# Therapeutic Potential of a Reduced-Intensity Preparative Regimen for Allogeneic Transplantation with Cladribine, Busulfan, and Antithymocyte Globulin against Advanced/Refractory Acute Leukemia/Lymphoma

**Takeshi Saito, Yoshinobu Kanda, Masahiro Kami, Kazunori Kato, Nahoko Shoji, Sachiyo Kanai, Toshihiro Ohnishi, Yoshifumi Kawano, Kunihisa Nakai, Toshie Ogasawara, Hiroshi Matsubara, Atsushi Makimoto, Ryuji Tanosaki, Kensei Tobinai, Hiro Wakasugi, Yoichi Takaue,[1] and Shin Mineishi**

Hematopoietic Stem Cell Transplantation and Hematology Division, National Cancer Center Hospital, Tokyo 104-0045 [T. S., Y. Kan., N. S., K. N., T. Og., H. M., A. M., R. T., K. T., Y. T., S. M.]; Pharmacology Division, National Cancer Center Research Institute, Tokyo [K. K., S. K., H. W.]; Department of Pediatrics, University of Tokushima, Tokushima 770–8503 [S. K., T. Oh., Y. Kaw.]; and National Kyushu Cancer Center, Fukuoka [Y. Kaw.], 811–1395 Japan

## ABSTRACT

***Purpose:*** **Cladribine (2-CdA) is a purine analogue that exhibits activity against a variety of hematological malignancies and has a potent immunosuppressive effect. We therefore performed a pilot study to evaluate the feasibility of a novel 2-CdA-based reduced-intensity stem cell transplantation (RIST) regimen.**

***Experimental Design:*** **A total of 16 scheduled patients with hematological malignancies were enrolled for comparison of their data with conventional stem cell transplantation ($n = 19$). The regimen for RIST consisted of 2-CdA (0.11 mg/kg/day for 6 days), busulfan (4 mg/kg/day for 2 days), and rabbit antithymocyte globulin (2.5 mg/kg/day for 4, 2, or 0 days). The underlying diseases included acute myelogenous leukemia ($n = 6$), chronic myelogenous leukemia ($n = 2$), myelodysplastic syndrome ($n = 6$), and non-Hodgkin's lymphoma ($n = 2$).**

***Results:*** **After RIST, four patients died before day 100 as a result of acute graft-*versus*-host disease ( $n = 1$), bacteremia ($n = 1$), disseminated candidasis ($n = 1$) and congestive heart failure ($n = 1$). Another patient died of cerebral infarction on day 140. Thus, acute-phase regimen-related toxicities >grade III were observed in only one patient. Engraftment and complete donor chimerism were achieved by day 28 in 14 evaluable patients, and 6 of them (43%) experienced grade II–IV acute graft-*versus*-host disease. With a median follow-up of 328 days (range, 231–633 days), the actuarial 1-year overall and disease-free survival rates were 69% and 50%, respectively. Notably, among seven high-risk patients (five patients had been in complete remission two or more times and two not in complete remission with refractory disease at transplant), only two patients developed leukemia relapse after RIST. Although the recovery of CD4+ cells was significantly slower ($P = 0.02$) in RIST than in conventional stem cell transplantation, the incidence of clinically documented infections was not significantly different between the two groups.**

***Conclusion:*** **The results suggest that this novel regimen containing 2-CdA is well tolerated and induces early complete donor chimerism. The unexpected durable remission achieved in patients with advanced disease at transplant suggests the presence of an acceptable antileukemia/lymphoma effect, which would warrant a further clinical trial.**

## INTRODUCTION

RIST[2] has recently been explored as an alternative to CST in myeloablative regimens. Patients who have been unable to undergo CST because of advanced age or organ dysfunction have been successfully treated with RIST regimens (1–8). Moreover, accumulating evidence of a GVT effect against solid tumors has facilitated the wider application of this procedure (9). At present, most RIST regimens contain fludarabine, a purine analogue that has strong immunosuppressive and moderate myelosuppressive activities, as a backbone agent (10). On the other hand, 2-CdA, another form of purine analogue, has immunosuppressive and myeloablative profiles similar to those of fludarabine (11–13). Although fludarabine-containing regimens are frequently used as a first-line or salvage therapy for leukemias and lymphomas (14, 15), there has been only limited experience with regimens that contain 2-CdA. Moreover, 2-CdA

Received 8/27/2001; revised 12/7/2001; accepted 12/18/2001.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] To whom requests for reprints should be addressed, at Hematopoietic Stem Cell Transplantation Unit, National Cancer Center Hospital, 1-1 Tsukiji 5-chome, Chuo-ku, Tokyo 104-0045, Japan. E-mail: ytakaue@ncc.go.jp.

[2] The abbreviations used are: RIST, reduced-intensity stem cell transplantation; CST, conventional stem cell transplantation; GVT, graft-*versus*-tumor; 2-CdA, cladribine (2-chlorodeoxyadenosine); ATG, antithymocyte globulin; G-CSF, granulocyte colony-stimulating factor; GVHD, graft-*versus*-host disease; CsA, cyclosporin A; MTX, methotrexate; SMX/TMP, sulfamethoxazole/trimethoprim; aGVHD, acute GVHD; RRT, regimen-related toxicities; CR, complete remission; AML, acute myeloid leukemia; NHL, non-Hodgkin's lymphoma; DLI, donor lymphocyte infusion.

has been shown to have therapeutic activity against a variety of hematological malignancies (11, 12). Hence, we conducted a pilot study to evaluate the feasibility of a regimen consisting of 2-CdA, busulfan, and rabbit ATG in the setting of RIST, which is a modification of the well-established Slavin format (3).

