cryopreservation were 2.50 (range, 1.83-4.39) x 10^7/kg and 0.76 (range, 0.27-1.52) x 10^5/kg, respectively. Anti-HLA antibodies were screened before transplant in 6 patients using a FlowPRA method (One Lambda), and LAB Screen PRA or Single Antigen (One Lambda) was used to identify HLA antibody specificities [7,8]. All patients were conditioned with fludarabine 25 mg/m^2 daily for 5 days, melphalan 40 mg/m^2 daily for 2 days, and 4 Gy of TBI in 2 fractions in 1 day. GVHD prophylaxis consisted of cyclosporine in 2, tacrolimus in 2, and tacrolimus plus mycophenolate mofetil in 8. Assessment of engraftment, GF, chimerism, GVHD and supportive care during transplantation were performed as previously reported [9] [10]. Karnofsky performance status score was assessed as surrogate for Quality of life of the survivors. Overall survival (OS) was estimated using the Kaplan-Meier method.

Results and discussion

Patients' outcomes are summarized in Table 1B. Eleven of the twelve patients achieved primary neutrophil and platelet engraftment. The median times to achieve neutrophil engraftment and platelet count >20 $\times 10^9$/L were 18 (range, 12-28) and 42 (range, 26-64) days, respectively. All patients who achieved engraftment had complete hematological recovery and were free from transfusion, and they showed complete donor chimerism at the time of the first chimerism analysis (median, 14 days; range,

11-73 days). One patient developed primary GF and was later found to have antibody against mismatched HLA on donor cells. Another patient developed secondary GF 3 years after UCBT. Both patients underwent a second RI-UCBT, and obtained rapid donor engraftment. The negative impact of multiple transfusions before transplant was not detected (Table 1A and 1B). Among 11 evaluable patients, 2 developed grade I and 5 developed grade II acute GVHD. Of the 9 patients who survived longer than 100 days post-transplant, 3 developed limited type of chronic GVHD. No patients developed grade III-IV acute GVHD and extensive type of chronic GVHD. Two of the twelve patients died from idiopathic pneumonia syndrome, and the remaining 10 patients are alive, having survived for a median of 36 months (range, 14-91 months). The probability of OS at 3 years was 83.3% (Figure 1). The surviving patients had high KPS score with the median of 90% (range; 60-100%).

The present study demonstrated that our RIC regimen allows a sufficient sustained engraftment of UCB in adult SAA patients. The RIC regimen was originally developed in our institute for UCBT for various hematological malignancies [9]. Eleven of the twelve patients achieved primary engraftment, which compares favorably with previously reported engraftment rates of UCBT for SAA [11-14 15 16]. Our RIC regimen would be more potent than the others to overcome immunological barriers for

engraftment. Cell dose has been known to significantly influence the rate of engraftment after UCBT [14]. In the present study, although the cell dose was not very large, sufficient engraftment was seen. Any significant relationship between cell dose (TNC; ≥ 2.5 vs $<2.5\times10^7$/ kg, CD34 $^+$; ≥ 0.8 vs $<0.8\times10^5$/ kg) and engraftment kinetics were observed (data not shown). Thus, not just cell dose but other factors, such as the intensity of the conditioning regimen and post-transplant immunosuppression, may be important to achieve better engraftment after UCBT for SAA patients. Interestingly, all 6 patients who were screened for HLA antibodies before transplant had HLA antibodies, and the one case, who had positive HLA antibodies against an HLA on a transplanted UCB unit, was the only one who failed primary engraftment. Recently, Takanashi et al. reported that, in large number of UCBT for various hematological malignancies, the patients with anti-HLA antibodies, when the specificity corresponding to mismatched antigen in UCB graft, showed significantly lower neutrophil or platelet recovery than those with antibodies-negative or –positive but not corresponding to UCB graft [17]. Although the observations may differ from that of diverse populations and warrants further investigation, if possible, the use of a UCB unit with corresponding HLA antibodies in the recipient should be avoided.

Three-year survival in the studied patients was 83.3%. In addition to high rate of

engraftment, the low risk of severe GVHD might contribute to high survival rate with good QOL, and which seems to be one of the important advantages of using a UCB unit for SAA patients. The other advantage of the use of UCB units is rapid availability. In the present study, 2 patients with fulminant type could be rescued by urgent hematopoietic stem cell transplantation using UCB units. More than 90% of recipients can find a suitable UCB unit in Japan, and thus, UCB expands the chance to receive transplantation for those who need it urgently.

In conclusion, this retrospective study strongly suggests the feasibility and effectiveness of RI-UCBT for adult SAA patients. RI-UCBT may become a viable therapeutic option for those who lack suitable HLA-matched donors and who fail or relapse after IST. Although our results should be interpreted with caution because of the small number of patients and still short follow-up duration, we believe that RI-UCBT with the conditioning regimen presented here deserves further evaluation in a prospective trial, hopefully in a multi-center setting.

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Authors' contribution

H.Y. and D.K. performed transplantation and analyzed extracted data and contributed to writing the paper; A.Y. reviewed histopathological section; H.Y. and N.M. performed statistical analysis; N.U. K.Izutsu. and S. Taniguchi reviewed study design and methods; K.Ishiwata., H.A., S. Takagi, M.T., N.N., Y.A-M., K.M., A.W., S.M performed transplantation and contributed to writing the paper.

Conflict-of-interest disclosure

The authors declare no competing financial interests.

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Table 1A. Characteristics of Patient, Grafts, and GVHD prophylaxis

Case	Age (years)	Previous treatment	Interval Dx to UCBT (months)	Previous transfusion (RBC / platelet) (times)	Disease status at UCBT	HLA match	HLA Ab (reactive to CB)	ABO group (R/D)	TNC ($\times 10^7$/kg)	$CD34^+$ ($\times 10^5$/kg)	GVHD prophylaxis
1	70	CSA	3	11 / 14	SAA	4 / 6	N.T	A/A	4.00	1.23	CSA
2	20	ATG+CSA	78	>20 / >20	VSAA	4 / 6	N.T	B/O	2.65	1.07	CSA
3	22	ATG+CSA, PSL	157	>20 / >20	SAA	4 / 6	N.T	A/O	2.26	0.27	Tac
4	26	ATG+CSA	3	>20 / >20	VSAA	5 / 6	N.T	A/A	2.65	0.70	Tac
5	59	ATG+CSA	8	>20 / >20	SAA	5 / 6	Positive (no)	O/O	2.15	1.52	Tac+MMF
6	49	ATG+CSA, PSL	12	>20 / >20	VSAA	3 / 6	N.T	A/A	2.04	0.62	Tac+MMF
7	70	None	1	5 / 8	Fulminant	4 / 6	Positive (yes)	A/O	4.39	1.29	Tac+MMF
8	52	None	1	4 / 6	Fulminant	4 / 6	N.T	AB/A	3.20	0.49	Tac+MMF
9	46	ATG+CSA	45	>20 / >20	VSAA	4 / 6	Positive (no)	AB/O	1.83	0.42	Tac+MMF
10	49	ATG+CSA, PSL	327	>20 / >20	VSAA	6 / 6	Positive (no)	B/O	2.34	0.82	Tac+MMF
11	65	CSA	6	16 / >20	VSAA	6 / 6	Positive (no)	A/A	3.31	0.56	Tac+MMF
12	31	ATG+CSA, PSL	215	>20 / >20	SAA	4 /6	Positive (no)	B/O	2.09	1.26	Tac+MMF

