | ≥90 | ≥90 | ≥90 | | Gr II (S, G, L) | Limited | Yes (fungal susp., unknown) | – | Dead (GvHD and its complication) | 187 |
| 15 | 85 | 88 | ≥90 | | Gr III (S, G) | – | – | – | Alive | 702 |
| 16 | 84 | 88 | ≥90 | | – | Limited [a] | Yes (CMV antigenemia) | – | Alive | 642 |
| 17 | 80 | ≥90 | D | Yes (d98, mixed chimerism) | Gr II (S, G)[b] | – | – | – | Dead (GvHD and its complication) | 149 |
| 18 | ≥90 | 88 | ≥90 | | Gr II (S) | Extensive | Yes (CMV antigenemia) | – | Alive | 729 |
| 19 | ≥90 | ≥90 | ≥90 | | Gr I (S) | Limited | – | – | Alive | 737 |
| 20 | ≥90 | ≥90 | ≥90 | | Gr II (G) | – | Yes (CMV antigenemia) | Yes (d147)[c] | Alive | 688 |
| 21 | 78 | 77 | 75 | | Gr II (S, L) | Extensive | – | Yes (d364) | Dead (BOOP) | 593 |
| 22 | ≥90 | ≥90 | ≥90 | | Gr II (S, G) | Extensive | Yes (unknown) | Yes (d98) | Dead (progressive disease) | 306 |
| 23 | ≥90 | ≥90 | D | | – | Extensive | Yes (unknown) | – | Dead (GvHD and its complication) | 151 |
| 24 | ≥90 | ≥90 | ≥90 | | Gr II (G) | Extensive | – | Yes (>d365)[d] | Dead (recurrent disease) | 825 |
| 25 | ≥90 | 87 | ≥90 | Yes (d186, d238, relapse) | Gr II (S) | Extensive | Yes (CMV antigenemia) | Yes (d156) | Dead (recurrent disease) | 401 |
| 26 | ≥90 | ≥90 | ≥90 | Yes (d204, relapse) | Gr I (S) | Extensive | Yes (unknown) | Yes (d141) | Dead (recurrent disease and its complication) | 412 |
| 27 | 84 | ≥90 | ≥90 | | – | Extensive | Yes (unknown) | | Alive | 371 |
| 28 | ≥90 | ≥90 | ≥90 | | Gr II (S) | Extensive | Yes (viral susp., unknown) | – | Alive | 365 |
| 29 | ≥90 | ≥90 | ≥90 | | Gr I (S) | Extensive | Yes (unknown) | – | Alive | 366 |
| 30 | ≥90 | ≥90 | ≥90 | | Gr I (S) | Limited | Yes (unknown) | Yes (d370) | Alive | 370 |

ND, not done; D, dead; DLI, donor lymphocyte infusion; Gr, grade; GvHD, graft-versus-host disease; GvHD site codes, S-skin, G-gut, L-liver; CMV, cytomegalovirus; susp., suspected; unknown, no microbiological evidence despite symptoms; IP, interstitial pneumonia.

[a]This patient developed a GvHD starting on day 112 after receiving DLI for mixed chimerism.

[b]This patient developed gut GvHD starting on day 92.

[c]CNS relapse without hematological relapse.

[d]This patient relapsed after day 365, but the exact date of relapse is unknown.

**TABLE III. Regimen-Related Toxicities Within 20 Days After HSCT According to the Seattle Criteria in 30 Patients**

| | Grade | | | |
|---|---|---|---|---|
| Toxicity | 1 | 2 | 3 | 4 |
| Heart | 1 | 0 | 0 | 0 |
| Bladder | 0 | 1 | 0 | 0 |
| Kidney | 5 | 1 | 0 | 0 |
| Lung | 2 | 0 | 0 | 0 |
| Liver | 8 | 0 | 0 | 0 |
| CNS | 1 | 0 | 0 | 0 |
| Stomatitis | 9 | 8 | 0 | 0 |
| GI toxicity | 4 | 1 | 0 | 0 |

HSCT, hematopoietic stem cell transplantation; CNS, central nervous system; GI, gastro-intestinal.

