Prefectural Hospital Koseikan (Saga), Osaka University (Osaka), Iwate Prefectural Central Hospital (Iwate), Tokushima Red Cross Hospital (Tokushima), Toyama Prefectural Central Hospital (Toyama), Akita University (Akita), Musashino Red Cross Hospital (Tokyo), Kyoto Prefectural University of Medicine (Kyoto), Kawasaki Medical School Hospital (Okayama), University of Yamanashi (Yamanashi), Kyushu Kosei Nenkin Hospital (Fukuoka), Northern Fukushima Medical Center (Fukushima), Okayama University (Okayama), Kagawa University (Kagawa), National Kyushu Cancer Center (Fukuoka), Hirosaki University (Aomori), Hamanomachi Hospital (Fukuoka), Shinshu University (Nagano), Tenri Hospital (Nara), Gifu University (Gifu), Niigata University (Niigata), Hokkaido University (Hokkaido), Tokyo Hitachi Hospital (Tokyo), Kanazawa University (Ishikawa), Yamaguchi University (Yamaguchi), Kumamoto Medical Center (Kumamoto), Mie University (Mie), Koga General Hospital (Miyazaki), St. Luke's International Hospital (Tokyo), Tohoku University (Miyagi), Tokushima University (Tokushima)

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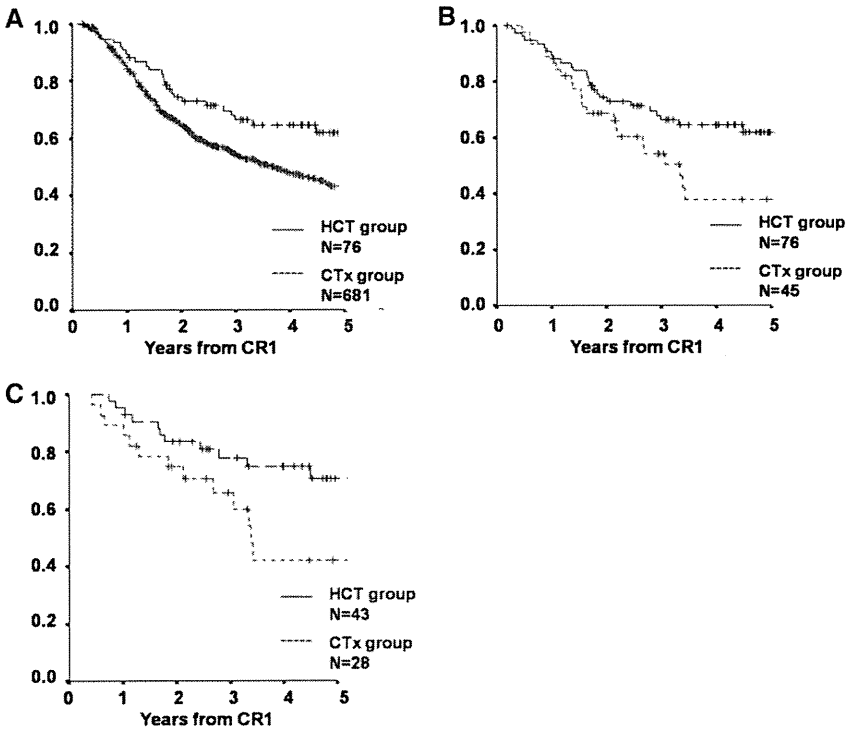
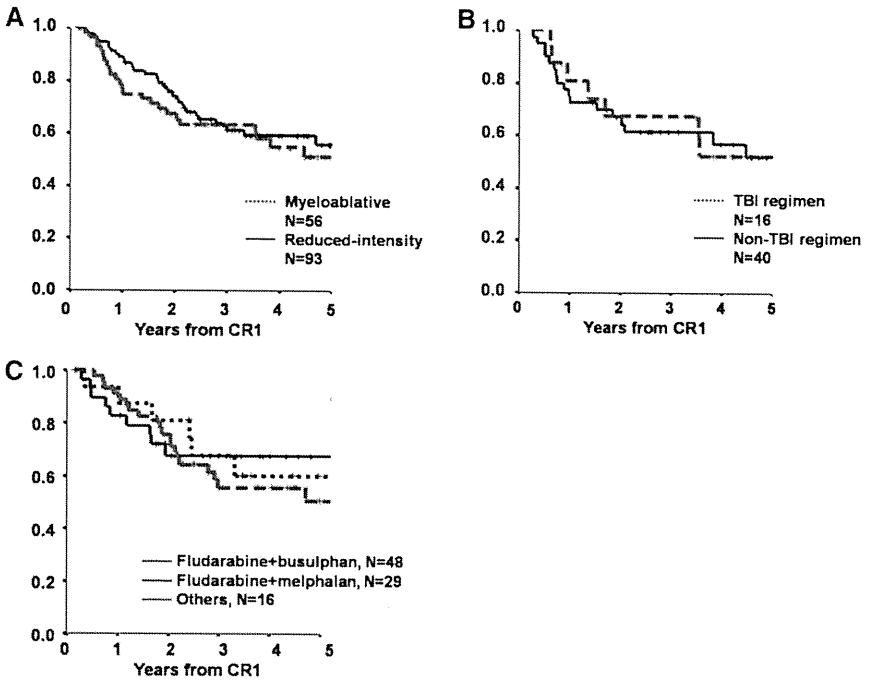