Dx, diagnosis; UCBT, unrelated cord blood transplantation; RBC, red blood cell; HLA, human leucocyte antigen; CB, cord blood; R, recipient; D, donor; TNC, total nucleated cells; GVHD, graft-versus-host disease; CSA, ciclosporin-A; ATG, antithymocyte globulin; PSL, prednisone; SAA, severe aplastic anemia; VSAA, very severe aplastic anemia; N.T, not tested; M, male; F, female; Tac, tacrolimus; MMF, mycophenolate mofetil

Table 1B. Outcomes of 12 patients after reduced-intensity unrelated cord blood transplantation

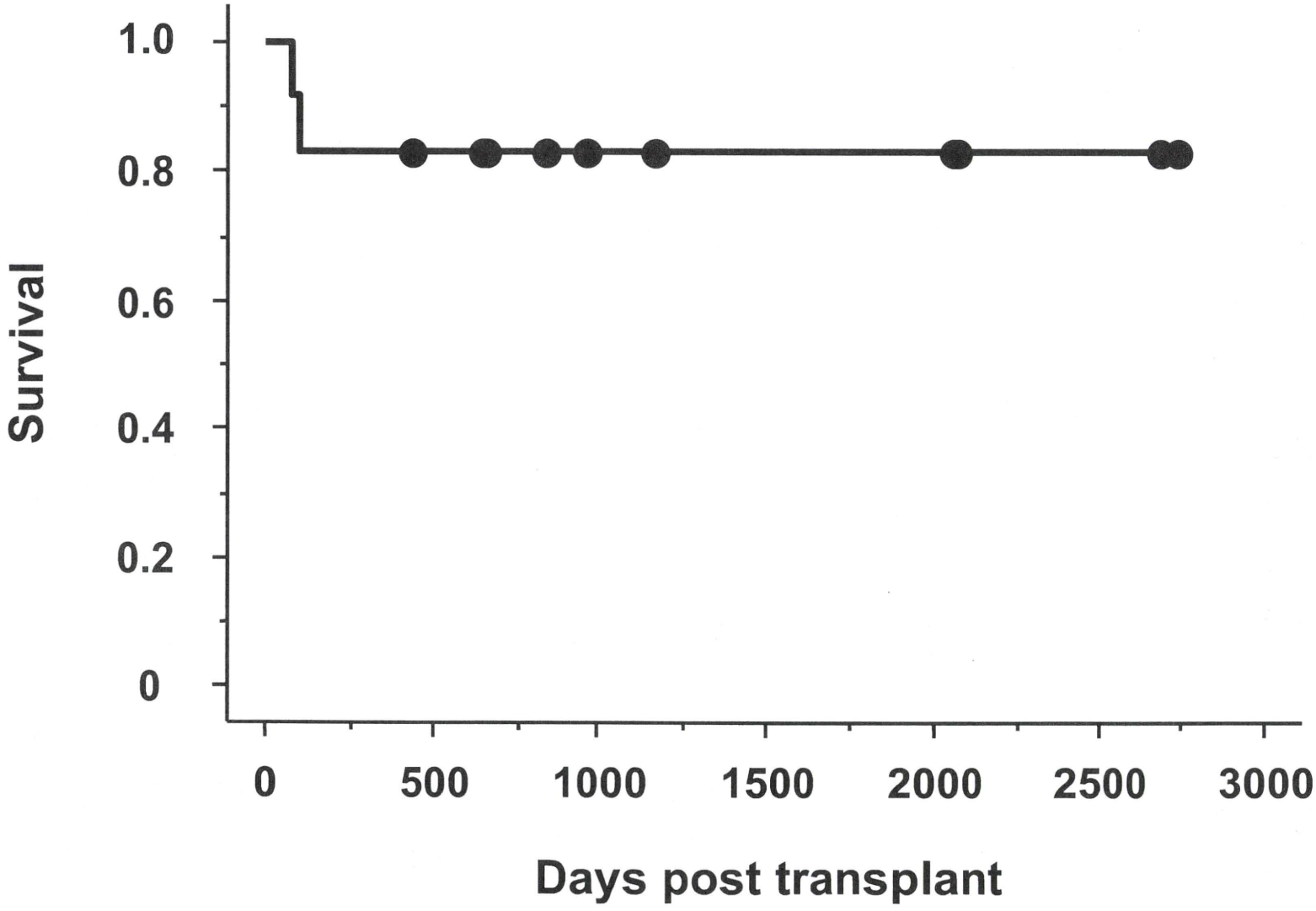
Case	Days to ANC $>0.5\times10^9$/l	Days to PC $>20\times10^9$/l	% Donor chimerism (Days tested, Methods)	aGVHD	cGVHD	Discontinuation of IS (months)	Complication	Survival (months)
1	12	52	100 (14, FISH)	gradeII(skin)	No	Yes (3)	Possible IPA	Alive (91)
2	20	64	90< (49, PCR-STR)	gradeII(skin)	limited	Yes (2)	No	Alive (90)
3	26	42	100 (26, FISH)	No	No	Yes (26)	Yes	Alive (69)
4	18	53	100 (18, FISH)	No	No	Yes (5)	Pneumocystis jirovecii, Late GF, rescued by 2nd UCBT	Alive (69)
5	16	26	96.6 (14, FISH)	gradeI(skin)	limited	Yes (14)	Norwalk virus colitis, EBV-PTLD	Alive (39)
6	28	64	99.6 (11, FISH)	No	N.E	No	IPS	Dead; IPS (3)
7	No	No	48.8 (10, FISH), 4.3 (15, FISH)	N.E	N.E	N.E	Primary GF, rescued by 2nd UCBT	Alive (32)
8	18	28	99.2 (13, FISH)	gradeII(skin, gut)	No	Yes (7)	CMV colitis, EBV-PTLD	Alive (28)
9	28	43	90< (14, PCR-STR)	gradeI(skin)	N.E	No	HSV pneumonia, IPS	Dead; IPS (3)
10	15	27	99 (73, FISH)	No	limited	No	No	Alive (22)
11	15	27	100 (20, FISH)	gradeII(skin, gut)	No	No	No	Alive (22)
12	13	28	100 (14, FISH)	gradeII(gut)	No	No	No	Alive (14)

