Journal of Antimicrobial Chemotherapy (2006) **57**, 1004–1007
doi:10.1093/jac/dkl089
Advance Access publication 21 March 2006

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Pharmacokinetics of ganciclovir in haematopoietic stem cell transplantation recipients with or without renal impairment

Yuki Asano-Mori[1], Yoshinobu Kanda[1,2]*, Kumi Oshima[1,2], Takuro Watanabe[1], Eriko Shoda[1], Toru Motokura[1], Mineo Kurokawa[1,2] and Shigeru Chiba[1,2]

[1]*Department of Hematology & Oncology, University of Tokyo Graduate School of Medicine and Hospital, Tokyo, Japan;*[2]*Department of Cell Therapy & Transplantation Medicine, University of Tokyo Hospital, Tokyo, Japan*

Received 3 October 2005; returned 22 November 2005; revised 25 January 2006; accepted 24 February 2006

***Objectives*: We investigated the pharmacokinetics of ganciclovir in 12 haematopoietic stem cell transplantation (HSCT) recipients to evaluate the validity of a 50% reduction in the ganciclovir dosage for mild renal impairment.**

***Patients and methods*: Ganciclovir at 5 mg/kg/day was pre-emptively infused in patients with estimated $CL_{CR} \geq 70$ mL/min (Group A), whereas the dose was reduced to 2.5 mg/kg/day in patients with CL_{CR} between 50 and 70 mL/min (Group B).**

***Results*: The peak concentration was significantly higher in Group A ($P < 0.01$). However, the decrease in the plasma ganciclovir concentration was slower in Group B ($P = 0.09$), and the AUC of all patients in both groups was distributed within a narrow range (25.6 ± 4.77 μg·h/mL), when two patients with exceptionally high AUC values were excluded.**

***Conclusions*: A 50% reduction in ganciclovir appeared to be appropriate for patients with mild renal impairment. Measuring the ganciclovir concentration at 4 h after starting infusion may be adequate for evaluating AUC.**

Keywords: cytomegalovirus, CMV, antigenaemia, antiviral therapy

Introduction

Ganciclovir is the mainstay of antiviral agents in pre-emptive therapy against cytomegalovirus (CMV) disease after allogeneic haematopoietic stem cell transplantation (HSCT).[1] Ganciclovir is mainly excreted from the kidney and about 90% of the administered dose is recovered unchanged in the urine after intravenous (iv) administration.[2] Therefore, total body clearance correlates well with CL_{CR}.[3,4] In HSCT settings, patients frequently develop renal impairment caused by the use of nephrotoxic drugs. A 50% reduction of ganciclovir is recommended in the drug information leaflet for patients with mild renal impairment of CL_{CR} between 50 and 70 mL/min in order to achieve an unchanged AUC. However, the pharmacokinetic profiles of ganciclovir have not yet been fully evaluated in such patients. Therefore, we investigated the validity of this dose reduction by serial evaluation of the plasma ganciclovir concentration.

Patients and methods

Twelve patients (nine men and three women) aged between 23 and 61 years were enrolled in a 12 h pharmacokinetic study of intravenous ganciclovir after ethical approval. The median age and weight were 50.5 years (range 23–61) and 57.5 kg (range 36.7–80.0), respectively. All patients provided informed consent to participate in this study. The underlying disease was acute leukaemia in three patients, chronic myelogenous leukaemia in three patients, myelodysplastic syndrome in two patients and pancreatic cancer in four patients. Five patients received a graft from an HLA-matched relative and seven received a graft from an alternative donor defined as an HLA-mismatched relative or a matched unrelated donor. We calculated CL_{CR} weekly, based on a 24 h urine collection. Patients were classified into two groups according to CL_{CR} evaluated within 1 week before the initiation of ganciclovir administration: Group A included seven patients with $CL_{CR} \geq 70$ mL/min (mean 98.1 mL/min, range 74.9–142.0 mL/min) and Group B included five patients

*Corresponding author. Tel: +81-3-3815-5411 ext. 35602; Fax: +81-3-5804-6261; E-mail: ycanda-tky@umin.ac.jp

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Table 1. Pharmacokinetic parameters of ganciclovir in Groups A and B

	Group A (CL_{CR} ≥ 70 mL/min)	Group B (CL_{CR} 50–70 mL/min)	*P* value
C0.5	6.56 (4.39–11.33) μg/mL	4.92 (2.90–10.80) μg/mL	0.37
C1	9.20 (5.50–19.03) μg/mL	4.75 (3.32–6.61) μg/mL	<0.01
C2	4.76 (2.72–12.09) μg/mL	2.38 (2.30–2.73) μg/mL	<0.01
C4	2.58 (1.25–6.30) μg/mL	1.57 (1.37–1.80) μg/mL	0.17
C6	1.69 (0.79–4.89) μg/mL	1.15 (0.90–1.30) μg/mL	0.29
C8	1.22 (0.40–3.99) μg/mL	0.91 (0.64–1.09) μg/mL	0.57
C12	0.62 (0.23–2.88) μg/mL	0.58 (0.39–0.81) μg/mL	0.94
LogC4/C1	–0.66 (–0.73– –0.48)	–0.42 (–0.68– –0.33)	0.09
AUC	29.8 (20.2–111.0) μg·h/mL	24.6 (22.5–28.3) μg·h/mL	0.57
$t_{1/2}$	3.57 (3.36–7.94) h	5.76 (5.05–8.87) h	0.03
CL_{TOT}	3.04 (0.73–4.31) mL/min/kg	1.66 (1.50–1.81) mL/min/kg	0.12

CL_{CR}, creatinine clearance; AUC, area under the concentration curve; $t_{1/2}$, elimination half-life; CL_{TOT}, total body clearance.
C0.5–C12 represent plasma ganciclovir concentrations at 30 min, and 1, 2, 4, 6, 8 and 12 h after start of infusion, respectively.
The values of each parameter are reported as the median and range.

with CL_{CR} between 50 and 70 mL/min (mean 59.1 mL/min, range 51.3–67.4 mL/min).

Antigenaemia assay for CMV infection was performed weekly after engraftment as described previously.[5] Ganciclovir was pre-emptively started when 20 or more positive cells were detected per two slides in patients who received a graft from an HLA-matched relative, whereas it was started when three or more positive cells were detected per two slides in patients who received a graft from an alternative donor. The starting dose of ganciclovir was once daily at 5 and 2.5 mg/kg/day in Groups A and B, respectively, which was infused at a constant rate over 1 h.[6] Venous blood samples were obtained before infusion (C0), 30 min (C0.5) and 1 (C1), 2 (C2), 4 (C4), 6 (C6), 8 (C8) and 12 (C12) h after starting the first-dose infusion. After the blood sample was centrifuged, the plasma was separated and stored at –20°C until measurement of the ganciclovir concentration.

The plasma ganciclovir concentration was measured after solid-phase extraction (SPE) and dilution in mobile phase by reversed-phase HPLC. In brief, plasma samples were heated at 58°C for 30 min to inactivate the virus prior to handling. These samples were then diluted with 0.1 M phosphate buffer (pH 8.0) and applied to disposable C_{18} SPE columns (Bond Elut C18-OH; Varian, Palo Alto, CA, USA) conditioned with methanol and water. The column was washed with 0.1 M phosphate buffer (pH 8.0) and water, and ganciclovir was then eluted by 1.5 mL of 15% methanol. After 0.1 mL of 10 μg/mL guanosine was added as an internal standard, the eluent was injected into the HPLC system (C_{18} column, CAPELL PAK C18 SG 120; Shiseido, Tokyo, Japan; mobile phase: a mixture of 20 mM KH_2PO_4 (pH 2.6) containing 5 mM sodium 1-octanesulfonate and acetonitrile (95 : 5, v/v)]. The flow rate of the mobile phase and the column temperature were 0.8 mL/min and 40°C, respectively. The HPLC was equipped with a photo diode array detector (SPD-M10A vp, Shimadzu, Kyoto, Japan) set at a detection wavelength of 254 nm. This quantitative assay provided a high selectivity for determining a compound in biological samples. It was available for 0.02–5 μg/mL of an analyte in plasma samples. The precision expressed as a coefficient of variation was less than 2.5%, and the accuracy expressed as an error per cent was <±3%. Endogenous sources of interference were not detected from blank plasma.

Pharmacokinetic parameters were calculated by non-compartment modelling using WinNonlin software (version 4.0; Pharsight Corporation). CL_{CR} was normalized to 1.73 m^2 body surface area and AUC was calculated using the linear trapezoidal rules with extrapolation to infinity by standard techniques. The decline ratio was calculated as Log C4/C1 for the evaluation of the decrease in plasma ganciclovir concentration in the distribution phase and early elimination phase, whereas the elimination half-life was calculated from the terminal portion of the slope after C4. The differences between groups were compared using the Wilcoxon (Mann–Whitney)-test. *P* values of less than 0.05 were considered statistically significant. The relationship between the total AUC and plasma ganciclovir concentration at each point after starting infusion was investigated by calculating correlation coefficients r^2 using linear regression analysis after logarithmic transformation because they did not fit a normal distribution.