## PATIENTS AND METHODS

**Patients.** This protocol was approved by the Institutional Review Board of the National Cancer Center Hospital (Tokyo, Japan). Patients with hematological malignancies who were not candidates for CST because of advanced age or organ dysfunction were eligible, and written informed consent was obtained from all patients/donors.

All patients were seen and transplanted at the National Cancer Center Hospital. Patients must have fulfilled at least one of the following criteria: 50–70 years of age; serum creatinine of 2.0–4.0 mg/dl or creatinine clearance of 25–50 ml/min, serum total bilirubin of 2.0–4.0 mg/dl, serum aspartate aminotransferase more than 6-fold above the upper limit of normal, cardiac ejection fraction <50% by echocardiogram, and $PaO_2$ at room air <50 mm Hg. In addition, patients must have had a HLA-identical or one antigen-mismatched related donor.

**Stem Cell Collection.** All patients received G-CSF-mobilized PBSCs. Donors received s.c. injections of G-CSF at 5 μg/kg twice daily starting 3 days before the first collection of PBSCs until the end of collection. Leukapheresis was performed daily until $>3.0 \times 10^6$ CD34+ cells/kg of recipient body weight were collected. Collected cells were then cryopreserved by standard techniques.

**Conditioning Regimen and Transplant Procedure.** The conditioning regimen consisted of 0.11 mg/kg/day 2-CdA [Leustatin; Ortho Biotech, Raritan, NJ; 2-h i.v. infusion for 6 days (from day −10 to day −5 in regimen A, and from day −8 to day −3 in regimen B)], 4 mg/kg oral busulfan for 2 days (days −6 and −5 in regimen A and days −4 and −3 in regimen B), and 2.5 mg/kg rabbit ATG (Thymoglobulin; IMTIX-SANGSTAT, Lyon, France) for 4 days (from day −4 to day −1) in regimen A or for 2 days (days −2 and −1) in regimen B, infused i.v. over 12 h on each day of administration. Regimen A, which was used initially, was replaced with regimen B for patients with a HLA-identical sibling donor beginning in April 2000, after stable engraftment was confirmed with the initial dose of ATG. These modifications were made in an attempt to further reduce the toxicity of the regimen because we observed a profound delay in the recovery of CD4+ cells with the addition of ATG. Furthermore, we omitted ATG for HLA-identical sibling pairs beginning in September 2000, when 14 of the scheduled 16 patients were registered. On day 0, after the conditioning regimen was completed, the cryopreserved PBSCs were thawed and infused.

GVHD prophylaxis was performed with CsA alone, except for the first two patients, who also received short-course MTX (10 mg/m$^2$ on day 1 and 7 mg/m$^2$ on days 3 and 6; Ref. 16). CsA was started on day −1 at a dose of 3 mg/kg/day by continuous i.v. infusion to maintain a serum level of ~250 ng/ml. CsA was changed to an oral form when it could be tolerated by the patient. Patients received antibacterial and antifungal prophylaxis that included oral ciprofloxacin at 200 mg three times a day and fluconazole at 200 mg/day, beginning 3 days before the beginning of the conditioning regimen. SMX/TMP was given for at least 14 consecutive days (1600 mg of SMX and 320 mg of TMP daily) before transplant as prophylaxis against *Pneumocystis carinii* pneumonia. SMX/TMP was resumed after engraftment on a 2 days/week schedule. Acyclovir at a dose of 200 mg five times a day was given p.o. (or 250 mg three times a day i.v.) from day −7 to day +35 and 400 mg a day thereafter for herpes virus prophylaxis. All patients received G-CSF at a dose of 5 μg/kg/day by i.v. infusion beginning on day 6 post-transplant until the neutrophil counts recovered to $>0.5 \times 10^9$/liter for 2 consecutive days. Patients who developed grade II–IV aGVHD were treated with 1 or 2 mg/kg i.v. methylprednisolone together with CsA.

**Chimerism Analysis.** Chimerism analyses were performed by the short tandem repeat method on days 14, 21, and 28 after transplant, and every 2–4 weeks thereafter as described previously (17). Briefly, DNA was extracted from peripheral blood mononuclear cells using a commercially available kit (Wizard Genomic DNA purification kit; Promega, Madison, WI). Multiplex PCR was performed using primer sets (AmpFISTR; Applied Biosystems, Foster City, CA). Four-color fluorescence detection was performed on an ABI 310 automated DNA sequencer (Applied Biosystems). For each short tandem repeat allele, the area under the curve for the corresponding signal was automatically processed by GeneScan 3.1 software (Applied Biosystems). The percentage of donor cells was calculated as: area signal donor/(area signal donor + area signal recipient).