Appendix 1. Overall survival from CR1 are compared between the patients who received allogeneic transplantation in first complete remission and those who did not among the group of patients who had a suitable related donor. (A) Comparison of the two groups when 622 patients who did not have their HLA typed (those who were not known to have a suitable related donor) were included in the chemotherapy group (66% versus 54%, $P = .011$). (B) Comparison of the two groups when landmark was extended to 5 months from CR1 (66% versus 54%, $P = .068$). (C) Comparison of the two groups limited to intermediate-risk AML patients (78% versus 63%, $P = .048$).



Appendix 2. (A) Overall survival (OS) rates from CR1 are compared between myeloablative and reduced-intensity conditioning regimens. There were no significant differences between myeloablative and reduced-intensity conditioning regimens (63% versus 61%, $P = .571$). (B) OS did not differ significantly according to the application of total-body irradiation among patients who received myeloablative regimen (TBI regimen versus non-TBI: 67% versus 61%, $P = .932$). (C) Among patients who received reduced-intensity conditioning regimen, OS from CR1 did not differ significantly among different regimens (fludarabine + busulfan-based, 56%; fludarabine + melphalan-based, 67%; others, 68%, $P = .862$).

Mycophenolate and Tacrolimus for Graft-Versus-Host Disease Prophylaxis for Elderly After Cord Blood Transplantation: A Matched Pair Comparison With Tacrolimus Alone

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Background. The optimal graft-versus-host disease (GVHD) prophylaxis after umbilical cord blood transplantation has not been established. Our previous observation using single calcineurin inhibitors revealed a high incidence and severity of early immune-mediated complications, especially for older patients or those with poor performance status.
Methods. We conducted a single institute pilot study assessing the safety and effectiveness of mycophenolate mofetil (MMF) and tacrolimus (FK) combination as a GVHD prophylaxis for 29 patients (FK+MMF), and the results were compared with matched-pairs extracted from our historical database who received FK alone as GVHD prophylaxis (control).
Results. FK+MMF group showed superior engraftment rate compared with control group (cumulative incidence until day 60 posttransplant; 90%±0% vs. 69%±1%, $P=0.02$). A cumulative incidence of severe type preengraftment immune reactions was significantly decreased in FK+MMF group (16%±1%) compared with that of control group (52%±2%, $P=0.03$), and, remarkably, there was no nonrelapse mortality (NRM) observed up to day 30 posttransplant in FK+MMF group, whereas 21%±1% of NRM was observed in the control group. However, the incidences of acute and chronic GVHD, estimated overall and progression-free survivals were comparable between two groups.
Conclusions. MMF and FK in combination was well tolerated and decreased early NRM possibly by better control of preengraftment immune reactions. Subsequent NRM or disease progression needs to be overcome to further improve survival.