ANC, absolute neutrophil count; PC, Platelet count; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; IS, immunosuppressant; FISH, fluorescent in situ hybridization; PCR-STR, PCR of short tandem repeat; N.E, not evaluable; IPA, invasive plumonary aspergillosis; GF, graft failure; EBV-PTLD, EBV associated posttransplantation lymphoproliferative disorders; IPS, idiopathic pneumonia syndrome

Figure legend

Figure 1. Survival of 12 patients with SAA undergoing unrelated cord blood transplantation.

Figure 1


1.0
0.8
0.6
0.4
0.2
0
Survival
0
500
1000
1500
2000
2500
3000
Days post transplant

Bone Marrow Transplantation (2010) 45, 1372–1374

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LETTER TO THE EDITOR

T-cell post-transplant lymphoproliferative disorder in a patient with chronic idiopathic myelofibrosis following allogeneic PBSC transplantation

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Post-transplant lymphoproliferative disorder (PTLD) is a well-recognized complication after solid organ and hematopoietic SCTs (HSCTs). The majority are of B-cell origin and EBV related.[1] Most of the T-cell PTLD cases have been described as occurring after solid organ transplantations;[2] T-cell PTLD cases following HSCT are exceedingly rare. There are only three reported cases of T-cell PTLD following allogeneic HSCT[3] and four cases following autologous HSCT.[4–7] Here we report a case of T-cell PTLD after allogeneic-PBSC transplantation (allo-PBSCT) in a patient with chronic idiopathic myelofibrosis (CIMF).

A 44-year-old Japanese woman with anemia and fever was diagnosed with CIMF in November 2006. At the time of her diagnosis, her WBC count was 900/μl, Hb 6.9 g/dl, plt count 39 000/μl with no morphologically abnormal cells in her peripheral blood, and an abdominal CT scan showed mild splenomegaly without hepatomegaly, lymphadenopathy or liver tumor. A specimen of her biopsied BM showed diffuse fibrosis and a decreased number of hematopoietic cells. No abnormal cell proliferation was observed. In December 2006, she underwent allo-PBSCT from an HLA-identical brother. Neutrophil engraftment was achieved on day 17 after transplant, and BM analysis showed full hematological recovery with 100% donor-type chimerism assessed by Y chromosome-based FISH analysis. As grade II acute GVHD involving the skin and subsequently an extensive type of chronic GVHD (cGVHD) developed; continued immunosuppressive therapy with cyclosporine and prednisolone was required for several months after the transplant. At 5 months after transplant, a liver tumor, 2 cm in diameter, was detected by an abdominal CT scan. Although PTLD was raised as a differential diagnosis, biopsied liver tissue was inadequate for pathological examination. Immunosuppressive therapy was reduced, resulting in a decrease in liver tumor size to 1.6 cm in 2 months. However, a subsequent flare-up of cGVHD required more intensive immunosuppressive therapy, and the liver tumor's diameter increased twice in size. A liver tumor biopsy performed at this time showed a diffuse proliferation of atypical lymphoid cells (Figure 1a). Immunohistochemically, these tumor cells were positive for LCA, CD3, CD7 and CD8, and negative for CD4, CD5, CD34, CD79a, MPO, CD30, CD56 and TdT (Figure 1b). These pathological findings are compatible with peripheral T-cell lymphoma-unspecified (Figure 1c). EBV infection was not detected by *in situ* hybridization. Y chromosome-based FISH analysis revealed the tumor cells were of recipient origin. She suffered from fever, pancytopenia and decreased liver function, and was hospitalized for further therapy in November 2007. BM examination showed infiltration of 4% abnormal lymphoid cells and the proliferation of macrophage with hemophagocytosis, with no sign of CIMF recurrence. Chromosome analysis of the BM cells showed 44, X, der(X)t(X;7)(q13;q11.2), add(2)(q21), add(4)(p11), add(4)(p16), der(9;17)(q10;q10), −10, −13, add(15)(p11), +mar [2/20]. An abdominal CT scan showed that the liver tumor grew rapidly to a size of $12 \times 6\,cm^2$ (Figure 1d). Serological tests for HIV, HBV, HCV and HTLV-1 were negative, and the EBV VCA IgG was positive but negative for IgM. Analyses by real-time PCR were negative for human herpesvirus-6, VZV, CMV and EBV in her peripheral blood. She was diagnosed with T-cell PTLD with lymphoma-associated hemophagocytic syndrome. CHOP therapy was started, but the disease progressed within 2 weeks after this. She underwent urgent unrelated cord blood transplantation (UCBT) from an HLA two antigen-mismatched donor. Her post-transplant course was complicated by sepsis, renal failure and respiratory failure. She died on day 6 after UCBT. An autopsy was not performed.

To our knowledge, there have been only four cases of T-cell PTLD following allo-SCT, including our case (Table 1). Time to T-cell PTLD diagnosis ranges from 2 to 43 months after a transplant. Although the type of PTLD was not consistent, ranging from precursor to peripheral T-cell neoplasms, none of them were associated with EBV infection. Our case was negative for EBV, and the type was peripheral T-cell lymphoma-unspecified.

There have been a few reports describing myelofibrosis in association with T-cell lymphoma.[8] In these cases, PDGF and tumor growth factor β, which may have been secreted by neoplastic T lymphocytes, had an important role in the development of myelofibrosis. In our case, there was no clinical evidence of T-cell lymphoma at the time of CIMF diagnosis, and no sign of myelofibrosis recurrence at the onset of T-cell lymphoma. Thus, the development of T-cell lymphoma in this case was considered to be independent of the CIMF.