**Evaluation of Immune Reconstitution.** Immunophenotypic analyses of peripheral blood mononuclear cells were performed on days 30, 60, 90, 120, 180, and 240. Fluorochrome-labeled monoclonal antibodies against CD3 (Leu 4; peridinin chlorophyll protein), CD4 (Leu-3a; FITC), CD8 (Leu-2a; phycoerythrin) and CD20 (Leu-16; phycoerythrin) were purchased from Becton Dickinson (San Jose, CA) or Immunotech (Marseilles, France). Acquisition and analysis of two- and three-color studies were performed using a flow cytometer (FACSCalibur; Becton Dickinson, San Jose, CA). To calculate the absolute numbers in each lymphocyte subset, the percentage of cells that were stained positive was multiplied by the absolute peripheral blood lymphocyte count.

**Outcome Measures and Statistical Considerations.** The primary end point was achievement of complete donor chimerism (>90% donor cells) without early (before day 100) transplant-related mortality, which was defined as "success." In addition, we evaluated the time to engraftment, RRTs, and the incidence of infections and aGVHD. RRT was evaluated using the criteria of Bearman *et al.* (18), and aGVHD was graded based on the 1994 consensus conference on aGVHD grading (19). The data were compared with those obtained for 19 patients who received allogeneic CST, which was performed in the same study period with a HLA-identical or one antigen-mismatched related donor.

To reject any treatment with a success rate <50% with a 10% α error and to accept any treatment with a success rate >80% with a 10% β error, we planned to have seven and nine patients in the first and second stages of this study, respectively, according to Simon's minimax two-stage design (20). If there

*Table 1* Characteristics of patients undergoing RIST

| UPN[a] | Age (yrs) | Sex | Disease | Status at transplant | Duration of preceding CR | Reasons for RIST | Dose of ATG | RRT grade (site) | aGVHD grade (onset) | Relapse | Outcome |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 50 | M | MDS | RAEB | NE | Liver dysfunction | 4 | 1 (H) | –[b] | | Alive and DF (day 633) |
| 2 | 55 | F | AML | 3rd CR | 15 mo | Age | 4 | | –[b] | Yes (day 357) | Alive on salvage therapy (day 609) |
| 3 | 29 | M | AML | Non-CR | 3 mo | Cardiac dysfunction | 4 | II (H) | II[c] (day 52) | | Alive and DF (day 470) |
| 4 | 46 | M | CML | 1st CP | NE | Renal dysfunction | 2 | II (R), I (H) | IV (day 26) | | Died of GVHD (day 66) |
| 5 | 56 | M | MDS | RA | NE | Age | 2 | II (H) | | | Alive and DF (day 377) |
| 6 | 37 | M | LG-NHL | Non-CR | 4 mo | Cardiac dysfunction | 2 | | IV (day 79) | | Alive and DF (day 370) |
| 7 | 53 | F | AML | 1st CR | NE | Age | 2 | | | | Alive and DF (day 328) |
| 8 | 58 | M | AML | 2nd CR | 18 mo | Age | 2 | | | Yes (day 136) | Alive on salvage therapy (day 312) |
| 9 | 62 | M | MDS | RA | NE | Cardiac dysfunction | 2 | IV (C) | | | Died of congestive heart failure (day 77) |
| 10 | 50 | F | CML | 1st CP | NE | Age | 2 | | | | Alive and DF (day 285) |
| 11 | 44 | F | LG-NHL | 4th CR | 4 mo | Multiorgan dysfunction | 0 | | III (day 13) | | Died of cerebral infarction (day 140) |
| 12 | 53 | M | AML | 2nd CR | 16 mo | Age | 2 | | | | Alive and DF (day 272) |
| 13 | 19 | F | MDS | RA | NE | Multiorgan dysfunction | 4 | II (S) | III[c] (day 13) | | Died of fungal infection (day 68) |
| 14 | 15 | M | AML | 3rd CR | 52 mo | Cardiac dysfunction | 4 | | –[c] | | Alive with autologous recovery (day 239) |
| 15 | 66 | M | MDS | RAEB | NE | Age | 4 | | –[c] | | Died of bacterial infection (day 7) |
| 16 | 56 | M | MDS | RAEB | NE | Age | 0 | | II (day 12) | | Alive and DF (day 231) |

[a] UPN, unique patient number; MDS, myelodysplastic syndrome; RAEB, refractory anemia with excess of blast; NE, not evaluable; H, hepatic toxicity; DF, disease free; CML, chronic myeloid leukemia; R, renal toxicity; RA, refractory anemia; LG-NHL, low-grade NHL; C, cardiac toxicity; S, stomatitis.

[b] MTX added to GVHD prophylaxis.

[c] One-antigen mismatch.

were fewer than four successes among the first seven patients, the treatment was to be rejected as being of little interest. Otherwise, accrual was planned to continue for a total of 16 patients. Finally, the treatment would be rejected if the success rate was $\leq$10 of 16.

We compared the time to engraftment and the numbers of infused CD34 or CD3+ cells between the two groups, using the Mann-Whitney *U* test. The incidences of RRT, aGVHD, and infections were compared using Fisher's exact test. Overall survival was estimated using the Kaplan-Meier technique. Disease-free survival was also calculated from the day of transplantation because all of the recipients were in CR when they achieved engraftment. The recoveries of immunological parameters were compared using repeated-measures ANOVA tests.