Results

The median pharmacokinetic parameters and the concentration versus time profile are shown in Table 1 and Figure 1(a). The peak plasma concentration (C_{max}) ranged from 3.32 to 19.03 μg/mL. The C_{max} in Group A was significantly higher than that in Group B (9.20 versus 4.75 μg/mL, $P < 0.01$). There was a borderline significance in the decline ratio between the two groups (–0.66 versus –0.42, $P = 0.09$). Total body clearance in Group B was lower than that in Group A (1.66 versus 3.04 mL/min/kg, $P = 0.12$). Also, the elimination half-life in Group B was significantly longer than that in Group A (5.76 versus 3.57 h, $P = 0.03$). There was no significant difference in AUC between the two groups (29.8 versus 24.6 μg·h/mL, $P = 0.57$). The AUCs of the patients in both groups were distributed within a narrow range (25.6 ± 4.77 μg·h/mL, Figure 1b), when we excluded two patients with exceptionally high AUC values (48.18 and 110.99 μg·h/mL). The CL_{CR} values of these two patients were 74.9 and 87.2 mL/min, respectively. Among the serial ganciclovir concentration measurements, C4 most strongly correlated with AUC ($r^2 = 0.95$, Figure 1c).

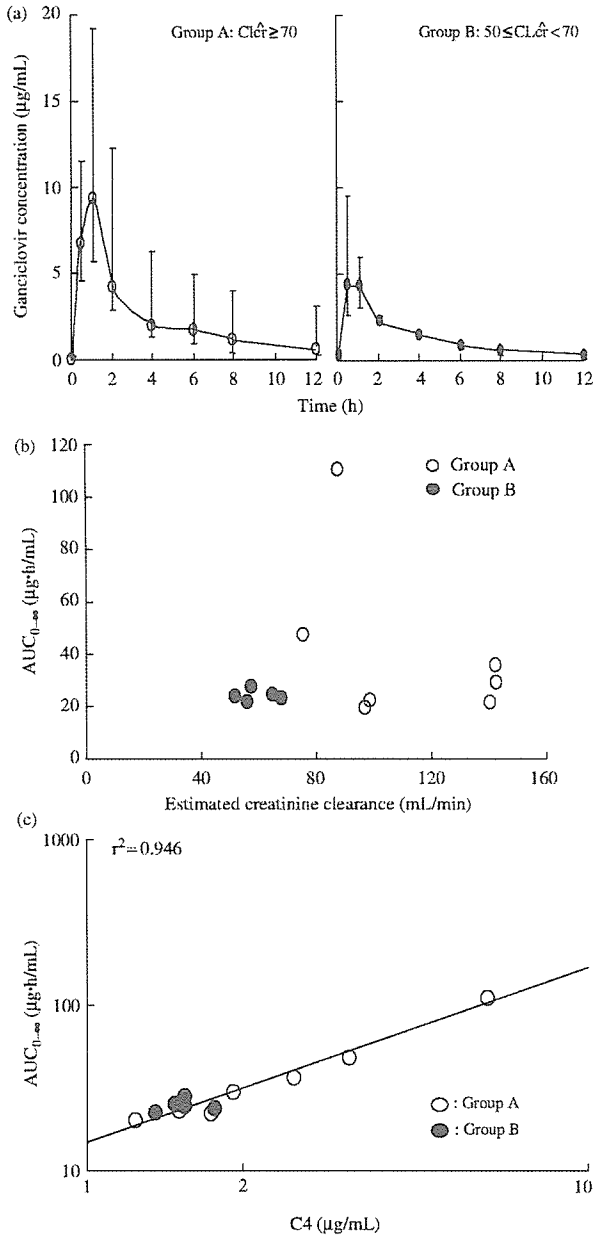

Figure 1. (a) Median concentrations of ganciclovir after 1 h iv infusion of 5 mg/kg ganciclovir in Group A and of 2.5 mg/kg ganciclovir in Group B. Open and filled circles represent each median concentration point in Groups A and B, respectively. (b) The AUC in each patient. Open and filled circles represent individual measurements in Groups A and B, respectively. (c) Correlation between the AUC and the plasma concentration at 4 h after starting infusion (C4). Open and filled circles represent individual measurements in Groups A and B, respectively. The solid line represents the orthogonal regression line described by the equation AUC = 17.666 × C4 – 4.4555.

Discussion

The results demonstrated that a 50% reduction in the ganciclovir dosage was appropriate for HSCT recipients with mild renal impairment of CL_{CR} between 50 and 70 mL/min. In addition to the significant difference in the elimination half-life, we observed a difference in the decline ratio (Log C4/C1) between the two groups with a borderline significance, which might indicate that renal excretion had started within 4 h of infusion. AUC was not significantly different from that in patients with normal renal function, probably due to the prolonged elimination in patients with mild renal impairment, although the small sample size might be responsible for the lack of significant difference. When we excluded two patients whose AUC values were exceptionally high, the AUC ranged within 25.6 ± 4.77 μg·h/mL, which was similar to the values reported previously.[4] An exceptionally high AUC was observed in two patients with CL_{CR} values between 70 and 90 mL/min. The reason for the high AUC is not clear, but it may suggest that the dose of ganciclovir should be reduced in patients with CL_{CR} values between 70 and 90 mL/min after confirming that the AUC is significantly high in such patients. Drug interaction is also a possible explanation for the high AUC, but these two patients were not being given drugs that are known to interact with ganciclovir. Also, the exceptionally high AUC might result from a transient renal dysfunction, which could not be detected even by a weekly CL_{CR} examination.

The role of clinical pharmacokinetic monitoring in solid organ transplantation as well as in HSCT is unclear.[7] Previous studies failed to show a significant correlation between the ganciclovir concentration and its efficacy or toxicity.[7,8] A possible explanation for this lack of correlation is the small number of patients in these studies, since a significant correlation between the cumulative dose of ganciclovir and the incidence of neutropenia has been shown in large-scale clinical studies.[9,10] However, it is difficult to perform a large-scale study with pharmacokinetic monitoring because of the need for repeated blood sampling from patients. In this study, C4 most strongly correlated with AUC, with r^2 values of 0.95, although we should confirm this in a larger study. Another limitation of pharmacokinetic monitoring of ganciclovir is that only the intracellular phosphorylated ganciclovir is active and it is not known how its concentration relates to the plasma concentrations. Nevertheless, a prospective study with monitoring of C4 is warranted to evaluate the role of pharmacokinetic monitoring in HSCT.

In conclusion, a recommended reduction of ganciclovir dosage by 50% appeared to be appropriate for HSCT recipients with mild renal impairment. Measurement of the plasma ganciclovir concentration C4 could be an accurate predictor of AUC. Further studies are necessary to validate these findings in a larger number of patients and to clarify the relationship among plasma concentrations, AUC and responses.

Transparency declarations

None to declare.

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American Journal of Hematology 81:875–879 (2006)

Pharmacokinetics of Alemtuzumab after Haploidentical HLA-Mismatched Hematopoietic Stem Cell Transplantation Using in Vivo Alemtuzumab With or Without CD52-Positive Malignancies

Kumi Oshima, Yoshinobu Kanda,* Fumio Nakahara, Eriko Shoda, Takahiro Suzuki, Yoichi Imai, Takuro Watanabe, Takashi Asai, Koji Izutsu, Seishi Ogawa, Toru Motokura, Shigeru Chiba, and Mineo Kurokawa

Departments of Hematology & Oncology, Cell Therapy & Transplantation Medicine, University of Tokyo Graduate School of Medicine and Hospital, Tokyo, Japan

We recently reported that the addition of in vivo alemtuzumab to the conditioning regimen enables 2- or 3-locus-mismatched hematopoietic stem cell transplantation without an excessive risk of graft rejection or graft-versus-host disease. In a later series of patients, however, one patient with refractory chronic lymphocytic leukemia with large residual tumors at transplantation developed graft rejection. While the peak alemtuzumab concentration in the previous patients without graft rejection was higher than 5 μg/ml, the peak alemtuzumab concentration in this patient was only 1.44 μg/ml. We considered that alemtuzumab was bound to the large residual tumors, which resulted in a low blood concentration of alemtuzumab. Therefore, it is important to debulk tumors before the conditioning regimen for patients with refractory CD52-positive hematological malignancies, or the dose of alemtuzumab should be adjusted by monitoring the blood concentration, when alemtuzumab is used for in vivo T-cell depletion in 2- or 3-locus-mismatched transplantation. Am. J. Hematol. 81:875–879, 2006. © 2006 Wiley-Liss, Inc.

Key words: chronic lymphocytic leukemia; hematopoietic stem cell transplantation; alemtuzumab; serum concentration; rejection

INTRODUCTION

Alemtuzumab (Campath-1H) is a humanized monoclonal antibody directed against human CD52 that is expressed at a high density on B- and T-cells and dendritic cells, but not on hematopoietic stem cells [1]. Although alemtuzumab was approved for the treatment of fludarabine-refractory chronic lymphocytic leukemia (CLL) [2], it has also been used for in vivo T-cell depletion to prevent graft rejection and graft-versus-host disease (GVHD) in allogeneic hematopoietic stem cell transplantation (HSCT) [3,4]. The addition of alemtuzumab to a conditioning regimen decreases graft rejection by depleting host T-cells. In addition, it has a long terminal half-life (15–21 days) and the blood concentration is maintained at a lympholytic level for about 2 months after transplantation, which contributes to the prevention of GVHD [5]. We extended the use of in vivo alemtuzumab to 2- or 3-locus-mismatched transplantation and successfully reduced the incidence of grade III-IV acute GHVD to only 9% without graft rejection in the first 12 patients in a prospective study approved by the ethics commitee [6]. However, in a later series of patients, a patient with fludarabine-refractory CLL with large residual tumors at transplantation developed graft rejection after an initial neutrophil recovery. We describe here the clinical course and discuss the pharmacokinetics of alemtuzumab in patients with or without CD52-positive hematological malignancies.