All three patients reported as having T-cell PTLD following allo-SCT had severe GVHD and received a heavy dose of immunosuppressive agents, suggesting some viral agents in an immunosuppressed state may have an important role in the development of T-cell PTLD. However, we were unable to find any evidence of viral

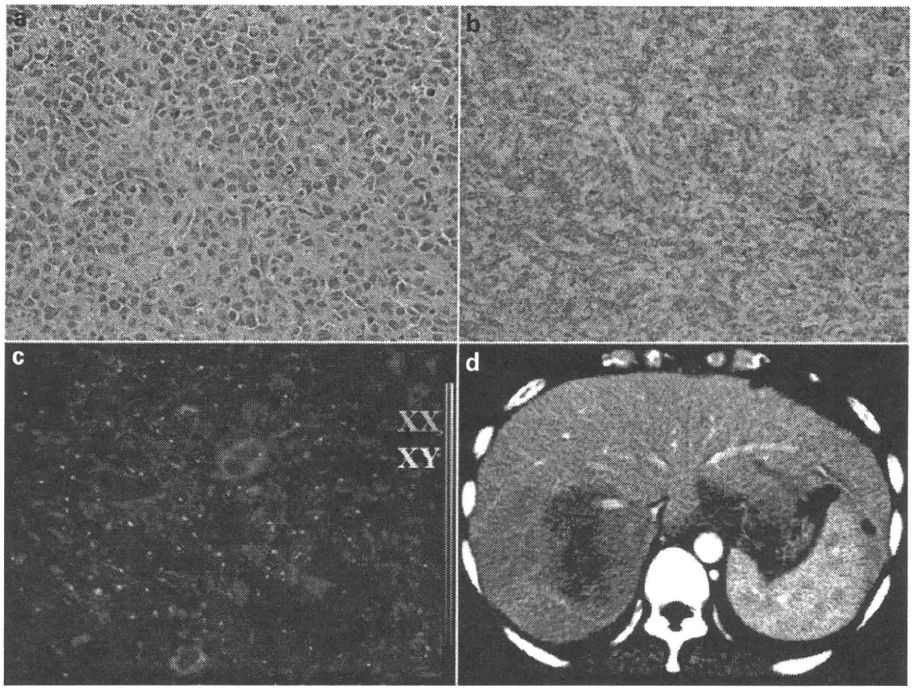

Figure 1 (**a**) Liver tumor biopsy shows monotonous infiltration of atypical lymphoid cells (H&E stain ×400). (**b**) Immunostaining for CD3 shows a large number of positive cells within the tumor. (**c**) Y chromosome-based FISH reveals the tumor cells are of recipient origin (XX signal). (**d**) Abdominal CT scan shows a low-density area with 12 cm diameter on the right side of the liver.

Table 1 T-cell post-transplant lymphoproliferative disorder after allogeneic stem cell transplantation

Authors	*Age/sex*	*Initial Dx*	*HSCT*	*Type of PTLD Dx (months after HSCT)*	*Origin*	*EBV*	*GVHD*	*Outcome (months after Dx)*
Zutter *et al.*[3]	14/M	AML	HLA-identical BM graft	T-lymphoblastic lymphoma (43)	Recipient	Neg	Mild aGVHD(S,L,Gut) Severe cGVHD(S,L,Gut)	Death (28)
	9/M	ALL	HLA-identical BM graft	T-lymphoblastic lymphoma (21)	Donor	Neg	Mild aGVHD(S) Severe cGVHD(S,L)	Death (6)
	2/F	ALL	HLA-2 mismatched BM graft	Polymorphic T-cell lymphoma (2)	Donor?	Neg	Severe aGVHD(S,L)	Death (0)
Present case	44/F	CIMF	HLA-identical allogeneic PBSC	PTCL-u (5)	Recipient	Neg	aGVHDII(S3,L0,Gut0) Extensive cGVHD(S,L)	Death (2)

Abbreviations: aGVHD = acute GVHD; cGVHD = chronic GVHD; CIMF = chronic idiopathic myelofibrosis; Dx = diagnosis; F = female; Gut = gastrointestinal tract; HSCT = hematopoietic stem cell transplantation; L = liver; M = male; neg = negative; PTCL-u = peripheral T-cell lymphoma-unspecified; PTLD = post-transplant lymphoproliferative disorder; S = skin.

infection and reactivation in our case and previously reported cases. It has been reported that only 15 of 76 cases of T-cell PTLD after solid organ transplantation were EBV positive,[9] and any other viral involvement has not been clearly demonstrated. These findings suggest that not only viral infection but also other factors, such as chronic antigenic stimulation, impaired immunoregulation and genetic factors, may be associated with the development of T-cell PTLD.[10]

The outcomes of reported T-cell PTLD so far are poor. All patients died because of the progression of the disease. In our patient, a transient response was observed by reducing immunosuppression, suggesting a graft-versus-lymphoma effect, which was necessitated to increase the immunosuppression. Standard cytotoxic chemotherapy led to a poor response in our patient, similar to the other cases previously described. More intensive chemotherapy, donor lymphocyte infusion or second HSCT should be considered at an early stage of the disease.

In conclusion, T-cell PTLD rarely occurs after allo-HSCT. Further research, however, is needed to fully characterize the clinicopathological features of this condition and to investigate the optimal therapy.

Conflict of interest

The authors declare no conflict of interest.

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LETTER TO THE EDITOR

What is the upper age limit for performing allo-SCT? Cord blood transplantation for an 82-year-old patient with AML

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Since morbidity and mortality associated with hematologic malignant diseases in elderly patients is higher than that in younger patients,[1] elderly patients are less likely to be candidates for allo-SCT, due to the facts that they are more likely to have comorbid organ conditions, either clinically or subclinically, which results in a higher rate of procedure-related mortality,[2] and that they are less likely to have HLA-matched related donors available, as siblings also tend to be elderly.

The development of reduced-intensity (RI) conditioning for transplants, which results in less toxicity and depends largely on GVL effects rather than high-dose therapy to eliminate leukemic cells, has been shown to allow elderly patients to undergo allo-SCT.[3–5] The use of umbilical cord blood transplantation (UCBT) for adults has been increasing due to the potential advantage of rapid availability and the lower risk of GVHD, thus permitting less stringent HLA matching.[4,5] RI-UCBT for adults, mostly elderly patients, has been increasingly reported and shown to be applicable.[6,7] However, there has been no clear description on the upper age limit of receiving allo-SCT, and it varies among institutes at this moment. We report here an 82-year-old man with refractory AML who had successfully treated with RI-UCBT.