Keywords: Cord blood transplantation, GVHD prophylaxis, Mycophenolate mofetil, Tacrolimus, Elderly patients.

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Although umbilical cord blood transplantation (UCBT) has been increasingly used as a curative treatment of hematological diseases, accompanying toxicity, especially early period posttransplant, has been a major problem (*1*, *2*). Our previous observation indicated that elderly patients were more vulnerable to early toxicity posttransplant, with nonrelapse mortality (NRM) being a major cause of treatment failure (*3*). Early immune-mediated complications, termed preengraftment immune reactions (PIR), were significant factors that negatively affected overall survival (OS) (*3–5*).

Various immunosuppressive drugs have been used for graft-versus-host disease (GVHD) prophylaxis in UCBT, including mycophenolate mofetil (MMF), (*6–8*) methotrexate (MTX), (*9–11*) corticosteroids, (*11*) anti-thymocyte globulin, (*12*, *13*), and sirolimus (*14*); mostly in combination with calcineurin inhibitors. So far, no available data indicate that one drug or combination is better than the other.

MMF is an inosine monophosphate dehydrogenase inhibitor that exerts its immunosuppressive effect by blocking the production of guanosine nucleotide synthesis through the

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N.U. performed research design, data analysis, and writing the manuscript; A.W., N.N., K.I., S.T., M.T., H.Y., D.K., N.M., K.M., H.A., Y.A.M., K.I., S.M., and A.Y. participated in the performance of the research; A.W. and S.T. contributed with data analysis and writing of the manuscript; and all authors reviewed the manuscript.

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de novo pathway (*15*). It has been extensively used in solid organ transplantations (*16*) and more recently, in hematopoietic cell transplantation (HCT) (*7, 17–19*). In HCT, less mucosal damage compared with MTX has been observed, (*19–21*) with a comparable incidence of GVHD, suggesting a potential advantage of MMF over MTX. It therefore seemed rational to incorporate MMF in reduced-intensity (RI) UCBT for patients at high risk for NRM. Since December 2005, MMF+tacrolimus (FK) combination was started to be used as GVHD prophylaxis in RI-UCBT as a pilot study for those who agreed to participate. The results were compared with that of those who performed RI-UCBT using FK alone extracted as matched pairs from our historical database.

RESULTS

Patients/Matched Controls

Table 1 shows the demography of the patient characteristics of two groups. A total of 89% of the control patients who had GVHD prophylaxis of FK alone were transplanted from 2004 to 2005, whereas 93% of the patients with FK+MMF were from 2006 to 2007 ($P<.0001$). The differences between the groups did not reach statistical significance in Eastern Cooperative Oncology Group (ECOG) performance status (PS), HCT-specific comorbidity index (HCT-CI) score, history of previous HCT, human leukocyte antigen (HLA) disparity to UCB, and conditioning regimen. The median FK concentrations (11.9±0.33 ng/mL in FK+MMF group vs. 12.6±0.47 ng/mL in control group, $P=0.46$) and the proportions of FK concentration more than or equal to 10 ng/mL during day 0 to the date of engraftment (72.4%±3.1% in FK+MMF group vs. 75.0%±4.0% in control group, $P=0.43$) were comparable in each group.