*Correspondence to: Yoshinobu Kanda, Department of Cell Therapy & Transplantation Medicine, The University of Tokyo Hospital, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan.
E-mail: ycanda-tky@umin.ac.jp

Received for publication 3 October 2005; Accepted 18 March 2006

Published online 21 July 2006 in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/ajh.20694

Clinical Course of a Patient Who Developed Graft Rejection

A 56-year-old woman with CLL, which was refractory to 10 courses of fludarabine (30 mg/m^2 × 5 days), 2 courses of rituximab (375 mg/m^2), and 4 courses of CVP therapy (cyclophosphamide 750 mg/m^2 × 1 day, vincristine 1.4 mg/m^2 × 1 day, prednisolone 60 mg/m^2 × 5 days), chose to participate in a clinical study of 2- or 3-locus-mismatched HSCT using in vivo alemtuzumab, since she did not have an available HLA-matched or 1-locus-mismatched donor among her family members and her disease status precluded a time-consuming donor coordination to identify an HLA-matched unrelated donor. Just before starting the conditioning regimen, she still had large residual tumors in the abdomen, although the peripheral blood lymphocyte count was decreased to 2.66 × 10^9/L. The conditioning regimen consisted of alemtuzumab (0.2 mg/kg/day from day −8 to −3), fludarabine (30 mg/m^2 from day −8 to −3), busulfan (4 mg/kg/day on days −5 and −4) and total body irradiation (TBI; 2 Gy twice daily on day −1). Peripheral blood mononuclear cells were collected from her 3-locus-mismatched son following a mobilization with filgrastim, cryopreserved without ex vivo manipulation, and infused on day 0. The number of infused CD34- and CD3-positive cells was 4.75 × 10^6 cells/kg and 0.86 × 10^8 cells/kg of recipient body weight, respectively. Posttransplantation prophylaxis against GVHD was performed with the continuous infusion of cyclosporine A (3 mg/kg) and short-term methotrexate (15 mg/m^2 on day 1 and 10 mg/m^2 on days 3, 6, and 11). Regimen-related toxicities were mild. Neutrophil engraftment, defined as the first of 3 consecutive days with an absolute neutrophil count of at least 0.5 × 10^9/L, was documented on day 15. On day 18, however, the granulocytic count began to rapidly decrease, associated with a high fever up to 104°F, disseminated intravascular coagulation, and a high lactate dehydrogenase level. We restarted filgrastim but the neutrophil count decreased to below 0.10 × 10^9/L. Eighty-two percent of the bone marrow cells were of donor origin on day 22, but donor cells became undetectable in both the bone marrow and peripheral blood on day 28. The abdominal CT scan on day 22 showed decreased but residual tumors. Flow cytometry analysis of the peripheral blood on day 26 showed that more than 90% of lymphocytes were CD8-positive T-cells. Although we waited for autologous hematopoietic recovery, the neutrophil count remained below 0.10 × 10^9/L. Therefore, we performed the second peripheral blood stem cell transplantation from the same donor on day 51 after the first transplantation following a conditioning regimen consisting of alemtuzumab (0.2 mg/kg/day from day −10 to −5), cyclophosphamide (30 mg/kg/day on days −7 and −6), and fludarabine (25 mg/m^2/day from day −5 to −1). The number of CD34- and CD3-positive cells infused at the second transplantation was 2.86 × 10^6 cells/kg and 0.53 × 10^8 cells/kg of recipient body weight, respectively. We started the continuous infusion of CsA on day −1. However, she developed acute renal failure and thus we replaced CsA with prednisolone at 1 mg/kg/day from day 2. At the same time, high fever, skin rash, fluid retention, weight gain, and noncardiogenic pulmonary edema rapidly progressed, which required mechanical ventilation from day 2 and continuous hemodiafiltration from day 9. Twice, we administered high-dose methyl-prednisolone at 1000 mg/day for 3 days (from days 9 and 15). Despite these treatments, capillary leak syndrome did not improve and she died on day 18 due to severe hypotension, although donor cell engraftment was confirmed on day 11. The clinical course of the CLL patient is summarized in Figure 1. Autopsy revealed no residual CLL cells and no microbiologically documented infections. Pathological finding of the skin showed the degeneration of epidermal cells and sweat gland cells with little lymphocyte infiltration, which were compatible with acute GVHD.

Blood Concentration of Alemtuzumab

The serum concentration of alemtuzumab was determined by indirect immunofluorescence using frozen sera as described in detail elsewhere [7,8]. The serial serum concentrations of alemtuzumab of the present patient in the first and second transplantations and those of three control patients who underwent haploidentical HSCT using alemtuzumab from a 3-locus-mismatched related donor and whose serum samples before and after transplantation were available are shown in Figure 2. The current patient and the three control patients participated in the same study to evaluate the safety of unmanipulated peripheral blood stem cell transplantation from 2- or 3-locus-mismatched related donors using alemtuzumab in vivo and received exactly the same alemtuzumab dosage schedule (0.2 mg/kg/day from day −8 to −3) and the same supportive care [6]. Of the three control patients, two (C1 and C2) had myeloid malignancies and the other (C3) had diffuse large B-cell lymphoma in partial remission. Patients C2 and C3 received the same conditioning regimen as the CLL patient did, whereas patient C1 received TBI at 2 Gy twice daily on days −7, −6, and −5, followed by cyclophosphamide at 60 mg/kg on days

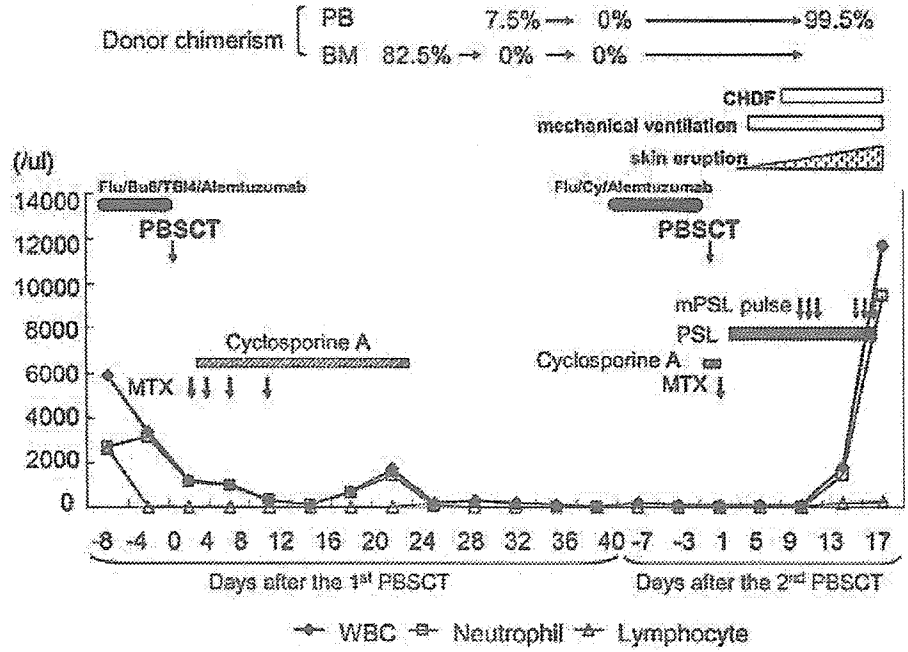

Fig. 1. Clinical course of the first and second haploidentical transplantation using in vivo alemtuzumab. PB, peripheral blood; BM, bone marrow; CHDF, continuous hemodiafiltration; Flu, fludarabine; Bu, busulfan; TBI, total body irradiation; Cy, cyclophosphamide; PBSCT, peripheral blood stem cell transplantation; MTX, methotrexate; PSL, prednisolone; WBC, white blood cell.

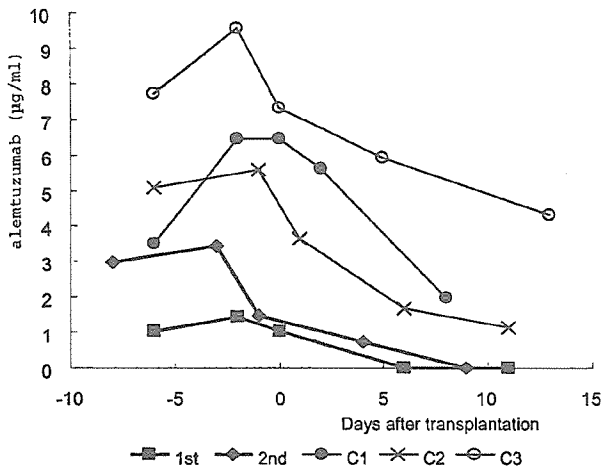

Fig. 2. Serial serum concentrations of alemtuzumab. Alemtuzumab activity was measured by indirect immunofluorescence. The lower limit of this assay for serum samples is 0.50 μg/ml and the upper limit is 20.00 μg/mL.

−3 and −2. Although the serum alemtuzumab concentrations in the three control patients were comparable to those in previous studies where alemtuzumab was used in a conditioning regimen [9,10], the serum alemtuzumab concentration in the CLL patient was persistently lower than 2.0 and 4.0 μg/ml in the first and second transplantations, respectively, and it quickly decreased to an undetectable level (<0.5 μg/ml) after transplantation. Therefore, the serum concentration of alemtuzumab before transplantation was too low to suppress host T-cells, which may have resulted in graft rejection after the first transplantation. In the second transplantation, host T-cells might have been sufficiently suppressed by the repeated conditioning regimen, but a strong reaction compatible with hyperacute GVHD occurred a few days after the infusion of donor graft, probably due to the insufficient alemtuzumab concentration on day 0 and thereafter.