The patient was diagnosed as AML (M5b) with adverse risk karyotype (46, XY, −7, +8) and complicated with disseminated intravascular coagulation (DIC). Although DIC was resolved soon after remission induction therapy consisted of idarubicin and cytarabine, and the patient achieved hematological remission, the disease subsequently progressed with lung infiltration and systemic skin tumor formation (Figure 1a). Immunohistochemical analysis of skin tumor showed positive for CD45, myeloperoxidase, and CD68 consistent with leukemic cell infiltration. Skin and lung infiltration was refractory to following high-dose Ara-C-containing chemotherapy. At 4 months after diagnosis of AML, following careful discussion and consent among the patient, his family and transplant staff, he received an RI-UCBT using two antigen- and three allele-mismatched CB in August 2007. His Eastern Cooperative Oncology Group (ECOG) performance status was 2, and HCT-CI score was 1. The preparative regimen consisted of i.v. fludarabine 25 mg/m^2 daily for 5 days (total dose 125 mg/m^2), i.v. melphalan 40 mg/m^2 daily for 2 days (total dose 80 mg/m^2) and 4 Gy of TBI fractionated by 2. GVHD prophylaxis consisted of tacrolimus by continuous infusion and 15 mg/kg twice daily of oral mycophenolate mofetil from day −1. CB unit contained 2.5×10^7 per kg of total nucleated cells and 0.98×10^5 per kg of CD34+ cells before cryopreservation. G-CSF 300 μg/m^2 was administered from day 1 until neutrophil engraftment. On day 14, the patient developed erythema, fever (39 °C) and diarrhea, and was diagnosed as having preengraftment immune reactions (PIR).[8] The symptoms disappeared immediately after initiation of methylprednisolone 0.5 mg/kg for 3 days. There was no episode of bacterial infection during neutropenia. ANC recovered to 0.5×10^9 per liter on day 25, and platelet count reached 2.0×10^9 per liter on day 64. Complete donor-cell chimerism was confirmed on day 27 by BM analysis using short tandem repeat-PCR method. Human herpesvirus-6 limbic encephalitis developed on day 17, which was successfully managed with foscarnet. The regimen-related toxicities observed were mucositis (grade 2), nausea (grade 2), renal dysfunction (grade 2) and diarrhea (grade 1), according to the National Cancer Institute Common Toxicity Criteria version 3.0. Acute GVHD of grade III (gut: stage 2) on day 46 was observed, but successfully managed with oral beclomethasone dipropionate. He finally achieved CR in BM, and his lung lesion and skin tumors also disappeared (Figure 1b). He was discharged from hospital on day 123 after RI-UCBT. To our surprise, his level of performance status got improved thereafter, almost as score 1 measured by ECOG PS scoring system, and returned to his work in 1 month after discharge. In the meantime, chronic GVHD of limited type developed, which was managed without treatment. One year after RI-UCBT, unfortunately, his disease relapsed and he died from disease progression 1 month later.

This remarkable case told us two important issues. First, some, may be not all, patients older than 80 years still can tolerate RI-UCBT. TRM has been shown to be correlated with several factors including age, or more comprehensively, the number of coexisting comorbidities.[9] According to our previous report, those older than 54 years showed cumulative incidence of TRM reaching to approximately 50%, and most of TRM occurred early period post-UCBT.[10] This patient had also faced life-threatening events, such as PIR or viral encephalitis, and was successfully managed by corticosteroid and foscarnet. In allo-SCT settings, there are always several factors that cannot be modulated intentionally, and there may have been good coincidences for him to reach this successful outcome. Nevertheless, this case strongly claims higher age should not be the single determinant of not performing allo-SCT. Second, the most powerful antileukemic activity was observed with RI-UCBT. Although, the patient had finally disease relapse, it was obvious that only RI-UCBT sufficiently suppressed leukemic cells and gave him a

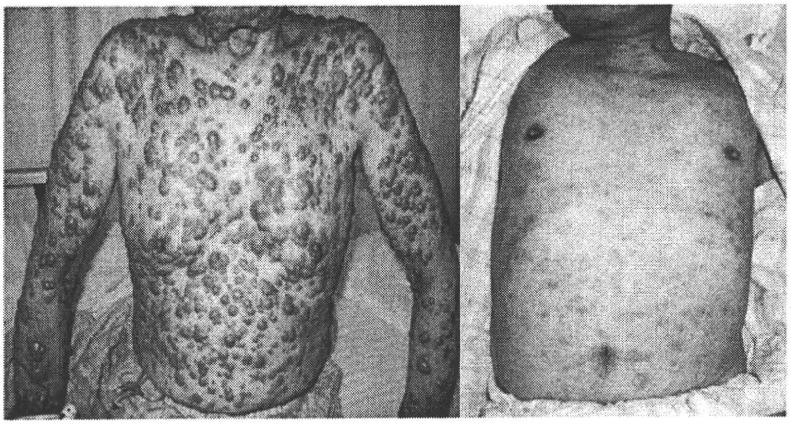

Figure 1 Skin tumors covering whole body of the patient just before RI-UCBT (**a**). Skin tumors of the patient had disappeared in 90 days after RI-UCBT (**b**).

sustained CR so that he had enough time to return to his job. Although CB has been shown to have functionally immature immune cells, it showed its extremely powerful anti-leukemic activity even from the early period post transplant, as the patient's skin lesion had never disappeared during induction chemotherapy including high-dose Ara-C.

Whether the clinical course of this case can be applicable to all aged patients or this is exceptional case needs to be investigated carefully. The indication of allo-SCT for those who are elderly has to be determined individually with extremely careful and repeated discussion with patients, families and transplant staff. Nevertheless, the indication of allo-SCT should not be determined by age as a sole factor. Otherwise, elderly patients may lose chance of cure or good disease control, by not performing toxic yet powerful treatment, such as transplant.

Conflict of interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

Pharmacokinetics of CsA during the switch from continuous intravenous infusion to oral administration after allogeneic hematopoietic stem cell transplantation

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We investigated the serial changes in the blood CsA concentration during the switch from continuous intravenous infusion to twice-daily oral administration in allogeneic hematopoietic stem cell transplant recipients ($n = 12$). The microemulsion form of CsA, Neoral, was started at twice the last dose in intravenous infusion in two equally divided doses. The area under the concentration–time curve during oral administration (AUC_{PO}) was significantly higher than the AUC during intravenous infusion (AUC_{IV}) (median 7508 vs 6705 ng/ml × h, $P = 0.050$). The median bioavailability of Neoral, defined as ($AUC_{PO}/DOSE_{PO}$) divided by ($AUC_{IV}/DOSE_{IV}$), was 0.685 (range, 0.45–1.04). Concomitant administration of oral voriconazole ($n = 4$) significantly increased the bioavailability of Neoral (median 0.87 vs 0.54, $P = 0.017$), probably due to the inhibition of gut CYP3A4 by voriconazole. Although the conversion from intravenous to oral administration of CsA at a ratio of 1:2 seemed to be appropriate in most patients, a lower conversion ratio may be better in patients taking oral voriconazole. To obtain a similar AUC, the target trough concentrations during twice-daily oral administration should be halved compared with the target concentration during continuous infusion.