## RESULTS

**Patient Characteristics.** The study was initiated in July 1999. Six of the seven initially registered patients achieved success as defined above, and the study was allowed to move to the second stage. Finally, a total of 16 scheduled patients were recruited, and the study was closed in October 2000 with 11 successes. The reasons for eligibility included age >50 ($n = 8$) and organ dysfunction ($n = 8$), and the patients' characteristics are summarized in Table 1. Among patients with AML or NHL, five were in their second or higher CR (the duration of the preceding CR was <2 years in all but one) and two had refractory disease. The donor was a HLA-identical sibling in 11, a phenotypically HLA-matched daughter in 1, and a related donor with one serologically mismatched antigen in 4.

*Table 2* Patient characteristics

| | RIST[a] | CST[b] |
|---|---|---|
| Total number | 16 | 19 |
| Age, median (range) | 51.5 (15–66) | 41 (18–51) |
| Sex (F/M) | 5/11 | 11/8 |
| Disease | | |
| AML/MDS[c] | 6/6 | 5/1 |
| ALL/NHL | 0/2 | 4/3 |
| CML | 2 | 6 |
| HLA | | |
| 6/6 | 12 | 18 |
| 5/6 | 4 | 1 |

[a] In the RIST group, ATG was administered 4 days in six patients, 2 days in eight patients, and 0 days in two.

[b] In the CST group, 9 received busulfan/cyclophosphamide and 10 received cyclophosphamide/total body irradiation.

[c] MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukemia; CML, chronic myeloid leukemia.

Nineteen patients who received CST during the same period were used as controls; 18 received grafts from a HLA-identical sibling donor, and 1 received graft from an antigen-mismatched donor (Table 2). The conditioning regimen used was busulfan/cyclophosphamide (4 mg/kg/day busulfan for 4 days and 60 mg/kg/day cyclophosphamide for 2 days) in 9 and

*Table 3* Graft composition and engraftment

| | RIST | CST | *P* |
|---|---|---|---|
| Infused cells per kg, median (range) | | | |
| CD34 $\times$ $10^6$ | 4.6 (1.6–6.6) | 5.27 (3.2–9.4) | 0.024 |
| CD3 $\times$ $10^8$ | 3.3 (1.4–7.8) | 4.18 (2.0–6.7) | 0.35 |
| Cell Recovery Day, median (range) | | | |
| ANC[a] $>$ 0.5 $\times$ $10^9$/liter | 11 (4–14) | 14 (11–22) | 0.0004 |
| Lymphocytes $>$ 0.5 $\times$ $10^9$/liter | 13 (7–31) | 14 (12–17) | 0.83 |
| PLT $>$ 20 $\times$ $10^9$/liter | 9.5 (0–12) | 14 (9–26) | 0.0001 |

[a] ANC, absolute neutrophil count; PLT, platelets.

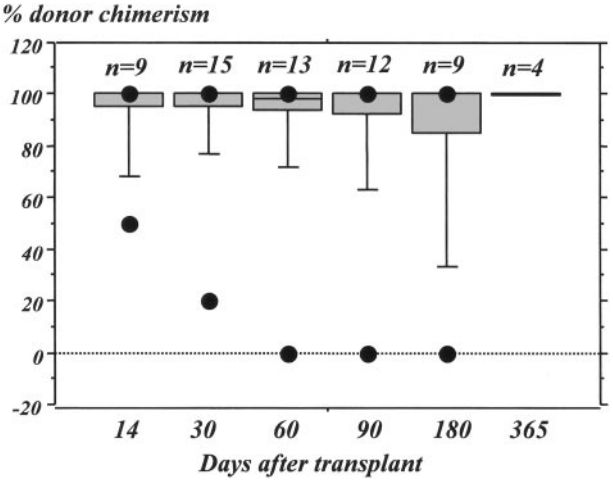


*Fig. 1* Percentage of donor chimerism after RIST. The *box* and *whisker plots* show 10, 25, 50, 75, and 90 percentile values. Outliers are indicated by ●.

cyclophosphamide/total body irradiation (60 mg/kg/day cyclophosphamide for 2 days and total body irradiation of 12 Gy in six fractions) in 10 patients. Prophylaxis against GVHD was exclusively CsA and MTX.

**RRTs.** The RIST regimen was generally well tolerated without significant early RRT, except for one patient who developed congestive heart failure (grade IV) on day 14 after transplant (patient UPN 9); this patient's cardiac function had been impaired before transplant. Hence, the relationship between the conditioning regimen and heart failure was unclear. There was no significant difference in the incidence of RRT related to hepatic, cardiac, or bladder involvement between the RIST and CST groups, except for stomatitis, which was significantly less frequent in the RIST group ($P = 0.0001$). Most RIST patients could maintain oral intake throughout the transplant course. Four patients subsequently died before day 100 as the result of aGVHD ($n = 1$), bacteremia ($n = 1$), disseminated candidasis ($n = 1$), or congestive heart failure ($n = 1$). Another patient died of cerebral infarction on day 140.