DISCUSSION

The use of in vivo alemtuzumab in an HSCT setting enables durable engraftment and a significant reduction of GVHD, even in 2- or 3-locus-mismatched HSCT [6,10]. Pharmacokinetic studies of alemtuzumab at a dose of 20 mg/day for 5 days before transplantation with a reduced-intensity conditioning regimen have demonstrated that the serum alemtuzumab concentration was higher than the level that was required to kill infused donor T-cells at the time of transplantation and remained at

TABLE I. The Incidence of Graft Failure after Allogeneic Transplantation Using Fludarabine, Melphalan, and Alemtuzumab

Underlying disease	*n*	Donor R/U	Dose of alemtuzumab	Primary graft failure	Secondary graft failure
CLL	41	24/17	100 mg in 27 60 mg in 6 50 mg in 7 40 mg in 1	3	5
AML/MDS	76	35/41	100 mg	2	0
MM	25	0/25	100 mg	0	0
NHL	88	65/23	100 mg	1	3
HL	49	31/18	100 mg	0	0

Note. CLL, chronic lymphocytic leukemia; AML, acute myeloblastic leukemia; MDS, myelodysplastic syndrome; MM, multiple myeloma; NHL, non-Hodgkin's lymphoma; HL, Hodgkin's lymphoma; R, related donor; U, unrelated donor.

a potentially lympholytic level for approximately 2 months after transplantation [5].

The pharmacokinetics of alemtuzumab in the treatment of CLL are quite different. The peak blood concentration after the first infusion of alemtuzumab at 30 mg for refractory CLL patients showed a wide variation among patients [7]. In addition, the blood concentrations of alemtuzumab showed a modest negative correlation with the starting lymphocyte counts [7]. These results suggest that the pharmacokinetics of alemtuzumab in CLL patients were affected by the number of tumor cells. The alemtuzumab concentration tends to be lower when the patient has bulky tumor cells, since alemtuzumab may bind to tumor cells.

In a transplantation setting, only a cumulative dose of 100 mg or less, which is far lower than that in the treatment of CLL, is highly immunosuppressive and clinically effective for the prevention of graft rejection and GVHD [11]. However, the cumulative dose of 1.2 mg/kg (66 mg/body) might have been insufficient to prevent graft rejection in this patient with bulky CD52-positive residual tumor cells. On the other hand, previous transplantation studies have not pointed out any differences in the pharmacokinetics of alemtuzumab between patients with CD52-positive lymphoid malignancies and those with myeloid malignancies [5]. However, Delgado et al. recently reported the results of 41 consecutive allogeneic hematopoietic cell transplantation for CLL using fludarabine, melphalan, and alemtuzumab [12]. They showed a higher incidence of primary or secondary graft failure (8 of 41) than that in transplantation for the other hematological malignancies using the same regimen (Table I) [13–16]. The alemtuzumab concentrations in these patients were assumed to be lower than those in transplantation for the other hematological malignancies, because they had CLL and/or the dose of alemtuzumab was reduced in 14 of the 41 patients. These data further support our hypothesis that the low alemtuzumab concentration might have resulted in graft rejection in the current CLL patient.

In conclusion, a residual CD52-positive tumor may strongly affect the blood concentration of alemtuzumab. It may be worthwhile to decrease tumor cells before the conditioning regimen or to increase the dose of alemtuzumab in a conditioning to prevent graft rejection and GVHD. More data on the pharmacokinetics of alemtuzumab are needed to determine an optimal dose for HSCT, especially from a 2- or 3-locus-mismatched donor.

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American Journal of Hematology 81:838–844 (2006)

Effect of Blood Cyclosporine Concentration on the Outcome of Hematopoietic Stem Cell Transplantation From an HLA-Matched Sibling Donor

Yoshinobu Kanda,[1]* Rie Hyo,[2] Takuya Yamashita,[3] Katsumichi Fujimaki,[4] Kumi Oshima,[1] Masahiro Onoda,[5] Takehiko Mori,[6] Toru Sakura,[7] Masatsugu Tanaka,[2] Miwa Sakai,[3] Jun Taguchi,[2] Mineo Kurakawa,[1] Atsuo Maruta,[2] Shinichiro Okamoto,[6] and Hisashi Sakamaki,[3] for the Kanto Study Group of Cell Therapy

[1] Departments of Hematology and Oncology, Cell Therapy and Transplantation Medicine, University of Tokyo, Graduate School of Medicine and Hospital, Tokyo, Japan
[2] Department of Hematology, Kanagawa Cancer Center, Kanagawa, Japan
[3] Tokyo Metropolitan Komagome Hospital, Tokyo, Japan
[4] Department of Internal Medicine and Clinical Immunology, Yokohama City University, Graduate School of Medicine, Kanagawa, Japan
[5] Chiba Aoba Municipal Hospital, Chiba, Japan
[6] Division of Hematology, Keio University School of Medicine, Tokyo, Japan
[7] Saiseikai Maebashi Hospital, Gumma, Japan

We retrospectively evaluated the effect of the blood cyclosporine (CsA) concentration on the outcome of allogeneic hematopoietic stem cell transplantation from an HLA-matched sibling donor in 171 patients who received a continuous infusion of CsA and short-course methotrexate to prevent graft-versus-host disease (GVHD). CsA was started at 3.0 mg/kg/day and the dose was adjusted to maintain the blood CsA concentration between 250 and 350 ng/ml. The actual dose of CsA averaged 1.9 mg/kg/day at the 3rd week after transplantation. The incidence of grade II–IV acute GVHD was 29.9%. Patient age and sex were identified as independent significant risk factors for acute GVHD. The CsA concentration during the 3rd week after transplantation most strongly affected the incidence of grade II–IV acute GVHD (RR 0.995 for an increase in CsA concentration by 1 ng/ml, $P = 0.037$) adjusted for other risk factors. The incidence of acute GVHD was significantly lower in patients with a 3rd-week CsA concentration higher than 300 ng/ml than in those with values between 200 and 300 ng/ml (20% vs. 35%, $P = 0.036$). We concluded that the blood CsA concentration at peri-engraftment period may be important in preventing acute GVHD. Am. J. Hematol. 81:838–844, 2006. © 2006 Wiley-Liss, Inc.

Key words: hematopoietic stem cell transplantation; cyclosporine; graft-versus-host disease; continuous infusion; blood concentration

INTRODUCTION

The combination of cyclosporine (CsA) and methotrexate (MTX) is widely used for the pharmacologic prevention of graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation. However, the dose, target blood level, and schedule of administration vary among protocols [1]. Previously, we retrospectively compared two methods of CsA infusion: continuous infusion versus twice-daily infusions early after transplantation [2]. The incidence of grade II–IV acute GVHD was significantly higher in patients who received a continuous infusion of CsA, although these patients showed a significantly lower incidence of renal dysfunction. The actual dose of CsA in the first 4 weeks after

*Correspondence to: Yoshinobu Kanda, MD, Department of Cell Therapy and Transplantation Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan.
E-mail: ycanda-tky@umin.ac.jp

Received for publication 30 September 2005; Accepted 22 April 2006

Published online 3 August 2006 in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/ajh.20710

transplantation, which was adjusted to maintain the blood CsA concentration between 250 and 400 ng/ml, was significantly lower in patients who received a continuous infusion of CsA. Therefore, this target concentration might be inappropriate. In this study, we retrospectively evaluated the effect of the blood CsA concentration on the incidence of acute GVHD and the other transplantation outcome in patients who received a continuous infusion of CsA and short-course MTX.

PATIENTS AND METHODS

Patients

We retrospectively analyzed the records of 171 adult patients who underwent allogeneic hematopoietic stem cell transplantation from an HLA-matched sibling donor using a GVHD prophylaxis regimen consisting of CsA and MTX for acute leukemia, chronic myelogenous leukemia, or myelodysplastic syndrome at seven centers that participated in the Kanto Study Group for Cell Therapy (KSGCT) between 1995 and 2002.

Transplantation Procedure

The conditioning regimen was mainly a combination of cyclophosphamide (120 mg/kg) with either busulfan (16 mg/kg/day) or fractionated total body irradiation (12 Gy). CsA was started at 3 mg/kg/day as a continuous infusion on day 1. The whole blood CsA concentration was measured at least once a week by fluorescence polarization immunoassay with a specific monoclonal antibody [3]. The sensitivity of this assay was 25 ng/ml. The dose of CsA was adjusted to maintain the blood CsA concentration between 250 and 350 ng/ml. The route of CsA administration was converted to oral at a ratio of 1:2 or 1:3 when patients were able to tolerate oral intake at least 3 weeks after transplantation. MTX was administered intravenously at 10–15 mg/m^2/day on day 1 and 7–10 mg/m^2/day on days 3 and 6 at the discretion of each center. Bone marrow or peripheral blood stem cells were harvested from an HLA-matched sibling and administered without ex vivo manipulation.

Statistical Considerations

Standard-risk disease was defined as acute leukemia in first or second complete remission, chronic myelogenous leukemia in first or second chronic phase, and myelodysplastic syndrome without leukemic transformation, while others were considered high-risk diseases [4]. The primary endpoint was the incidence of grade II–IV acute GVHD, which was evaluated based on clinical and pathological findings among patients who achieved engraftment [5]. Disease-free survival (DFS) and the cumulative incidences of acute GVHD, relapse, and nonrelapse mortality were the secondary endpoints. DFS and the cumulative incidence of acute GVHD were calculated using the Kaplan-Meier method. The incidences of relapse and nonrelapse mortality were calculated using Gray's method considering each other event as a competing risk [6]. Potential confounding factors considered in the analysis were age, sex, performance status, disease risk, stem cell source, conditioning regimen, and prophylactic use of fluconazole and granulocyte-colony stimulating factor (G-CSF). Factors that showed at least borderline significance ($P < 0.15$) in univariate analyses were included in the multivariate analyses and then stepwisely deleted from the model. The blood CsA concentration was added in the final model. Finally, factors with P values less than 0.05 were considered significant. There were missing data in several factors as shown in Table I. Although the number of patients with missing data was small, it might have affected the results of statistical analyses.