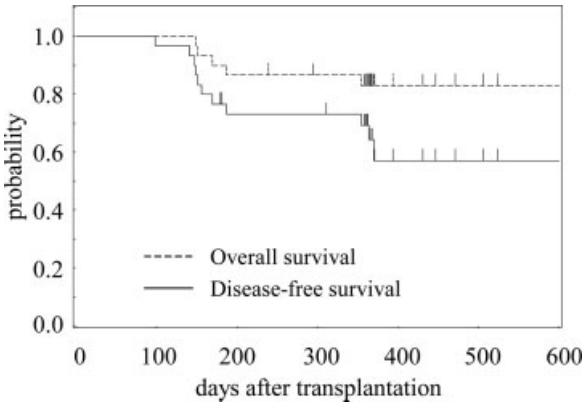


**Figure 2. Kaplan–Meier product estimates of overall survival and disease-free survival.**

gut GvHD, three of those developed extensive chronic GvHD and all were treated with corticosteroid.

The Kaplan-Meier estimated probability of OS and DFS at 1 year was, respectively, 83% and 62% (Fig. 2). Both patients age ($\leq$55 years versus >55 years) and CD34+ cell dose (>5.0 $\times$ $10^6$ $kg^{-1}$ versus $\leq$5.0 $\times$ $10^6$ $kg^{-1}$) were not associated with better outcomes by a stratified analysis (data not shown).

### Pharmacokinetic results for fludarabine and busulfan

2F-ara-A and busulfan PK parameters were calculated from data obtained from blood samples from six consenting patients (UPN 1, 3–7). After the start of the 4th infusion of fludarabine phosphate (30 mg/$m^2$/dose), the maximum plasma level of 2F-ara-A was 3.12 ± 1.08 nmol/ml, with a subsequent decline to $T_{1/2}$ of 8.59 ± 1.57 h. The AUC (0–24 hr) and CL were 17.7 ± 2.82 nmol hr/ml and 78.9 ± 13.1 ml/min/$m^2$, respectively. After the 6th administration of busulfan (1 mg/kg/dose for eight times), the maximum plasma level of busulfan was 1.37 ± 0.34 nmol/ml, with a subsequent decline to a $T_{1/2}$ of 2.88 ± 0.65 hr. The AUC (0–6 hr) and CL were 4.85 ± 1.07 nmol hr/ml and 3.60 ± 0.88 ml/min/$m^2$, respectively. Since these parameters are similar to those in a previous study with the repeated administration of fludarabine phosphate alone at 15, 20, and 25 mg/$m^2$/dose (data not shown), combination with busulfan seemed to have no effect on the pharmacokinetics of 2F-ara-A. The steady-state plasma level of busulfan (808 ± 178 ng/ml) was observed to remain within a therapeutic level (600–900 ng/ml) in adults [21].

### Discussion

In this prospective study, we showed that a combination of fludarabine (180 mg/$m^2$) and oral busulfan (8 mg/kg), despite the omission of antithymocyte globulin from the original regimen by Slavin et al. [6], can be successfully used to help prepare patients older than 50 years with hematological malignancies for HSCT from an HLA-matched related donor: All patients achieved sustained engraftment without graft failure, only an insignificant occurrence of RRT and treatment-related complications were seen, and PS and dietary intake were well maintained, which agrees with published observational studies on RIST with fludarabine and busulfan [16,22,23].

The rapid induction of complete donor-type chimerism was considered as an essential part of the RIST procedure. Although all of our patients rapidly developed conventional neutrophil and platelet engraftment, two of the 30 patients without preceding cytotoxic chemotherapy remained in mixed T-cell chimerism during the first 6 months after transplantation. A more rapid induction of T-cell chimerism has been observed in other studies of RIST in patients who had been previously treated with chemotherapy for diseases other than CML or MDS [24]. Although a close association between the occurrence of acute GvHD and the induction of higher levels of donor T-cell chimerism has been reported [14], in our experience over 50% of patients did not achieve complete chimerism at the onset of acute GvHD, demonstrating that mixed chimerism status did not provide absolute protection from GvHD, which is in agreement with data published by Baron et al. [15]. We speculate that differences in the conditioning regimen and GvHD prophylaxis may result in different observations.