**Engraftment and Chimerism.** All but two of the patients who received the RIST regimen achieved sustained engraftment (Fig. 1). One patient showed primary graft rejection after transplant from a HLA-mismatched donor, which was subsequently followed by autologous hematopoietic recovery (patient UPN 14). Another patient died of bacteremia before engraftment (patient UPN 15). The median times to an absolute neutrophil count $>0.5 \times 10^9$/liter and an unsupported platelet count $>20 \times 10^9$/liter were 11 days (range, 4–14 days) and 9.5 days (range, 0–12 days), respectively. Five patients never required platelet transfusions. Both neutrophil and platelet recoveries were significantly faster in RIST than CST ($P = 0.0004$ and 0.0001, respectively), although fewer CD34 cells were infused in the RIST group (Table 3). All engrafted patients ($n = 14$) achieved complete chimerism, defined as $>$90% donor cells, by day 28 after RIST without additional DLI. However, one patient (patient UPN 2), who was transplanted for AML in the third CR, developed a decrease in host cells to 10–15% after day 100. This patient did not receive DLI because of the donor's refusal and eventually relapsed on day 357. Another patient (patient UPN 8) had a relapse in the subsequent course without preceding mixed chimerism.

**GVHD.** Among 14 evaluable patients, 6 (43%) experienced grade II–IV aGVHD, including 2 who developed aGVHD after the rapid discontinuation of CsA, which started after the confirmation of engraftment and was completed by day 45, because they had refractory disease at transplantation. One patient (patient UPN 4) died of grade IV GVHD. Another patient (patient UPN 6) developed grade IV liver GVHD after discontinuation of CsA, which was improved with added ATG treatment. Although the background and prophylactic regimens of the two groups differed, the incidence of aGVHD in RIST was not significantly different from that in CST. Among 11 evaluable patients, 3 developed chronic GVHD.

**Infections.** The recovery of CD4+ cells was significantly slower in RIST than in CST ($P = 0.02$), whereas there was no significant difference in the recovery of CD8+ cells between the two groups ($P = 0.15$). The incidence of documented bacterial or fungal infection was not significantly different between the two groups. One patient (patient UPN 15) developed *Staphylococcus aureus* bacteremia during the conditioning regimen and eventually died of septic shock on day 7. Another patient (patient UPN 13) died of disseminated candidasis after high-dose corticosteroid therapy for grade III gut aGVHD. In a separate analysis, 9 (64%) in the RIST group and 16 (84%) in the CST group developed positive cytomegalovirus antigenemia, although none developed cytomegalovirus diseases.[3]

**Disease Status and Survival.** Eleven patients remained alive with a median follow-up of 324 days (range, 227–629 days) after transplantation. Actuarial 1-year disease-free survival and overall survival were 50% and 69%, respectively (Fig. 2). Among patients who underwent a HLA-matched transplant, overall survival was 74%. Notably, there were only two cases who had a relapse among seven high-risk patients (five patients had been in CR two or more times and two who were in non-CR with refractory disease). The two patients who had had refrac-

[3] Unpublished data.

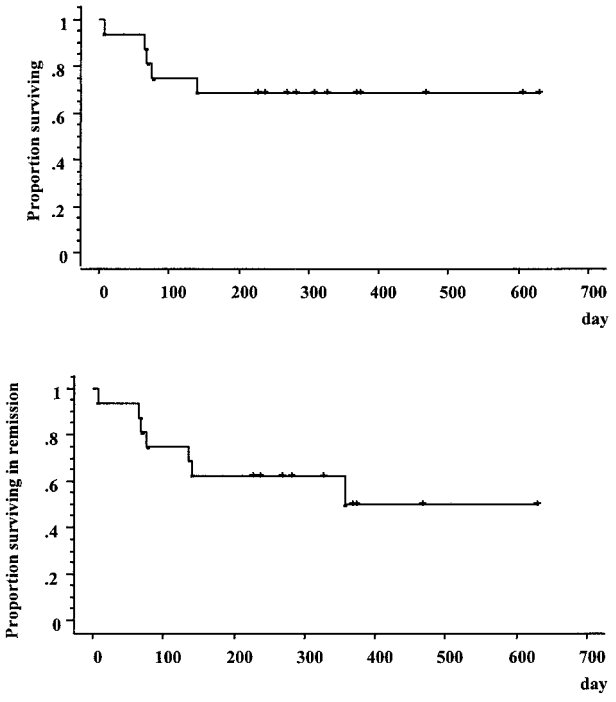


*Fig. 2* Overall (*top panel*) and disease-free (*bottom panel*) survival for all patients.

tory disease before RIST (patients UPN 3 and UPN 6) have survived for 466 and 366 days without disease, respectively.

## DISCUSSION

RIST is in an early phase of development as a novel therapeutic modality to expand the application of allogeneic stem cell transplantation to those who would not be eligible for CST because of advanced age or organ dysfunction and to those with solid tumors, including renal cell carcinoma. To accomplish RIST, various conditioning regimens have been used, but a suitable regimen still remains to be established. Most of the published regimens have contained fludarabine, a purine analogue. However, 2-CdA, which like fludarabine has potent immunosuppressive and relatively mild myeloablative effects, has also been used (10–12).