TABLE I. Characteristics of the Patients

Donor	
Median age	39 (range 9–65)
Sex	Male 87/female 82 (N.D. 2)
Patient	
Median age	40 (range 14–58)
Sex	Male 102/female 69
Performance status	0/1/2/3 113/32/7/0 (N.D. 19)
Underlying disease	AML 66, AUL 2, ALL 30, CML 50, MDS 23
Disease risk	Standard 111, high 53 (N.D. 7)
Transplantation procedure	
Total body irradiation	Yes 109/No 62
Stem cell	Bone marrow 101/peripheral blood 70
Fluconazole prophylaxis	Yes 121/No 50
G-CSF	Yes 124/No 38 (N.D. 9)

N.D., not described.

RESULTS

Patients' Characteristics

The characteristics of the 171 patients and their donors are summarized in Table I. The underlying disease was acute myeloblastic leukemia in 66, acute unclassified leukemia in 2, acute lymphoblastic leukemia in 30, chronic myelogenous leukemia in 50, and myelodysplastic syndrome in 23. Bone marrow

was used in 101 patients, whereas 70 patients received peripheral blood stem cell graft. Fluconazole and G-CSF were prophylactically administered in 121 and 124 patients, respectively.

Risk Factors for Grade II–IV Acute GVHD

Engraftment was observed in 166 patients a median of 16 days (range 7–47 days) after transplantation. The incidence of grade II–IV acute GVHD was 29.9%, with a median onset of 21 days (range 3–37 days) after transplantation (see Fig. 1). The incidence of grade III–IV acute GVHD was 9.8%. In univariate analyses to evaluate the impact of potential confounding factors on the incidence of grade II–IV acute GVHD, patient age was identified as the only significant risk factor (Table II, A). Patient sex, stem cell source, and the total dose of MTX were borderline significant. All of these factors were included in the multivariate analysis using the backward stepwise selection and only two factors, patient age (relative risk [RR] 1.04 for an increase in age by 1 year, 95% confidence interval [CI] 1.00–1.07, $P = 0.01$) and patient sex (RR 0.54, 95% CI 0.29–1.00, $P = 0.05$), were identified as independent significant risk factors (Table II, B).

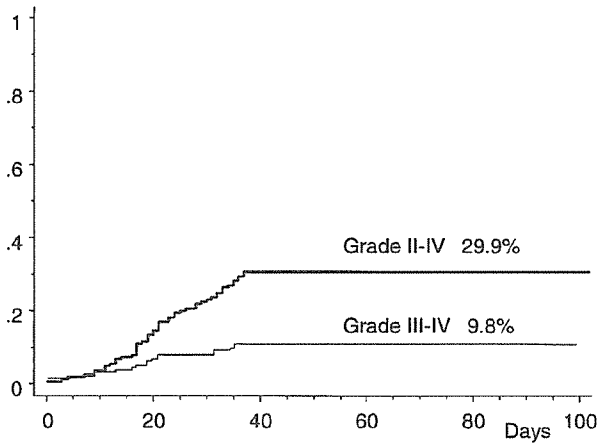

Fig. 1. The incidence of grade II–IV and grade III–IV acute GVHD.

Effect of CsA Concentration on the Incidence of Grade II–IV Acute GVHD

The dose of CsA was adjusted to maintain the blood CsA concentration between 250 and 350 ng/ml. This adjustment was successful and the mean CsA concentration was 375 ± 124, 310 ± 79, and 326 ± 130 ng/ml at the 1st, 2nd and 3rd week after transplantation, respectively (Fig. 2A). However, the actual dose of CsA was decreased every week and the mean dose of CsA was 2.5 ± 0.6, 2.0 ± 0.7, and 1.9 ± 0.7 mg/kg at the 1st, 2nd, and 3rd week after transplantation, respectively (Fig. 2B). In three of the four patients who developed early renal toxicity, CsA was stopped or the dose of CsA was decreased, but the mean dose of CsA was the same (1.80 ± 0.6 mg/kg) when we excluded patients who developed renal dysfunction within 21 days after

TABLE II. Univariate (A) and Multivariate (B) Analyses for the Incidence of Grade II–IV Acute GVHD

	Incidence (%)	Relative risk (95% CI)	*P* value
A. Univariate analysis			
Dichotomous variables			
Patient sex (male vs. female)	34 vs. 23		0.11
Patient PS (0 vs. 1–4)	30 vs. 30		0.89
Donor sex (male vs. female)	32 vs. 29		0.88
Sex mismatch (yes vs. no)	33 vs. 28		0.36
Disease risk (standard vs. high)	31 vs. 30		0.76
Total body irradiation (yes vs. no)	28 vs. 34		0.37
G-CSF (yes vs. no)	28 vs. 40		0.18
Stem cell (BM vs. PB)	35 vs. 23		0.12
Continuous variables			
Patient age		1.03 (1.00–1.06)	0.02
MTX dose		1.04 (0.99–1.09)	0.15
B. Multivariate analysis			
Patient age		1.04 (1.00–1.07)	0.01
Patient sex		0.54 (0.29–1.00)	0.05
C. Effect of cyclosporine concentration			
Cyclosporine concentration		0.995 (0.991–0.999)	0.023
1st week concentration		0.999 (0.996–1.001)	0.34
2nd week concentration		0.998 (0.994–1.002)	0.26
3rd week concentration		0.995 (0.991–1.000)	0.037

The effect of blood cyclosporine concentration was adjusted for independent significant risk factors (C).

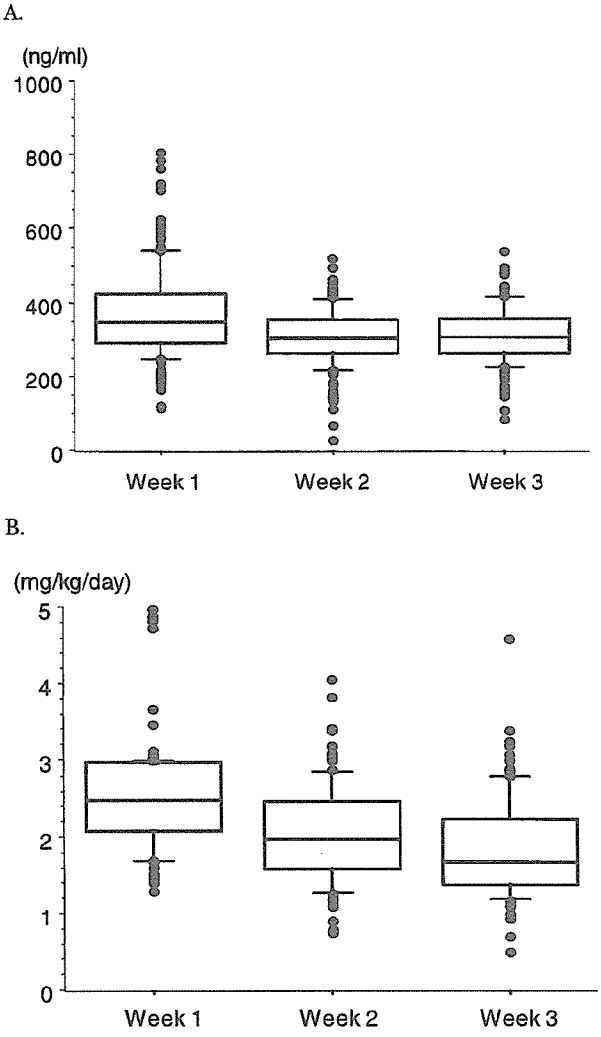

Fig. 2. A: Blood CsA concentration at the 1st, 2nd, and 3rd week after transplantation. B: The actual daily dose of CsA at the 1st, 2nd, and 3rd week after transplantation. The box-and-whisker plot shows 10, 25, 50, 75, and 90 percentile values. Outliers are indicated by dots.

transplantation. The ratio of the blood CsA concentration to the actual dose of CsA was not different between those who were receiving fluconazole and those who were not (194 vs. 189 [ng/ml]/[mg/kg], $P = 0.78$), which suggested that fluconazole did not significantly interact with the CsA concentration.

The mean CsA concentration in each patient within 3 weeks after transplantation significantly affected the incidence of acute GVHD (RR 0.995 for an increase in CsA concentration by 1 ng/ml, 95% CI 0.991–0.999, $P = 0.023$, Table II, C), adjusted for the two independent significant risk factors. Next, we evaluated the effect of the CsA concentration during the 1st, 2nd, and 3rd weeks after transplantation on the incidence of grade II–IV acute GVHD among patients who did not develop GVHD before each period. Only the CsA concentration during the 3rd week after transplantation significantly correlated with the incidence of acute GVHD (RR 0.995, 95% CI 0.991–1.000, $P = 0.037$). As shown in Fig. 3, the incidence of acute GVHD was different among the CsA-concentration groups during the 3rd week after transplantation (44% for patients with CsA concentration lower than 200 ng/ml, 35% for those with CsA concentration between 200 and 300 ng/ml, and 20% for those with CsA concentration higher than 300 ng/ml, $P = 0.035$). When we grouped patients with the 1st week CsA concentration lower than 300 ng/ml and the 3rd week CsA concentration higher than 300 ng/ml as "low–high" group and those with the 1st week CsA concentration higher than 300 ng/ml and the 3rd week CsA concentration lower than 300 ng/ml as "high–low" group, the incidence of grade II–IV acute GVHD was 23, 33, and 30% in the "low–high" group, the "high–low" group, and the others, respectively, although the difference was not statistically significant.