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Introduction

CsA is the most widely used immunosuppressive agent for the prophylaxis of GVHD after allogeneic hematopoietic stem cell transplantation (HSCT). It is usually administered by intravenous infusion for at least several weeks after allogeneic HSCT because of the damage done to the oral and gastrointestinal mucosa by the conditioning regimen. However, the dose, target blood level, and schedule of administration vary among protocols and have not been optimized.[1] It has been shown that the blood concentration of CsA affects the incidences of acute GVHD and adverse events,[2] and an increase in the target blood concentration from 300 to 500 ng/ml in the continuous infusion of CsA significantly decreased the incidence of acute GVHD.[3] On the basis of these results, we are currently administering CsA by continuous infusion with target concentrations of 500 ng/ml for standard-risk patients and 300 ng/ml in high-risk patients. When patients can tolerate oral intake, CsA is switched from intravenous to oral administration at a dose ratio of 1:2. Neoral, a microemulsion formulation of CsA, has improved bioavailability and is the most commonly used oral product.[4] However, the appropriateness of this conversion rate has been inconsistent among earlier studies.[5,6] Parquet *et al.* reported that doubling the last intravenous dose provided the best therapeutic range concentration, whereas the concentration/dose ratio was similar in intravenous administration and oral administration and thus, 1:1 conversion seemed appropriate in the McGuire's study. In addition, no data are available regarding the detailed pharmacokinetics in allogeneic HSCT recipients. Therefore, in this study, we investigated the serial changes in the CsA blood concentration during the switch from intravenous to oral administration and assessed the bioavailability of Neoral.

Patients and methods

Patients

Patients who underwent allogeneic HSCT with GVHD prophylaxis consisting of the continuous infusion of CsA and short-term MTX were included. This single-center prospective study was approved by the Institutional Review Board of Jichi Medical University, and each patient provided their written informed consent to be enrolled in the study.

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Transplantation procedure

The conditioning regimen was mainly a combination of cyclophosphamide (60 mg/kg for 2 days) and TBI (2 Gy twice daily for 3 days) ($n=8$). Patients with severe aplastic anemia ($n=3$) were prepared with fludarabine, cyclophosphamide, and anti-thymoglobulin with or without a low dose of TBI at 2 Gy.[7] A reduced-intensity regimen with fludarabine and melphalan was used for a 58-year-old patient with acute lymphoblastic leukemia ($n=1$). GVHD prophylaxis consisted of the continuous infusion of CsA with a starting dose of 3 mg/kg/day and short-term MTX (10–15 mg/m^2 on day 1 and 7–10 mg/m^2 on days 3 and 6, and optionally on day 11 in HSCT from a donor other than an HLA-matched sibling). The dose of CsA was adjusted to maintain the blood CsA concentration between 450 and 550 ng/ml in standard-risk patients ($n=9$) or 250 and 350 ng/ml in high-risk patients ($n=3$) according to the disease status.[3] Acute GVHD was graded as described earlier.[8] Prophylaxis against bacterial, fungal, and Pneumocystis jiroveci infection consisted of levofloxacin, fluconazole (FLCZ), and sulfamethoxazole/trimethoprim (ST) or inhalation of pentamidine. In three patients, micafungin (MCFG) was used instead of FLCZ because of persistent fever despite broad-spectrum antibiotic therapy, development of Candidemia, and high risk for invasive aspergillosis, respectively. As prophylaxis against herpes simplex virus infection, acyclovir (ACV) was given from days −7 to 35, followed by a long-term low-dose administration of ACV for varicella zoster reactivation.[9] Pre-emptive therapy with ganciclovir for cytomegalovirus infection was performed by monitoring cytomegalovirus antigenemia.[10]

Study schedule

When patients were able to tolerate oral intake, CsA was switched from continuous infusion to oral administration. Intravenous infusion was stopped just before the first oral administration. The initial dose of Neoral was twice the last daily dose of continuous infusion, and was given in two equally divided doses based on the reported bioavailability of Neoral of about 0.4 (40%) in allogeneic HSCT recipients.[5] On the last day of the continuous infusion of CsA (day −1), the serum CsA concentration was measured at 9:00, 15:00, and 21:00. After the patient was switched to Neoral, the CsA concentration was measured just before (C_0), and 1 (C_1), 2 (C_2), 3 (C_3), 4 (C_4), 6 (C_6), and 12 (C_{12}) hours after the oral administration of Neoral on the first day (day 0) and between day 3 and day 5. The CsA concentration was measured using the CYCLO-Trac SP-whole blood kit (DiaSorin, Inc., Stillwater, MN, USA).[11] In brief, 200 µl of whole blood sample was mixed with 800 µl of methanol and centrifuged at 1600 *g* for 5 min. The methanolic supernatant (50 µl in duplicate) was mixed with 100 µl of ^{125}I-ligand and 1 ml of anti-CYCLO-Trac Immune Sep (pre-mixed mouse monoclonal antibody, donkey anti-mouse serum, and normal mouse serum). After centrifuging, the ligand was discarded by decanting and the amount of radioactivity of the pellet was determined. Data were analyzed by logit-log reduction. The standard curve was obtained using the CsA standard sera provided in the kit. The intra-assay coefficient of variance was <15%. The inter-assay coefficient of variance was <14%. The limit of detection was 4.0 ng/ml. The results of this assay showed good correlation with those obtained by high-performance liquid chromatography ($r=0.98$).

During the study, the dose of CsA could be modified at the discretion of each physician. Vital signs and laboratory variables including renal and liver function tests were evaluated on days 0, 3, 7, and 14. Concomitant medications that could potentially interact with CsA were recorded.

Statistical considerations

The area under the concentration–time curve (AUC) (0–12 h) of CsA was calculated by the trapezoidal method. We estimated the bioavailability of Neoral by dividing ($AUC_{PO}/DOSE_{PO}$) by ($AUC_{IV}/DOSE_{IV}$). Toxicities after switching from intravenous to oral administration were evaluated compared with the baseline data on day 0. Renal toxicity was defined as an elevation of the creatinine (Cr) level above × 1.5 the baseline value. Liver dysfunction was defined as an elevation of alanine aminotransferase (ALT) above × 2 the baseline value, or elevation of the total bilirubin (T-bil) level by 2 mg per 100 ml compared with the baseline value. Comparisons were made using the Wilcoxon signed-rank test for continuous variables. The Pearson correlation coefficient was used to analyze the correlation between AUC and the CsA concentration at each measurement point after logarithmic transformation. The effect of concomitant medications on CsA pharmacokinetics was first analyzed by a univariate analysis with the Mann–Whitney *U*-test, and then those with at least borderline significance ($P<0.10$) were subjected to a multivariate analysis using multiple regression modeling. A *P*-value of <0.05 was considered to be significant.