Engraftment

Twenty-seven patients in FK+MMF group achieved neutrophil engraftment, and all except 1 showed complete donor chimerism. The cumulative incidence of primary engraftment until day 60 posttransplant was 90%±0%, whereas that of control group was 69%±1% ($P=0.02$). Median time to engraftment was 19 days after transplantation both in FK+MMF group (range, 13–32 days) and control group (range, 12–33 days). Among the two patients in FK+MMF group who failed to engraft, one experienced disease recurrence before day 28, and the other experienced rejection of donor cells and was later found to have anti-HLA antibodies against one of the antigens expressed on donor cells. One patient in FK+MMF group who showed mixed chimerism on neutrophil engraftment, when 87.2% of total bone marrow (BM) cells were of donor origin, experienced early BM relapse of leukemia on day 30 posttransplant. There were three patients in control group who experienced hemophagocytic syndrome (HPS) early after transplant and resulted in early death before engraftment, whereas there was no such cases observed in FK+MMF group. Platelet recovery more than 20×10^9/L was observed in 17 patients, with a cumulative incidence of 59%±1% at day 100 posttransplant (median, 40 days; range, 25–70 days), whereas in control group, the cumulative incidence was 52%±1% (median, 40 days; range, 26–62 days, $P=0.69$).

TABLE 1. Patient, treatment, and donor umbilical cord blood characteristics

Characteristic	N (%) of patients FK+MMF	Control	*P*
Sex			0.38
Male	21 (72)	23 (79)	
Female	8 (28)	6 (21)	
Age (yr)			0.67
Median (range)	62 (52–70)	63 (56–69)	
Age distribution (yr)			
51–55	5 (17)	0	
56–60	4 (14)	9 (31)	
61–65	12 (41)	13 (45)	
66–70	8 (28)	7 (24)	
Diagnosis			0.11
AML/MDS	19 (66)	16 (55)	
ALL	2 (7)	5 (17)	
ML	5 (17)	5 (17)	
CML	0	3 (10)	
AA	3 (10)	0	
ECOG performance status			0.37
0	0	0	
1	22 (76)	17 (59)	
2	5 (17)	9 (31)	
3	2 (7)	3 (10)	
HCT-CI			0.25
0	9 (31)	18 (62)	
1	12 (41)	7 (24)	
2	1 (3)	1 (3)	
≥3	7 (24)	3 (10)	
Disease status			0.78
Standard risk	10 (34)	9 (31)	
High risk	19 (66)	20 (69)	
History of prior HCT			0.16
None	22 (76)	26 (90)	
Autologous	4 (14)	3 (10)	
Allogeneic	3 (10)	0	
Year of transplant			<0.0001
2004	0	11 (38)	
2005	2 (7)	12 (41)	
2006	7 (24)	6 (21)	
2007	20 (69)	4 (14)	
Conditioning regimen[a]			
Flu/Mel 140	8 (28)	1 (3)	
Flu/Mel 80-140/TBI 2-8	13 (45)	25 (86)	
Flu/Mel 80/Tespa 10	0	1 (3)	
Flu/Mel 80-140/Bu 8-16	4 (14)	0	
Flu/Bu 16	0	1 (3)	
Flu/Bu 8-16/TBI 2-4	3 (10)	1 (3)	
Flu/Bu 8/VP-16 450	1 (3)	0	
HLA disparity to UCB			0.22
0 antigen mismatch	1 (3)	1 (3)	
1 antigen mismatch	5 (17)	1 (3)	
2 antigen mismatch	23 (79)	27 (93)	
Total nucleated cell number			0.66
Median ($\times10^7$/kg)	2.4	2.31	
Range ($\times10^7$/kg)	2.0–4.5	1.91–4.76	
$CD34^+$ cell number			0.15
Median ($\times10^5$/kg)	0.9	0.81	
Range ($\times10^5$/kg)	0.11–2.32	0.11–1.9	

[a] Units for each number are as follows: Mel (mg/m^2), TBI (Gy), Tespa (mg/kg), Bu doses: oral (1 dose=1 mg/kg) or iv (1 dose=0.8 mg/kg), and VP-16 (mg/m^2).

AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukemia; ML, malignant lymphoma; CML, chronic myeloid leukemia; AA, aplastic anemia; HCT-CI, hematopoietic cell transplantation-specific comorbidity index; Flu, fludarabine; Mel, melphalan; TBI, total body irradiation; Bu, busulfan; VP-16, etoposide; and UCB, umbilical cord blood; FK, tacrolimus; MMF, mycophenolate mofetil; ECOG, Eastern Cooperative Oncology Group; HLA, human leukocyte antigen.

TABLE 2. Incidence of PIR and GVHD

	FK+MMF (N)	Control (N)
PIR (n=29)		
No. of evaluable[a]	29	28
Yes	22	23
Severe type	4	10
Acute GVHD		
No. of evaluable[b]	27	20
Grade I	4	4
Grade II	7	2
Grade III	7	5
Grade IV	4	3
Chronic GVHD		
No. of evaluable[c]	13	11
Limited	1	2
Extensive	1	2

[a] Those who showed clinical symptoms characteristic to PIR, and those who survived longer than 27 d posttransplant without PIR.

[b] Those who engrafted without disease progression.

[c] Those who survived beyond day 100 posttransplant without disease progression.

PIR, preengraftment immune reactions; GVHD, graft-versus-host disease; FK, tacrolimus; MMF, mycophenolate mofetil.

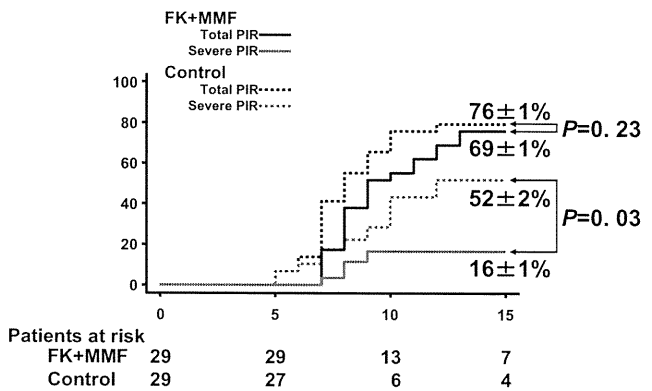

FIGURE 1. Cumulative incidences of preengraftment immune reactions (PIR) after RI-UCBT according to tacrolimus (FK)+mycophenolate mofetil (MMF) or FK alone graft-versus-host disease (GVHD) prophylaxis. The overall incidences of PIR in FK+MMF group (*black solid line*), in control group (*black dotted line*), and the incidences of severe type of PIR in FK+MMF group (*gray solid line*), and in control group (*gray dotted line*) were plotted. There was significant reduction of severe type of PIR in FK+MMF group compared with that in control group (P=0.03).

PIR and GVHD

In FK+MMF group, 22 of 29 patients experienced clinical symptoms defined as PIR, whereas in control group, 23 of 28 evaluable patients did (Table 2). Cumulative incidences of PIR in both groups were comparable each other (76%±1% in control group and 69%±1% in FK+MMF group, P=0.23, Fig. 1) and were similar to that reported in our previous publication (*3*). However, the cumulative incidence of severe type of PIR, defined by the criteria described in materials and methods section, in the FK+MMF group was lower (16%±1%) than that of control group (52%±2%) with statistical significance (P=0.03, Fig. 1).

TABLE 3. Causes of death

	FK+MMF, N (%)	Control, N (%)
NRM	9 (45)	11 (65)
GVHD	5 (25)	3 (18)
IPS	4 (20)	1 (6)
Infection	0	5 (29)
CNS complication	0	2 (12)
Relapse/disease progression	11 (55)	6 (35)
Total	20	17

FK, tacrolimus; MMF, mycophenolate mofetil; NRM, nonrelapse mortality; GVHD, graft-versus-host disease; IPS, idiopathic pneumonia syndrome; CNS, central nervous system.