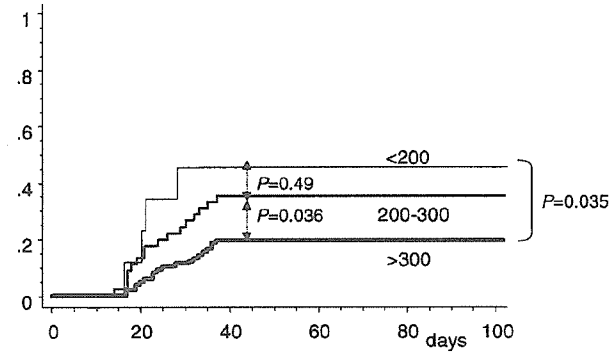

Fig. 3. Incidence of grade II–IV acute GVHD grouped according to the blood CsA concentration (ng/ml) during the 3rd week after transplantation among patients who did not develop GVHD before the period.

Effect of CsA Concentration on Transplantation Outcome

DFS for the entire population was 56% at 3 years after transplantation. Patient age, performance status, and disease risk were identified as independent significant factors for DFS (Table III). We evaluated the effect of the CsA concentration on DFS adjusted for these factors and did not find a significant effect of CsA concentration on DFS (RR 1.00, 95% CI 0.994–1.004, $P = 0.57$). The cumulative incidences of relapse and non-relapse mortality at 3 years were 24 and 20%, respectively. The blood CsA concentration did not significantly affect the incidence of these events after adjusting for other significant risk factors (RR 0.998, 95% CI 0.993–

TABLE III. Univariate (A) and Multivariate (B) Analyses for Disease-Free Survival (DFS)

	3-year DFS	Relative risk (95% CI)	*P* value
A. Univariate analysis			
Dichotomous variables			
Patient sex (male vs. female)	55 vs. 59		0.42
Patient PS (0 vs. 1–4)	61 vs. 41		0.02
Donor sex (male vs. female)	60 vs. 52		0.24
Sex mismatch (yes vs. no)	58 vs. 54		0.43
Disease risk (standard vs. high)	67 vs. 30		<0.0001
Total body irradiation (yes vs. no)	58 vs. 56		0.95
G-CSF (yes vs. no)	64 vs. 54		0.62
Stem cell (BM vs. PB)	59 vs. 53		0.47
Continuous variables			
Patient age		1.02 (1.00–1.04)	0.07
MTX dose		1.00 (0.96–1.04)	0.96
B. Multivariate analysis			
Patient PS (0 vs. 1–4)		0.57 (0.33–0.97)	0.04
Patient age		1.03 (1.00–1.05)	0.03
Disease risk (standard vs. high)		0.41 (0.25–0.68)	0.0005
C. Effect of cyclosporine concentration			
Cyclosporine concentration		1.00 (0.994–1.004)	0.57

The effect of the blood cyclosporine concentration was adjusted for independent significant risk factors (C).

TABLE IV. Univariate (A) and Multivariate (B) Analyses for the Incidence of Relapse

	Incidence	Relative risk (95% CI)	*P* value
A. Univariate analysis			
Dichotomous variables			
Patient sex (male vs. female)	21 vs. 28		0.35
Patient PS (0 vs. 1–4)	19 vs. 35		0.07
Donor sex (male vs. female)	25 vs. 23		0.64
Sex mismatch (yes vs. no)	19 vs. 29		0.28
Disease risk (standard vs. high)	21 vs. 40		0.06
Total body irradiation (yes vs. no)	26 vs. 21		0.40
G-CSF (yes vs. no)	26 vs. 14		0.29
Stem cell (BM vs. PB)	22 vs. 27		0.51
Continuous variables			
Patient age		1.00 (0.97–1.03)	0.87
MTX dose		0.99 (0.94–1.04)	0.71
B. Multivariate analysis			
Disease risk (standard vs. high)		2.31 (1.14–4.66)	0.02
C. Effect of cyclosporine concentration			
Cyclosporine concentration		0.998 (0.993–1.003)	0.41

The effect of the blood cyclosporine concentration was adjusted for independent significant risk factors (C).

1.003, $P = 0.41$ for relapse adjusted for disease risk and RR 0.998, 95% CI 0.991–1.005, $P = 0.57$ for nonrelapse mortality adjusted for patient sex, sex mismatch, and patient age, Tables IV and V).

Renal Toxicity

Renal dysfunction, defined as an elevation of the serum creatinine level above 2.0 × baseline, was observed in 18.9% of the patients, with a median onset of 36 days (range 5–151 days) after transplantation. Among them, only four developed renal dysfunction within the first 3 weeks after transplantation and thus we could not evaluate the effect of the blood CsA concentration on the incidence of renal dysfunction.

DISCUSSION

The results of this study clearly demonstrated the importance of the blood CsA concentration in the

TABLE V. Univariate (A) and Multivariate (B) Analyses for the Incidence of Nonrelapse Mortality (NRM)

	Incidence	Relative risk (95% CI)	*P* value
A. Univariate analysis			
Dichotomous variables			
Patient sex (male vs. female)	24 vs. 13		0.06
Patient PS (0 vs. 1–4)	20 vs. 24		0.55
Donor sex (male vs. female)	15 vs. 25		0.11
Sex mismatch (yes vs. no)	27 vs. 14		0.05
Disease risk (standard vs. high)	18 vs. 30		0.19
Total body irradiation (yes vs. no)	18 vs. 22		0.33
G-CSF (yes vs. no)	20 vs. 22		0.23
Stem cell (BM vs. PB)	19 vs. 21		0.74
Continuous variables			
Patient age		1.04 (1.01–1.08)	0.02
MTX dose		1.01 (0.95–1.07)	0.77
B. Multivariate analysis			
Patient sex (male vs. female)		2.46 (1.08–5.60)	0.03
Sex mismatch (yes vs. no)		2.46 (1.19–5.12)	0.02
Patient age		1.05 (1.01–1.08)	0.01
C. Effect of cyclosporine concentration			
Cyclosporine concentration		0.998 (0.991–1.005)	0.57

The effect of the blood cyclosporine concentration was adjusted for independent significant risk factors (C).

incidence of acute GVHD, especially during the 3rd week after transplantation from an HLA-matched sibling donor. The median time to engraftment was 16 days and thus the blood CsA concentration during the peri-engraftment period may be important for preventing acute GVHD [7]. The incidence of acute GVHD was significantly lower in patients with a 3rd-week CsA concentration higher than 300 ng/ml than in those with values between 200 and 300 ng/ml. Although a target range around 300 ng/ml is widely used as daily practice, it may be worthwhile to explore a higher target range, since the actual dose of CsA was decreased to 1.9 mg/kg/day and only four patients developed renal dysfunction within the first 3 weeks after transplantation. In this regard, we considered that the results of previous studies that compared the continuous infusion of CsA and tacrolimus as GVHD prophylaxis should be interpreted with caution [8–10]. The comparison between CsA at higher-dose and tacrolimus at less-toxic dose is warranted to evaluate their true efficacy to prevent GVHD.

Elevating the target concentration of CsA may result in an increased incidence of relapse. In this study, we did not observe a relationship between the CsA concentration and relapse incidence. However, the CsA concentration was kept at a significantly lower level in high-risk patients than in standard-risk patients (319 ng/ml vs. 346 ng/ml, $P = 0.03$), probably to enhance a graft-versus-leukemia effect, and this might have biased the effect of the CsA concentration on the incidence of relapse. In fact, we previously showed that the incidence of relapse was significantly lower after the continuous infusion of CsA with this low target CsA concentration when compared with twice-daily infusions, which resulted in better DFS in high-risk patients [2]. Therefore, we are currently evaluating the feasibility and efficacy of the continuous infusion of CsA with a target CsA concentration of between 450 and 550 ng/ml only in standard-risk patients. When the dose of CsA is adjusted to this target concentration, we can expect that the actual dose is maintained around 3 mg/kg [11].

In conclusion, the blood CsA concentration significantly affected the incidence of grade II–IV acute GVHD after allogeneic hematopoietic stem cell transplantation from an HLA-matched sibling donor. The CsA concentration during the 3rd week after transplantation appeared to be most important. Therefore, the CsA concentration at peri-engraftment period should be monitored carefully to prevent acute GVHD.

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Graft-versus-Tumor Effect Against Advanced Pancreatic Cancer after Allogeneic Reduced-Intensity Stem Cell Transplantation

Yoshinobu Kanda,[1,5] Yutaka Komatsu,[2] Masaaki Akahane,[3] Shigeyuki Kojima,[4] Yuki Asano-Mori,[1] Minoru Tada,[2] Kumi Oshima,[1] Hiroyuki Isayama,[2] Seishi Ogawa,[1] Toru Motokura,[1] Shigeru Chiba,[1] Kuni Ohtomo,[3] Masao Omata,[2] and Hisamaru Hirai[1]

Background. The prognosis of advanced pancreatic cancer is extremely poor and therefore a novel treatment strategy is desired. The authors thus started a prospective study of allogeneic reduced-intensity hematopoietic stem cell transplantation (RIST) for patients with advanced pancreatic cancer to evaluate the feasibility and efficacy of this approach for such patients.

Methods. Only patients with pathologically proven pancreatic cancer that was locally advanced or metastatic and not amenable to curative resection were included. The conditioning regimen consisted of gemcitabine, fludarabine, and busulfan.

Results. In the first stage of this study, the authors treated seven patients. Treatment-related mortality before day 100 was observed in one patient. The median survival after RIST was 229 days. An objective response on computed tomographic scan was observed in two patients and another had a tumor marker response. Marked tumor shrinkage was observed in one of the remaining patients after donor lymphocyte infusion. These antitumor effects appeared after the effect of the conditioning regimen had disappeared. In addition, some of these responses were associated with an increase in the serum anticarcinoembryonic antigen antibody level.