Results

Patients

Between January 2008 and April 2009, 12 patients were enrolled in the study. There were 7 males and 5 females with a median age of 34.5 years (range, 16–58). Underlying diseases included acute myeloblastic leukemia ($n=4$), acute lymphoblastic leukemia ($n=3$), severe aplastic anemia ($n=3$), chronic myelogenous leukemia ($n=1$), and myelodysplastic syndrome ($n=1$). Five patients received bone marrow graft from an unrelated donor, whereas 1 and 6 patients, respectively, received bone marrow and peripheral blood stem cell graft from a related donor. There was an HLA mismatch in three donor-recipient pairs.

Pharmacokinetic analysis

The median duration from transplantation to the switch from intravenous to oral administration was 40 days (range, 27–60). The dose of CsA and the pharmacokinetic parameters during intravenous and oral administration are shown in Table 1. Neoral was started at approximately twice the last dose of intravenous infusion, except that 1 patient (No. 8) received Neoral at the same dose as in intravenous infusion, as the mean CsA concentration on the last day of intravenous infusion was >700 ng/ml.

Table 1 Dose of CsA and pharmacokinetic parameters during the intravenous and oral administration of CsA

Patient no.	*Day −1*			*Day 0*					*Steady state (Days 3–5)*				
	$DOSE_{IV}$ (mg/day)	C_{mean} (ng/ml)	AUC_{IV} (ng/ml × h)	$DOSE_{PO}$ (mg/day)	C_{max} (ng/ml)	T_{max} (h)	C_{min} (ng/ml)	AUC_{IV-PO} (ng/ml × h)	$DOSE_{PO}$ (mg/day)	C_{max} (ng/ml)	T_{max} (h)	C_{min} (ng/ml)	AUC_{PO} (ng/ml × h)
1	96	590	7110	200	1300	2	370	9525	160	1400	3	550	10625
2	140	643	7680	280	1600	3	480	10860	250	1000	2	320	7080
3	130	553	6630	260	2700	3	360	12555	160	1200	2	290	7790
4	173	663	7950	360	1900	2	340	11785	360	2500	1	420	12420
5	192	677	7920	400	1500	3	240	8685	400	1500	2	280	8355
6	125	577	6780	260	1200	2	360	8300	260	1200	3	360	8450
7	80	527	6330	160	650	0	390	5725	160	800	2	280	6105
8	192	717	8730	200	930	2	360	8100	200	990	4	300	7225
9	240	477	5820	500	1600	3	280	9035	500	2400	2	290	11265
10	125	357	4350	260	840	2	210	5285	260	880	2	210	5310
11	58	257	3090	120	720	2	130	3375	120	360	4	110	2860
12	77	303	3690	160	1100	2	190	6025	160	1000	1	260	6590

Abbreviations: AUC_{IV} = area under the concentration–time curve (AUC) during continuous infusion; AUC_{PO} = AUC during oral administration; $DOSE_{IV}$ = dose of CsA during continuous infusion; $DOSE_{PO}$ = dose of CsA during oral administration.

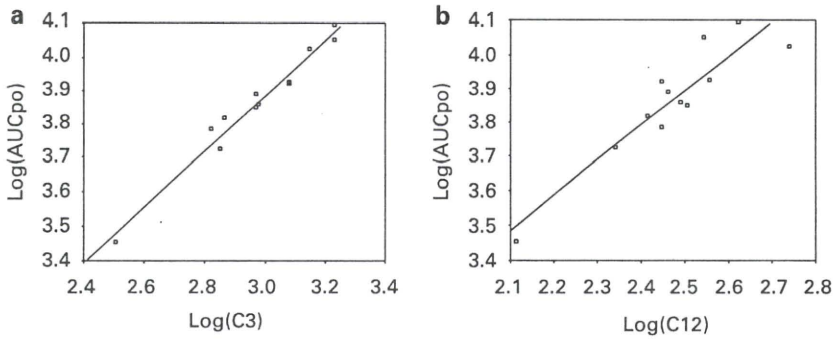

Figure 1 Correlation between the AUC and the CsA peak (**a**: C_3) and trough (**b**: C_{12}) levels.

In three patients (Nos. 1, 2, and 3), the dose of CsA was reduced on day 1 due to the high CsA concentration on day 0 (the day when Neoral was started).

The median AUC value was 6705 ng/ml × h (AUC_{IV}; range, 3090–8730) before the conversion from intravenous to oral administration (day −1), 8493 ng/ml × h (AUC_{IV-PO}; range, 3375–12 555) on day 0, and 7508 ng/ml × h (AUC_{PO}; range, 2860–12420) on days 3–5, respectively. AUC_{PO} was considered to be the AUC of Neoral in the steady state, as AUC_{IV-PO} was affected by the intravenous administration of CsA and at least 3 days are required for the CsA concentration to stabilize after a change in the administration route. As a result, not only AUC_{IV-PO} but also AUC_{PO} was significantly higher than AUC_{IV} ($P = 0.050$), even though the dose of Neoral was reduced in three patients and the conversion ratio was 1:1 in another patient. The median bioavailability of Neoral was 0.685 (range, 0.45–1.04).