In FK+MMF group, 22 of 27 evaluable patients developed acute GVHD, and 18 of them were grade II and higher. In control group, 14 of 20 evaluable patients had acute GVHD, and 10 of them were grade II and higher (Table 2). Cumulative incidences of grade II and higher acute GVHD at day 100 posttransplant were 63%±1% in FK+MMF and 35%±1% in control group (P=0.09). Chronic GVHD was observed in two of 13 FK+MMF group and four of 11 control group patients who survived longer than 100 days posttransplant without disease progression (Table 2). Cumulative incidences of chronic GVHD at 2 years posttransplant were 7%±0% in FK+MMF and 16%±1% in control group (P=0.35).

Survival, Disease Progression, and NRM

At the time of analysis, 9 FK+MMF group patients survived for a median of 980 days (range, 145–1430 days) after transplantation, whereas 12 control group patients were alive for a median of 1073 days (range, 49–2071 days). The Kaplan-Meier estimates of OS and progression-free survival (PFS) at 2 year posttransplant in FK+MMF group were 33%±9% and 21%±8%, whereas those in control group were 45%±10% and 34%±9%, respectively. The differences were not statistically significant (P=0.83 for OS, and P=0.75 for PFS).

Thirteen patients in FK+MMF group showed progression of the underlying disease at a median of 84 days (range, 19–344 days) after transplantation, and 11 of these patients died of the disease (Table 3). In control group, 9 patients did so at a median of 126 days (range, 12–1084 days) and 6 died of the disease. The cumulative incidences of disease progression at 2 years were 46%±1% in FK+MMF group and 29%±1% in control group, respectively (P=0.29).

Nine in FK+MMF group died of nonrelapse causes, whereas in control group patients, 11 NRM were observed (Table 3). GVHD and noninfectious pulmonary complications were observed in both groups as cause of death. None of the FK+MMF group died from infections as a sole reason of death, whereas five of the control group did. There was no death before day 30 posttransplant in FK+MMF group, whereas six in control group did. The cumulative incidences of NRM at day 30, 100, 365 were 0%±0%, 21%±1%, 28%±1% in FK+MMF group, and 21%±1%, 35%±1%,

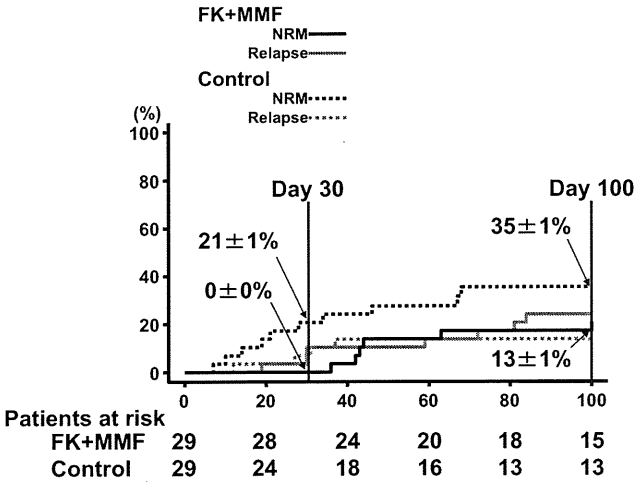

FIGURE 2. Day 100 nonrelapse mortality (NRM) and disease progression. Cumulative incidence estimates of NRM (*black line*) and disease progression (*gray line*) up to day 100 posttransplant for tacrolimus (FK)+mycophenolate mofetil (MMF) group (*solid line*) and control group (*dotted line*) were plotted. There were no NRM within 30 days posttransplant in FK+MMF group, whereas 21%±1% NRM were estimated in control group (P=0.01).

39%±1% in control group, respectively (P=0.01, P=0.17, P=0.29, Fig. 2).