2-CdA is used as a first-line therapy for hairy cell leukemia (21) and has also been shown to have definite activity against AML, although this is associated with considerable dose-limiting neurotoxicity (22). In an RIST setting, Giralt *et al.* (2) administered 2-CdA at a dose of 12 mg/m$^2$/day combined with 1-β-D-arabinofuranosylcytosine at 1 g/m$^2$/day for 5 days in seven patients with advanced myeloid malignancies that were refractory to chemotherapy or who relapsed after previous treatment including fludarabine. CR was achieved in five of the seven patients, suggesting that 2-CdA was active against fludarabine-resistant leukemia. Thus, these findings suggest that 2-CdA may be an attractive alternative agent for use in an RIST regimen, particularly for advanced or refractory leukemia/lymphoma.

Nevertheless, the Anderson trial disclosed excessive toxicities after conditioning with 2-CdA and melphalan (23). In our study, therefore, we decreased the dose of 2-CdA to 0.11 mg/kg/day for 6 days, which was equal to the dose used in the single-agent treatment of lymphoid malignancies (24) and approximately half of that used by Giralt *et al.* (2) The severity of RRT with our regimen was comparable to that of other types of RIST regimens that include fludarabine, although one patient (patient UPN 9), who had impaired cardiac function before transplant, died of congestive heart failure on day 77. The direct pathogenetic relationship between our regimen and the progression of heart failure is not clear. In the remaining 15 patients, no significant RRTs were observed. Thus, a reduced dose of 2-CdA could be one of the reasons for reduced toxicities. The observed clinical response also suggested that this regimen may have therapeutic potential toward advanced or refractory leukemia/lymphoma.

Regarding the primary end point, we achieved complete donor chimerism without early transplant-related mortality in 11 of the 16 patients at high risk of RRT. Notably, 10 of the 12 HLA-matched patients achieved successful donor chimerism without added DLI, whereas 3 of the 4 patients transplanted from a HLA-mismatched donor did not. Serial chimerism analyses revealed that most of our patients, including two who did not receive ATG, achieved complete donor chimerism by day 28 after transplant. Bornhauser *et al.* (25) reported that they achieved similar early complete donor chimerism using a regimen of fludarabine/busulfan, which has the same backbone as ours. On the other hand, Childs *et al.* (7) reported the kinetics of donor chimerism after RIST using the fludarabine/cyclophosphamide regimen. Although the two types of regimens cannot be compared, complete donor chimerism was achieved more slowly and DLI was occasionally required. Therefore, our results suggest that busulfan contributed to engraftment.

Because it has been hypothesized that the cytokine release caused by RRT enhances aGVHD (26), it was speculated that the use of a RIST regimen may reduce the incidence of aGVHD. In the present series, with the use of ATG we observed aGVHD in 43% of the patients, and two deaths were closely related to aGVHD (patients UPN 4 and UPN 13). Although we did not see any meaningful difference in the incidence of GVHD with a conventional transplant, within the context of RIST, most studies, including ours, may have actually encouraged GVHD by reducing the intensity of prophylaxis by omitting MTX and/or through the early discontinuation of CsA (2–4, 8). Moreover, more older patients have been registered to RIST protocols. Hence, the question of whether GVHD is reduced in a RIST procedure should be tested in a prospective study, and we plan a randomized Phase II study to compare RIST regimens with different GVHD prophylaxes. A higher dose of ATG may offer an advantage in achieving stable engraftment and preventing GVHD, but this will be associated with an increased risk of infection and an impaired GVT effect. We found that T-cell immune reconstitution was delayed after our RIST regimen, although this did not directly translate to an increased incidence of clinically documented infections. Assuming that this delay was attributed to ATG, we subsequently decreased the dose of ATG in HLA-matched pairs.

It has also been proposed that a RIST strategy would not work against a rapidly growing tumor because the clinically

significant manifestation of a GVT effect may take time or may realistically be too weak to overcome a growing tumor. However, only two patients relapsed after RIST in this study, although the follow-up period is still too short. The most impressive antileukemic effect was observed in patient UPN 3, who had a relapse of AML 3 months after the first bone marrow transplantation from an unrelated donor and who was prepared with cyclophosphamide/total body irradiation. Although his disease was refractory to reinduction chemotherapy, CR was obtained and maintained for 1 year after RIST. Considering the cytoreductive intensity of the regimens used in the first and second transplants, an added GVT effect might have prevented the recurrence of leukemia. Nevertheless, an antitumor effect associated with our RIST procedure should be evaluated in a prospective study with a larger number of patients.

In conclusion, we have shown that the combination of 2-CdA-busulfan-ATG enables the early achievement of complete donor chimerism without excessive toxicities in patients who would not be able to tolerate conventional transplant regimens. The RIST regimen is associated with a narrow window of RRT, immune suppression, occurrence of GVHD, and opportunistic infections. The development of a formula for GVHD prophylaxis should be balanced against the risk of GVHD and the merit of a GVT effect, and GVHD prophylaxis will need to be fine-tuned for transplants in which there is a high risk for GVHD. The unexpected durable remission achieved after RIST in patients with advanced or refractory disease warrants a further clinical trial to compare this novel regimen with conventional myeloablative or fludarabine-based RIST regimens.