Relationship between AUC and the CsA concentration at each measurement point

Although the CsA concentration at each measurement point significantly correlated with AUC_{PO} after logarithmic transformation, the strongest correlation was observed between C_3 and AUC_{PO} (Figure 1a and Table 2, correlation coefficient 0.984, $P < 0.001$). The AUC_{PO} could be predicted from the trough concentration (C_0 or C_{12}), which is widely measured in daily practice, by the following formula based on the linear regression model: $\text{Log}(AUC_{PO}) = 1.020 \times \text{Log}(C_{12}) + 1.344$ (Figure 1b). Accordingly, each trough concentration between 50 and 250 ng/ml corresponds to the CsA concentration during the continuous intravenous infusion of CsA with the same AUC, calculated by dividing the predicted AUC by 12, between 99 and 514 ng/ml (Table 3). Thus, when the continuous intravenous administration of CsA with a target concentration of 500 ng/ml was switched to twice-daily oral administration, the target trough level should be about 250 ng/ml to obtain the same AUC. Also, the target blood concentration of 300 ng/ml during continuous infusion corresponds to the target trough concentration at 150 ng/ml during twice-daily oral administration. This estimation was different from that in kidney transplantation by Nakamura *et al.* (Table 3).[12]

Influence of possible confounding factors on the bioavailability of Neoral

With regard to laboratory data, there were no statistically significant correlations between the bioavailability of Neoral and the serum Cr level, ALT level, and T-bil level

Table 2 Correlation coefficients between the AUC and the cyclosporine concentration at each measurement point

	Correlation coefficient	P-*value*	*Conversion formula*
C0	0.869	<0.001	$Log(AUCPO) = 0.846 \times Log(C0) + 1.747$
C1	0.874	<0.001	$Log(AUCPO) = 0.465 \times Log(C1) + 2.539$
C2	0.953	<0.001	$Log(AUC_{PO}) = 0.718 \times Log(C_2) + 1.693$
C3	0.984	<0.001	$Log(AUC_{PO}) = 0.821 \times Log(C_3) + 1.424$
C4	0.918	<0.001	$Log(AUC_{PO}) = 0.876 \times Log(C_4) + 1.319$
C6	0.961	<0.001	$Log(AUC_{PO}) = 1.314 \times Log(C_6) + 0.258$
C12	0.921	<0.001	$Log(AUC_{PO}) = 1.020 \times Log(C_{12}) + 1.344$

Abbreviation: AUC_{PO} = area under the concentration–time curve during oral administration.

Table 3 Target cyclosporine concentration during continuous infusion to obtain a similar AUC during twice-daily oral administration with each target trough concentration

Trough level of CsA during twice-daily oral administration (ng/ml)	*Corresponding CsA concentration during continuous infusion*	
	Nakamura et al.[12]	*Current study*
50	128	99
100	255	202
150	383	305
200	510	409
250	638	514

Abbreviation: AUC = area under the concentration–time curve.

($P = 0.867$, $P = 0.159$, and $P = 0.770$, respectively). Four patients had developed acute GVHD before the change in the route of CsA administration, but all of them had stage 1 skin GVHD that was successfully controlled by topical steroid. None of the patients had gastrointestinal involvement and thus the influence of gut GVHD on the bioavailability of Neoral could not be evaluated.

With regard to drug interactions, the effects of the following drugs on the bioavailability of Neoral were evaluated; antifungal agents including FLCZ, itraconazole (ITCZ), voriconazole (VRCZ), and MCFG, antibacterial agents including ST, vancomycin, fluoroquinolones (FQ), and cefepime, antiviral agents including ACV and ganciclovir (DHPG), and other drugs including amlodipine, sulpiride, gabapentin, and prednisolone (PSL) (Table 4). FLCZ ($n = 3$), ITCZ ($n = 3$), and VRCZ ($n = 4$) were exclusively administered orally. These agents had been started at least 7 days before the change in the route of CsA administration. By the Mann–Whitney *U*-test, VRCZ, FQ, and ST were shown to have significant effects with at least borderline significance ($P = 0.048$, $P = 0.061$, and $P = 0.100$, respectively). Among these, only VRCZ was identified as an independent significant factor by a multivariate analysis ($P = 0.017$). The median bioavailability of Neoral in patients taking VRCZ was 0.87 (range, 0.76–1.04), whereas it was only 0.54 (range, 0.45–0.94) in those without VRCZ.

Clinical course after the change in the route of CsA administration

One patient (No. 2) developed liver dysfunction with an elevation of ALT from 28 IU/l at baseline to 300 IU/l 2

Table 4 Clinical and laboratory data at the conversion that could influence the cyclosporine pharmacokinetics

Patient no.	AUC_{IV} / $DOSE_{IV}$	AUC_{PO} / $DOSE_{PO}$	*Bioavailability*	*aGVHD*			*Cr (mg per 100 ml)*	*Liver function*		*Concomitant medications*	
				Grade	*Stage*			*ALT (IU/l)*	*T-bil (mg per 100ml)*	*Antifungal agents*	*Others*
					Skin	*Liver*	*Gut*				
1	74	66	0.89	I	(1	0	0)	1.14	40	0.24	VRCZ 400 mg po	VCM, ST, ACV, PPI
2	55	28	0.51	0	(0	0	0)	0.65	28	0.9	ITCZ 200 mg po	ACV, PPI, FQ
3	47	49	1.04	0	(0	0	0)	0.81	182	0.77	VRCZ 400 mg po	ST, ACV, PPI, amlodipine gabapentin
4	46	35	0.76	I	(1	0	0)	0.98	28	1.06	VRCZ 400 mg po	ST, ACV, PPI, PSL
5	41	21	0.51	0	(0	0	0)	0.89	43	0.33	FLCZ 200 mg po	ACV, PPI
6	54	33	0.61	0	(0	0	0)	0.65	92	0.79	ITCZ 200 mg po	DHPG, PPI, amlodipine
7	79	38	0.48	I	(1	0	0)	0.72	85	0.59	ITCZ 200 mg po	DHPG, PPI, amlodipine
8	45	36	0.8	0	(0	0	0)	0.60	78	0.78	FLCZ 200 mg po	ACV, PPI
9	24	23	0.94	0	(0	0	0)	0.66	96	0.65	MCFG 150 mg iv	CFPM, ACV, PPI, amlodipine
10	35	20	0.57	0	(0	0	0)	1.43	46	0.37	FLCZ 200 mg po	CFPM, ACV, PPI
11	53	24	0.45	I	(1	0	0)	0.84	16	0.53	MCFG 150 mg iv	ACV, PPI, FQ, sulpiride
12	48	41	0.85	0	(0	0	0)	1.19	20	0.55	VRCZ 400 mg po	ACV, PPI

Abbreviations: ACV = acyclovir; ALT = alanine aminotransferase; AUC_{IV} = area under the concentration–time curve (AUC) during continuous infusion; AUC_{PO} = AUC during oral administration; CFPM = cefepime; DHPG = ganciclovir; $DOSE_{IV}$ = dose of CsA during continuous infusion; $DOSE_{PO}$ = dose of CsA during oral administration; FLCZ = fluconazole; FQ = fluoroquinolones; ITCZ = itraconazole; MCFG = micafungin; PPI = proton pump inhibitors; PSL = predonisolone; ST = sulphametoxazole-trimetoprim; VCM = vancomycin; VRCZ = voriconazole.