While our less intensive regimen was associated with less toxicity, this strategy will only work if modifications to the conditioning regimen intensity that allow early clinical benefits do not also lead to reduced induction of GvL effect or other complications that increase relapse rate or result in worse survival in later time period [25]. A recent observational study from European Group of Blood and Marrow Transplantation Registry compared treatment-related mortality (TRM) and other outcomes between 315 RIST recipients and 407 CIST recipients, who were over 50 years and transplanted from a HLA matched sibling donor [26], and suggested that lower TRM but higher relapse rate were seen in RIST recipients. Given the fact that all three patients, who relapsed within 6 months after transplantation, were MDS with poor prognostic factors, the incidence of relapse in our study seems to be no higher than that in published data for CIST [27–30]. Taussig et al. evaluated the feasibility and safety of the fludarabine based RIST regimen in 16 patients with standard risk diseases [31]. In this study, TRM rate within 100 days was 0%, however, OS and DFS at 1 year read from Fig. 2 were 69% and 56%, respectively, where most of the patients included in this study had early stage diseases and over 30% of patients were aged less than 50 years. Despite the older patient population, our data showing no treatment-related mortality (TRM) within the first 100 days after transplantation and OS and DFS at 1 year of 83% and 62%, respectively, was encouraging.

In a previous report, we suggested that the development of GvHD is not essential for the control of low-risk myeloid malignancies, and that GvHD and infection, rather than relapse, are more important problems to be addressed in these patients [25]. Although our data showed favorable outcomes, six patients with four low risk disease and three patients aged less than 55 years died of GvHD or its complication within the first year should be interpreted with care. The incidence of Grade II–IV acute GvHD in this

study was somewhat higher than that in published literature and our own observational data with elder patients and high risk diseases [25]. However, Grade III–IV acute GvHD was infrequent and none died from acute GvHD. The incidence of chronic GvHD was higher than that in our previous experience (56%) [32] or in other reports [31,33] even after considering inevitable differences in the ethnicity, GvHD prophylaxis and matching practice of HLA, or disease risk. G-CSF mobilized peripheral blood stem cells may have been associated with an increased incidence of GvHD, particularly in its chronic form [34,35]. Conditioning regimen excluded antithymocyte globulin was also a possible explanation of this finding [23]. Most importantly, patients undergoing RIST are usually older than those undergoing CIST, which leads to a higher risk for GvHD [36,37]. Early CyA withdrawal regulation to get speedy achievement of complete donor chimerism after RIST in our protocol might have influenced the increased incidence of Grade II–IV acute GvHD, which might have affected the rate of chronic GvHD [33,35,38,39]. Although severe GvHD will be unavoidable for some patients including MDS with poor prognostic factors [40,41], the balance between GvHD and GvL is a significant concern in RIST and we should seriously evaluate the type and tapering speed of immunosuppressive agents after RIST. Current findings suggested GvHD control might be improved simply by extending the duration of CyA administration. Additionally, we noticed that the clinical features of GvHD are different in RIST than in CIST, i.e. a syndrome compatible with acute GvHD occurs well after Day 100. Hence, the current grading system for GvHD, which was developed on the basis of experience in ablative settings, may not be an optimal tool for assessing GvHD after RIST. We observed a late onset of acute GvHD and an early onset of chronic GvHD, and therefore believe that a significant number of late-onset acute GvHD may have been judged as chronic GvHD in this study simply because the onset of GvHD was over 100 days after transplantation. Our results support the current proposition by Mielcarek and Storb concerning the abandonment of the traditional Day 100 cutoff for separating acute from chronic GvHD [35].

In this prospective study, we confirmed the short-term safety and efficacy of our RIST procedure for hematological malignancies in the elderly. Long-term follow-up of patients to evaluate disease control and the consequence of therapy is mandatory, and the development of optimal GvHD prophylaxis, with the use of novel assessment criteria, will be of primary importance for the wider application of the RIST procedure. RIST may also be beneficial in young patients, since organ damage, including infertility, might be milder and less frequent in RIST than in CIST, which should be confirmed by further prospective clinical trials. Although the number of patients studied was limited, the analysis of fludarabine pharmacokinetics has for the first time provided reliable information on the interaction of key drugs, and we found no evidence to suggest that synergic or specific toxicities were associated with increased exposure to the concomitant use of busulfan, or vice versa. This information should be useful in future studies in which different drugs are combined with fludarabine.


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## Appendix

The following institutions contributed data to this study: National Cancer Center Hospital, Kanazawa University Hospital, Toranomon Hospital, Imamura Bun-in Hospital, Ehime Prefectural Central Hospital, Ishikawa Prefectural Central Hospital, Osaka City University Hospital, and Toyama Prefectural Central Hospital.

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