## ACKNOWLEDGMENTS

We thank Mitori Kogure, Kazue Kamijyo, Makiko Shimada, Yukie Hotate, Akira Iizuka, and Yukie Harada for excellent technical support.

## REFERENCES

1. Carella, A. M., Champlin, R., Slavin, S., McSweeney, P., and Storb, R. Mini-allografts: ongoing trials in humans. Bone Marrow Transplant., *25:* 345–350, 2000.

2. Giralt, S., Estey, E., Albitar, M., Van Besien, K., Rondon, G., Anderlini, P., O'Brien, S., Khouri, I., Gajewski, J., Mehra, R., Claxton, D., Andersson, B., Beran, M., Przepiorka, D., Koller, C., Kornblau, S., Korbling, M., Keating, M., Kantarjian, H., and Champlin, R. Engraftment of allogenic hematopoietic progenitor cells with purine analog-containing chemotherapy: harnessing graft-*versus*-leukemia without myeloablative therapy. Blood, *89:* 4531–4536, 1997.

3. Slavin, S., Nagler, A., Naparstek, E., Kapelushnik, Y., Aker, M., Cividalli, G., Varadi, G., Kirschbaum, M., Ackerstein, A., Samuel, S., Amar, A., Brautbar, C., Ben-Tal, O., Eldor, A., and Or, R. 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, *91:* 756–763, 1998.

4. Khouri, I. F., Keating, M., Korbling, M., Przepiorka, D., Anderlini, P., O'Brien, S., Giralt, S., Ippoliti, C., Von Wolff, B., Gajewski, J., Donato, M., Claxton, D., Ueno, N., Andersson, B., Gee, A., and Champlin, R. Transplant-Lite: induction of graft-*versus*-malignancy using fludarabine-based nonablative chemotherapy and allogeneic blood progenitor-cell transplantation as treatment for lymphoid malignancies. J. Clin. Oncol., *16:* 2817–2824, 1998.

5. Sykes, M., Preffer, F., McAfee, S., Saidman, S. L., Weymouth, D., Andrews, D. M., Colby, C., Sackstein, R., Sachs, D. H., and Spitzer, T. R. Mixed lymphohematopoietic chimerism and graft-*versus*-lymphoma effects after non-myeloablative therapy and HLA-mismatched donor-bone marrow transplantation. Lancet, *353:* 1755–1759, 1999.

6. McSweeney, P. A., Niederwieser, D., Shizuru, J. A., Sandmaier, B. M., Molina, A. J., Maloney, D. G., Chauncey, T. R., Gooley, T. A., Hegenbart, U., Nash, R. A., Radich, J., Wagner, J. L., Minor, S., Appelbaum, F. R., Bensinger, W. I., Bryant, E., Flowers, M. E. D., George, G., E., Grumet, F. C., Kiem, H-P., Torok-Storb, B., Yu, C., Blume, K. G., and Storb, R. F. Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-*versus*-tumor effects. Blood, *97:* 3390–3400, 1999.

7. Childs, R., Clave, E., Contentin, N., Jayasekera, D., Hensel, N., Leitman, S., Read, E. J., Carter, C., Bahceci, E., Young, N. S., and Barrett, A. J. Engraftment kinetics after nonmyeloablative allogeneic peripheral blood stem cell transplantation; full donor T-cell chimerism precedes alloimmune responses. Blood, *94:* 3234–3241, 1999.

8. Kottaridis, P. D., Milligan, D. W., Chopra, R., Chakraverty, R. K., Chakrabarti, S., Robinson, S., Peggs, K., Verfuerth, S., Pettengell, R., Marsh, J. C. W., Schey, S., Mahendra, P., Morgan, G. J., Hale, G., Waldmann, H., Elvira, M. C., Devereux, S., Linch, D. C., Goldstone, A., H., and Mackinnon, S. *In vivo* CAMPATH-1H prevents graft-*versus*-host disease following nonmyeloablative stem cell transplantation. Blood, *96:* 2419–2425, 2000.

9. Childs, R., Chernoff, A., Contentin, N., Bahceci, E., Schrump, D., Leitman, S., Read, E. J., Tisdale, J., Dunbar, C., Linehan, W. M., Young, N. S., and Barrett, A. J. Regression of metastatic renal-cell carcinoma after nonmyeloablative allogeneic peripheral-blood stem-cell transplantation. N. Engl. J. Med., *343:* 750–758, 2000.

10. Keating, M. J., Kantarjian, H., Talpaz, M., Redman, J., Koller, C., Barlogie, B., Velasquez, W., Plunkett, W., Freireich, E. J., and McCredie, K. B. Fludarabine: a new agent with major activity against chronic lymphocytic leukemia. Blood, *74:* 19–25, 1989.

11. Seto, S., Carrera, C. J., Kubota, M., Wasson, D. B., and Carson, D. A. Mechanism of deoxyadenosine and 2-chlorodeoxyadenosine toxicity to nondividing human lymphocytes. J. Clin. Investig., *75:* 377–383, 1985.

12. Carrera, C. J., Terai, C., Lotz, M., Curd, J. G., Piro, L. D., Beutler, E., and Carson, D. Potent toxicity of 2-chlorodeoxyadenosine toward human monocytes *in vitro* and *in vivo*. J. Clin. Investig., *86:* 1480–1488, 1990.