Conclusions. Pancreatic cancer appeared to be sensitive to a graft-versus-tumor effect; therefore, a larger clinical study with a refined strategy is warranted.

Keywords: Reduced-intensity stem cell transplantation, Minitransplantation, Pancreatic cancer, Graft-versus-tumor effect, Graft-versus-host disease.

(*Transplantation* 2005;79: 821–827)

Pancreatic cancer is the fifth most common cause of cancer-related mortality in Japan and the United States. The median duration of survival in advanced pancreatic cancer is less than 6 months, even when treated with gemcitabine (*1*), and therefore a novel treatment strategy is desired. Allogeneic nonmyeloablative or reduced-intensity hematopoietic stem cell transplantation (RIST) is a recently developed treatment approach for obtaining a graft-versus-tumor (GVT) effect without the toxicity associated with a myeloablative conditioning regimen (*2–6*). This treatment strategy is suitable for patients with solid tumors, because patients with advanced solid tumors are generally clinically infirm, and also a strong antitumor effect cannot be expected with an intensification of chemotherapy. In addition, a GVT effect has been noted in several solid tumors after conventional hematopoietic stem-cell transplantation (*7, 8*). Based on this background, RIST has been investigated for use against solid cancers since the late 1990s and its feasibility has already been demonstrated in several studies (*9–14*). However, there is still little information available regarding its efficacy against individual solid cancers. Childs et al. showed an excellent response rate of 53% after RIST against metastatic renal cell cancer (*9*). A GVT effect against renal cell cancer was confirmed in trials by other centers. In contrast, RIST against metastatic melanoma, which has been considered to be a good candidate for immunotherapy, resulted in a miserable outcome (*15*). Therefore, it is difficult to predict whether a GVT effect can be achieved against an individual tumor. We started a prospective study to evaluate the feasibility and efficacy of RIST against advanced pancreatic cancer after ethical approval in April 2002.

PATIENTS AND METHODS

Patients

Eligible patients were younger than 70 years of age and had pathologically proven pancreatic adenocarcinoma, which was locally advanced or metastatic and not amenable to curative resection. Patients had a human leukocyte antigen (HLA)-matched sibling or a family donor with a single mismatched HLA antigen. Patients with a poor performance status (Eastern Cooperative Oncology Group 2–4) and those with severely impaired organ function were excluded. Patients and donors gave their written informed consent to participate in this study.

Twelve patients with pancreatic cancer fulfilled the inclusion criteria but lacked a suitable donor. They were considered control patients. Four of them had metastatic lesions, whereas eight had locally advanced disease. Nine received

[1] Department of Cell Therapy & Transplantation Medicine, University of Tokyo, Tokyo, Japan.
[2] Department of Gastroenterology, University of Tokyo, Tokyo, Japan.
[3] Department of Radiology, University of Tokyo, Tokyo, Japan.
[4] R & D Center, BML, Inc., Kawagoe, Japan.
[5] Address correspondence to: Yoshinobu Kanda, M.D., Department of Cell Therapy & Transplantation Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. E-mail: ycanda-tky@umin.ac.jp.

Received 28 September 2004. Revision requested 27 October 2004. Accepted 10 November 2004.

ISSN 0041-1337/05/7907-821
DOI: 10.1097/01.TP.0000153507.94980.A5

chemotherapy with gemcitabine, whereas three were observed without chemotherapy.

Transplantation Procedure

Donors received granulocyte colony-stimulating factor at 200 $\mu g/m^2$ administered subcutaneously twice daily starting 3 days before the first collection of peripheral blood stem cells until the end of collection. Leukapheresis was performed daily until more than 2.0×10^6 $CD34^+$ cells/kg of the recipient's body weight were collected. Collected cells were then cryopreserved using standard techniques without ex vivo manipulation.

The conditioning regimen consisted of gemcitabine (1,000 mg/m^2/day as a 90-min infusion on days −16, −9, and −2) (*16*), fludarabine (30 mg/m^2/day as a 30-min infusion on days −8 to −3), and busulfan (4 mg/kg/day administered orally in four divided doses on days −6 and −5) (*4*, *17*). Graft-versus-host disease (GVHD) prophylaxis was performed with cyclosporine (CsA) (3 mg/kg/day as a continuous infusion) and short-term methotrexate (10 mg/m^2 on day 1 and 7 mg/m^2 on days 3 and 6). Frozen peripheral blood stem cells were thawed and infused on day 0. CsA was decreased at 10% per week from day 30 and discontinued by day 100 unless the patient developed acute GVHD. In patients with progressive disease without any evidence of acute GVHD, CsA was rapidly tapered over a 4-week period. Acute GVHD was graded as previously described (*18*). Grade II to IV acute GVHD was treated with methylprednisolone at 1 mg/kg per day, except for grade II GVHD limited to the skin, which was treated with topical steroid ointment.

Prophylaxis against bacterial, fungal, and *Pneumocystis carinii* infection consisted of tosufloxacin, fluconazole, and sulfamethoxazole-trimethoprim. As prophylaxis against herpes simplex virus infection, acyclovir was administered at 500 mg per day intravenously or 1,000 mg per day orally from days −7 to 35, followed by long-term low-dose (200 mg/day) oral administration (*19*). Patients received granulocyte colony-stimulating factor (filgrastim) at 300 μg per day by 3-hr infusion beginning on day 7 until the neutrophil count recovered to 500/mm^3. Cytomegalovirus antigenemia assay using C10/C11 antibody was performed at least once per week after engraftment. Ganciclovir was started when more than two positive cells were detected on two slides (*20*, *21*).

Donor lymphocyte infusion (DLI) was performed for patients who had progressive disease and did not develop GVHD even after CsA was discontinued. The initial $CD3^+$ cell dose was 1 to 3×10^7 cells/kg and the dose was escalated every 4 weeks when the patient did not develop tumor response or GVHD.

Chimerism and Immunologic Analyses

Host-donor cell chimerism after transplantation was analyzed monthly by sex-chromosome fluorescent in situ hybridization or the short tandem repeat method after transplantation (*22*). The serum anti-carcinoembryonic antigen (CEA) antibody level was determined by enzyme-linked immunosorbent assay as previously described (*23*). Briefly, 96-well microplates were coated overnight at 4°C with a 5-μg/mL CEA preparation. The plates were washed and blocked for 2 hr at room temperature with 200 μL/well of a 0.1% Tween20, 5% nonfat dry milk, phosphate-buffered saline solution to prevent nonspecific binding. After the plates were washed further, 50 μL of 1:100 diluted patient sera was added per well and incubated for 2 hr at room temperature. After the plates were washed five times, 50 μL of horseradish peroxidase-labeled anti-human immunoglobulin G antiserum at 0.1 μg/mL in blocking buffer was added per well. The plates were incubated for 90 min at room temperature and then washed five times. The conjugated anti-CEA antibody was detected by adding 100 μL of tetramethylbenzidine substrate per well, incubating for 30 min at room temperature, adding 50 μL of 2N H_2SO_4 per well to terminate the reaction, and measuring the absorbance at 450 nm. The net anti-CEA antibody absorbance was determined by subtracting the absorbance of a noncoated well from the gross absorbance.

Outcome Measures

The primary endpoint of this study was transplant-related mortality within 100 days after transplantation. The secondary endpoint was the tumor response within 6 months after transplantation. Toxicities associated with the conditioning regimen were graded according to the criteria of Bearman et al. (*24*). Objective tumor response was evaluated by an independent radiologist using a monthly computed tomographic (CT) scan. Complete response was defined as disappearance of all clinical evidence of tumor for a minimum of 4 weeks. Minor and partial responses were defined as decreases of 25% to 50% and greater than 50%, respectively, in the sum of the products of the maximum diameter and its perpendicular diameter of all measurable lesions for a minimum of 4 weeks (*1*). The tumor marker response was evaluated by biweekly measurement of the serum CA19-9 level, because imaging modalities including ultrasonography and CT scan are

TABLE 1. Characteristics of the patients

Patient	Age/sex	Prior treatment	Duration from Dx to transplant (mo)	Meta	HLA	No. of $CD34^+$ cells
1	48/M	Gem	3	—	6/6	4.8×10^6/kg
2	40/M	Gem+RT, Gem	9	—	6/6	5.1×10^6/kg
3	57/F	Gem+CDDP	4	Liver	6/6	4.0×10^6/kg
4	36/F	Gem	3	Liver	6/6	2.9×10^6/kg
5	59/M	Gem	2	—	6/6	5.6×10^6/kg
6	66/F	Gem	2	Peritonitis	6/6	2.0×10^6/kg
7	61/M	Gem+RT	12	Liver	6/6	3.0×10^6/kg

Dx, diagnosis; Meta, metastatic lesion; Gem, gemcitabine; RT, local radiation; CDDP, cisplatin.

TABLE 2. Outcome after RIST

Patient	Donor chimerism (%)	aGVHD	Objective response	Final outcome
1	100	III	MR	Died as a result of bacteremia on day 192
2	100	II	SD	Died as a result of PD on day 293
3	100	II[a]	MR[d]	Died as a result of PD on day 262
4	100	II[a]	PD	Died as a result of PD on day 72
5	100	II	SD[c,d]	Died as a result of PD on day 587
6	100	III[b]	PD	Died as a result of PD on day 229
7	100	III[a]	SD	Died as a result of pneumonia on day 83

[a] GVHD that occurred after the rapid tapering of immunosuppressants.
[b] GVHD that occurred after DLI.
[c] Partial response on tumor marker evaluation.
[d] Morphine was discontinued.
aGVHD, acute graft-versus-host disease; MR, minor response; SD, stable disease; PD, progressive disease; DLI, donor lymphocyte infusion.

not sufficient to determine the accurate tumor size of pancreatic cancer (*25*). For patients with a normal value of CA19-9 before RIST, CEA, Dupan-II, or Span-I was measured instead. A complete marker response was defined as normalization of the tumor marker for a minimum of 4 weeks. Minor and partial responses were defined as decreases of 25% to 50% and greater than 50%, respectively, in the tumor marker for a minimum of 4 weeks.