DISCUSSION

The most remarkable observation in this study was that higher rate of neutrophil recovery and no early deaths before day 30 posttransplant were observed in FK+MMF group despite the patients' poor conditions before transplant, that is, all were older than 50 years and 69% of them had some comorbidities. Although the incidence of PIR in FK+MMF group was comparable with control group, the severity of PIR was less and thus did not result in severe organ damage early after transplant. There was no death directly caused by infections in FK+MMF group. We have reported higher incidence of HPS after RI-UCBT, which has been reasoned to be the delayed engraftment or graft failure (*22*). Interestingly, majority of the suffered BM cells were donor cell dominant, indicating HPS was mediated by donor-derived immune cells. Moreover, we have reported HLA mismatch in GVH direction, not in host-versus-graft direction, affected negatively to successful engraftment (*23*). All these facts fit well to the idea that hyperimmune reactions caused by donor cord blood (CB) cells may play crucial role in high rate of early NRM. Because there were no case of HPS in FK+MMF group, MMF may have promoted engraftment by sufficiently suppressing immune reactions of CB cells and preventing development of hemophagocytosis, which may also have reduced the incidence of severe infections. The presence of this type of hyperimmune reactions after UCBT has recently been recognized by others (*24*). The differences in incidence of PIR may have been affected by agents included in pretransplant conditioning, such as antithymocyte globulin, or by GVHD prophylaxis including corticosteroids or intravenous MMF.

Despite the present observation that the combination of MMF and FK succeeded in reducing early NRM, the incidence and severity of GVHD was not altered. Because most of the patients in the present study had advanced disease status, MMF was discontinued or started to be tapered on the day of neutrophil engraftment, which may have been responsible for this results. Much longer administration of MMF has been used in the setting of matched unrelated BM/peripheral blood (PB) transplantation (*7*). In addition, MMF was administered at 15 mg/kg twice daily in this study, which is the common dosing schedule in the settings of solid organ transplant (*16*). Several recent reports from Minnesota and Seattle considered 15 mg/kg three times daily as more appropriate based on pharmacokinetic data obtained from HCT recipients (*7, 17, 25*). A serum concentration measurement of mycophenolic acid, which was not assessed in this study, is needed to determine the optimal dosing of MMF.

Although NRM early after UCBT was significantly reduced in FK+MMF group, OS and PFS at 2 year posttransplant were still comparable with those of control group. Fifty-five percent of the deaths were from disease relapse or progression. Although MMF may have a beneficial effect on early survival after transplant by reducing severe immune reactions, it may increase the risk of disease progression for those who have active disease with a high risk of disease recurrence. According to previous publications, relapse rate is comparable in CB and unrelated BM/PB recipients despite lower incidences of chronic GVHD in CB recipients (*26, 27*), early immune reactions may have impact on reducing disease relapse. Because this is a relatively small sized, retrospective study, the presence of uncontrolled bias cannot be excluded. Prospectively conducted larger studies are warranted to further confirm the results.

In conclusion, MMF, used in combination with FK as GHVD prophylaxis in elderly patients with advanced hematologic diseases with or without comorbidities, may reduce early mortality posttransplant by regulating severe PIR and thus protecting patients from severe organ damage or HPS. An optimal dosing schedule of MMF needs to be determined prospectively using more homogenous populations.

MATERIALS AND METHODS

Patients

The initial pilot study included patients aged 51 years and older who underwent RI-UCBT using MMF+FK combination as GVHD prophylaxis at our institute from December 2005 through December 2007. Patients were eligible for this study if they had any hematologic malignancies at high risk for relapse or severe aplastic anemia refractory to standard immunosuppressive therapy and were unable to find suitable related or unrelated BM/PB donors within reasonable periods relative to their disease conditions. Patients with acute leukemia could be at first remission but at high risk for relapse due to adverse cytogenetic abnormalities, have a previous hematologic disorder, or be at any status beyond first remission. Patients with myelodysplastic syndrome (MDS) had to be refractory anemia (RA) with excess of blasts or chronic myelomonocytic leukemia, or have RA with transfusion dependency or severe neutropenia. Malignant lymphoma (ML) patients had to be beyond first remission. Patients who had end-stage cardiac dysfunction (left ventricular ejection fraction <35%), pulmonary dysfunction (SpO_2 <90% in room air), or active serious infection at the time of transplantation were not eligible. All patients gave written informed consent. Twenty-nine patients were enrolled and subjected to the matched pair analysis as below.