13. Liliemark, J., and Juliusson, G. On the pharmacokinetics of 2-chloro-2′-deoxyadenosine in humans. Cancer Res., *51:* 5570–5572, 1991.

14. Dinndorf, P. A., Avramis, V. I., Wiersma, S., Kralio, M. D., Liu-Mares, W., Seibel, N. L., Sato, J. K., Mosher, R. B., Kelleher, J. F., and Reaman, G. H. Phase I/II study of idarubicin given with continuous infusion fludarabine followed by continuous infusion cytarabine in children with acute leukemia: a report from the Children's Cancer Group. J. Clin. Oncol., *15:* 2780–2785, 1997.

15. Solal-Celigny, P., Brice, P., Brousse, N., Caspard, H., Bastion, Y., Haioun, C., Basly, A., Tilly, H., Bordessoule, D., Sebban, C., Harousseau, J. L., Morel, P., Dupas, B., Plassart, F., Vasile, N., Fort, N., and Leporrier, M. Phase II trial of fludarabine monophosphate as first-line treatment in patients with advanced follicular lymphoma. J. Clin. Oncol., *14:* 514–519, 1996.

16. Morishima, Y., Morishita, Y., Tanimoto, M., Ohno, R., Saito, H., Horibe, K., Hamajima, N., Naito, K., Yamada, K., Yokomaku, S., Hirabayashi, N., Yamada, H., Nakaide, Y., Kojima, S., Minami, S., Matsuyama, K., and Kodera, Y. Low incidence of acute graft-*versus*-host disease by the administration of methotrexate and cyclosporine in Japanese leukemia patients after bone marrow transplantation from human leukocyte antigen compatible siblings: possible role of genetic homogeneity. Blood, *74:* 2252–2256, 1989.

17. Thiede, C., Florek, M., Bornhauser, M., Ritter, M., Mohr, B., Brendel, C., Ehninger, G., and Neubauer, A. Rapid quantification of mixed chimerism using multiplex amplification of short tandem repeat markers and fluorescence detection. Bone Marrow Transplant., *23:* 1055–1060, 1999.

18. Bearman, S. I., Appelbaum, F. R., Buckner, C. D., Peterson, F. B., Fisher, L. D., Clift, R. A., and Thomas, E. D. Regimen-related toxicity in patients undergoing bone marrow transplantation. J. Clin. Oncol., *6:* 1562–1568, 1988.

19. Przepiorka, D., Weisdorf, D., Martin, P., Klingemann, H-G., Beatty, P., Hows, J., and Thomas, E. D. Consensus conference on acute GVHD grading. Bone Marrow Transplant., *15:* 825–828, 1995.

20. Simon, R. Optimal two-stage designs for Phase II clinical trials. Control Clin. Trials, *10:* 1–10, 1989.

21. Saven, A., and Piro, L. Newer purine analogues for the treatment of hairy-cell leukemia. N. Engl. J. Med., *330:* 691–697, 1994.

22. Vahdat, L., Wong, E. T., Wile, M. J., Rosenblum, M., Foley, K. M., and Warrell, R. P. Therapeutic and neurotoxic effects of 2-chlorodeoxyadenosine in adults with acute myeloid leukemia. Blood, *84:* 3429–3434, 1994.

23. Giralt, S., Thall, P. F., Khouri, I., Wang, X., Braunschweig, I., Ippolitti, C., Claxton, D., Donato, M., Bruton, J., Cohen, A., Davis, M., Andersson, B. S., Anderlini, P., Gajewski, J., Kornblau, S., Andreeff, M., Przepiorka, D., Ueno, N. T., Molldrem, J., and Champlin, R. Melphalan and purine analog-containing preparative regimens: reduced-intensity conditioning for patients with hematologic malignancies undergoing allogeneic progenitor cell transplantation. Blood, *97:* 631–637, 2001.

24. Tobinai, K., Ogura, M., Hotta, T., Kobayashi, Y., Narabayashi, M., Suzuki, R., Kinoshita, T., Kozuru, M., Uike, N., and Ohashi, Y. Phase I study of cladribine (2-chlorodeoxyadenosine) in lymphoid malignancies. Jpn. J. Clin. Oncol., *27:* 146–153, 1997.

25. Bornhauser, M., Thiede, C., Schuler, U., Platzbecker, U., Freiberg-Richter, J., Helwig, A., Plettig, R., Rollig, C., Naumann, R., Kroschinski, F., Neubauer, A., and Ehninger, G. Dose-reduced conditioning for allogeneic blood stem cell transplantation: durable engraftment without antithymocyte globulin. Bone Marrow Transplant., *26:* 119–125, 2000.

26. Antin, J. H., and Ferrara, J. L. M. Cytokine dysregulation and acute graft-*versus*-host disease. Blood, *80:* 2964–2968, 1992.

Clinical Cancer Research

# Therapeutic Potential of a Reduced-Intensity Preparative Regimen for Allogeneic Transplantation with Cladribine, Busulfan, and Antithymocyte Globulin against Advanced/Refractory Acute Leukemia/Lymphoma

Takeshi Saito, Yoshinobu Kanda, Masahiro Kami, et al.

*Clin Cancer Res* 2002;8:1014-1020.