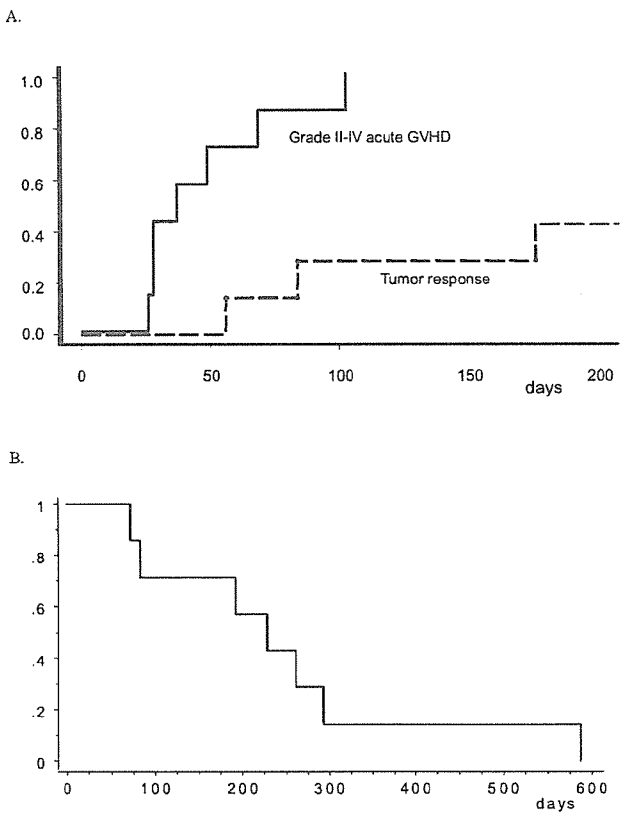

FIGURE 1. (A) Cumulative incidence of grade II to IV acute GVHD (*solid line*) and tumor response including both the objective response and the tumor marker response (*broken line*). (B) Overall survival after RIST against advanced pancreatic cancer.

Statistical Considerations

We defined success as the absence of early transplant-related mortality and planned seven and nine patients in the first and second stages of the study, with target and lower success rates of 80% and 50% and α and β errors of 10% and 10%, respectively (*26*). This is an analysis of the seven patients in the first stage. The cumulative incidence of tumor response was calculated by Gray's method considering death without response as a competing risk (*27*).

RESULTS

Patients

In the first stage of this study, seven patients with a median age of 57 years (range, 36–66 years) underwent RIST (Table 1). The duration from diagnosis to transplantation was 2 to 12 months. Four had metastatic disease and three had locally advanced disease. Prior treatment consisted of chemotherapy mainly with gemcitabine without an objective response, except for one patient (patient 2) who achieved a transient partial response after gemcitabine combined with local irradiation. All of the patients had progressive disease just before the conditioning regimen. All received a peripheral blood stem-cell graft from an HLA-identical sibling donor. The median number of $CD34^+$ cells in the graft was 4.0×10^6 cells/kg recipient body weight (range, 2.0–5.6 cells/kg). Three patients (patients 2, 5, and 6) underwent DLI for tumor progression 221, 336, and 69 days after transplantation, respectively. The dose of infused $CD3^+$ cells ranged between 2.7×10^7 and 1.8×10^8 cells/kg.

Regimen-Related Toxicity, Engraftment, and GVHD

Regimen-related toxicities were generally mild and well tolerated. Grade II to IV toxicity according to Bearman's grade was observed in two patients. One developed mild hepatic veno-occlusive disease, which recovered spontaneously. Another developed ileus caused by the pancreatic head tumor during the neutropenic period, which required nasogastric suction.

The median number of infused $CD34^+$ cells was 4.0×10^6 cells/kg (range, $2.0–5.6\times10^6$ cells/kg). Neutrophil engraftment was obtained within 12 days (range, 11–12 days)

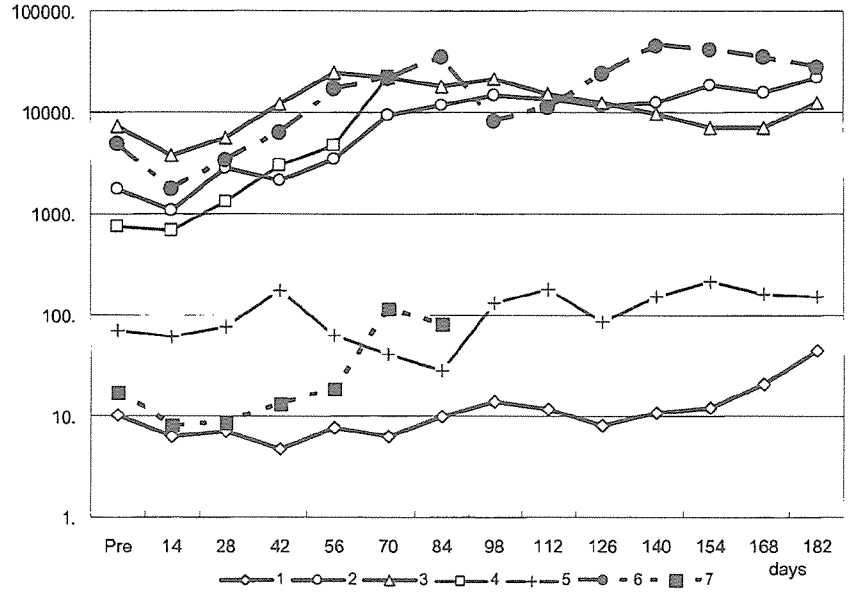

FIGURE 2. Serial changes in the values of tumor markers. CA19-9 was used all except patients 1 and 7, in whom CEA was measured instead because the CA19-9 level was within normal limits before transplantation. The units for the y-axis are units per milliliter for CA19-9 and nanograms per milliliter for CEA. A log scale was used for the y-axis to show serial data of all patients in one figure. Pre, pretransplant level.

after transplantation. Complete donor chimerism (>95%) was achieved in all patients by day 28 and maintained thereafter (Table 2). Grade II to III acute GVHD was observed in three patients (patients 1, 2, and 5) without rapid tapering of CsA, in three (patients 3, 4, and 7) after rapid tapering of CsA, and in one after DLI (Fig. 1A). Acute GVHD limited to the skin was cured with topical steroid only, whereas gut GVHD was successfully treated with systemic steroid.

Transplant-Related Mortality and Survival

Transplant-related mortality within 100 days after transplantation was observed in 1 patient (patient 7), who died as a result of pneumonia on day 83. Another patient (patient 1) died with bacteremia on day 192 caused by bacterial cholangitis. The other five patients died as a result of progressive disease. Median survival after RIST was 229 days (Fig. 1B), which was longer than the median survival of control patients after they were referred to our hospital (125 days), but this difference was not statistically significant.

Tumor Response

An objective minor response on CT scan was seen in two patients (patients 1 and 3) (Table 2 and Fig. 1A). Another patient (patient 5) achieved a partial tumor marker response. Two (patients 3 and 5) of the responders became free from all analgesics after achieving tumor regression.

As shown in Figure 2, the serum CA19-9 or CEA level generally increased within 6 to 8 weeks after transplantation after a transient decline associated with the conditioning chemotherapy. However, it stabilized (n=1) or began to decrease (n=3) thereafter, associated with the discontinuation of CsA or the development of GVHD. This suggests that the antitumor effect was caused by a GVT effect, not a chemotherapy effect. In particular, the serum CA19-9 level decreased from 25,180 U/mL on day 56 to 7,100 U/mL on day 154, with a tumor shrinkage on CT scan in patient 3 after the development of gut GVHD on day 69 (Fig. 3A). Evidence of a GVT effect against pancreatic cancer was also clearly seen in patient 6, who underwent DLI on day 69 for a progressive peritoneal metastatic lesion. The serum CA19-9 level decreased from 35,160 U/mL to 8,140 U/mL in 1 month, with an improvement of the peritoneal lesion on abdominal CT scan (Fig. 3B). However, these tumor responses were not durable and the response duration was between 28 and 98 days.

Serum Anti-CEA Antibody Level

Serum anti-CEA antibody levels were sequentially measured before and after RIST. The serum anti-CEA antibody level before RIST was higher than that in a normal population (n=3), with borderline significance (optical density, 0.109 ± 0.065 vs. 0.028 ± 0.030; $P=0.076$). It generally decreased 1 month after RIST with conditioning chemotherapy. Thereafter, an increase in the serum anti-CEA antibody level was observed in three patients. All three of these patients showed a tumor response, including one after DLI, whereas only one of the four patients without an increase in the serum anti-CEA antibody level showed a response. As shown in Figure 4, the increase in the serum anti-CEA antibody level was simultaneously associated with, or followed by, a decrease in the tumor marker level. However, this response was suppressed by the administration of high-dose steroid (Fig. 4A).

The increase in the serum anti-CEA level did not reflect nonspecific immune recovery, because we did not observe a significant relationship between serum anti-CEA antibody levels and antibody levels against other viral antigens (data not shown). In addition, the increase in the serum anti-CEA level did not result from a decrease in the serum CEA antigen that may absorb anti-CEA antibody, because we did not observe a negative relationship between them (data not shown).

DISCUSSION

In this study, we showed that RIST is a feasible treatment for patients with advanced pancreatic cancer. In addition, an objective response and a tumor marker response were observed in two and one of seven patients, respectively, who