Selection of Matched Controls and Matching Variables

A matched-pair control group (GVHD prophylaxis with FK alone) for 29 patients who used MMF+FK combination was obtained by selecting one of the most recently transplanted control patients from our historical RICBT database from 2004 to 2007 after excluding those who met exclusion criteria of the pilot study described earlier. Controls were individually matched to cases on a 1:1 ratio. Matching was attempted for the following criteria applied in the order listed: age at transplantation (51–60, 61–70 years), disease risk (standard risk vs. high risk, acute leukemia, chronic myeloid leukemia, or ML in complete remission, MDS RA, aplastic anemia patients were categorized as standard risk, and all the others were as high risk), ECOG PS (PS 0–1, 2–3), pretransplant conditioning (busulfan containing vs. others), number of serological HLA mismatch (0–1, 2), HCT-CI (0–1, ≥2), total nucleated cell dose infused (≤2.3, $>2.3\times10^7$/kg), and $CD34^+$ cell dose infused (≤0.8, $>0.8\times10^5$/kg). To avoid any potential selection bias, matching was blinded, and only the patient's initials and pretreatment variables were known. This retrospective analysis was approved by the institutional review board.

One hundred percent matching was achieved for age group; 97% for disease risk (high risk, 66% of FK+MMF patients vs. 69% of control patients; $P=0.78$); 83% for ECOG PS (≥2 score, 32% of FK+MMF patients vs. 41% of FK alone patients; $P=0.16$); 72% for HCT-CI (≥2 score, 28% of FK+MMF patients vs. 14% of control patients; $P=0.19$); and number of serological HLA mismatch (2 antigens, 79% of FK+MMF patients vs. 93% of control patients; $P=0.13$); 86% for pretransplant conditioning (inclusion of busulfan, 24% of FK+MMF patients vs. 7% of control patients; $P=0.07$); 69% for total nucleated cell dose ($\leq2.3\times10^7$/kg, 41% of FK+MMF patients vs. 45% of control patients; $P=0.79$); and 62% for $CD34^+$ cell dose ($\leq0.8\times10^5$/kg, 45% of FK+MMF patients vs. 48% of control patients; $P=0.79$). Characteristics of the studied patients in both groups were shown in Table 1. Patients' comorbidity was assessed by a previously reported scoring system (*28*).

Donor Selection

UCB units were obtained from the Japanese Cord Blood Bank Network. HLA-A, -B, and -DR antigens were identified by serologic typing. UCB grafts had at least four of six HLA-A, -B, and -DR antigens that were matched to the recipient and had a cryopreserved cell dose of at least 1.9×10^7 nucleated cells per kg of recipient body weight.

Conditioning Regimens and Postgrafting Immunosuppression

Pretransplant conditionings were primarily RI regimens including 125 to 180 mg/m^2 of fludarabine (25 mg/m^2 for 5 days or 30 mg/m^2 for 6 days). Antithymocyte globulin was not incorporated. Granulocyte colony-stimulating factor (G-CSF) was started on day 1 posttransplant. Detailed information is shown in Table 1. Immunosuppressive therapy with FK (0.03 mg/kg continuous infusion, aiming for 12 to 17 ng/mL by at least three times a week measurement) with or without MMF (15 mg/kg twice daily) were started on day −1. MMF was discontinued or started to taper down on the day of neutrophil engraftment in the absence of active GVHD.

Definition of Engraftment, Preengraftment Immune Reactions, and Endpoints

Engraftment was defined as absolute neutrophil count more than 0.5×10^9/L for 3 consecutive days. Chimerism was assessed using fluorescent in situ hybridization in sex-mismatched donor-recipient pairs, or polymerase chain reaction for a variable number of tandem repeats with donor cells detected at a sensitivity of 10% in sex-matched pairs. Whole blood or BM cells were assessed at the time of granulocyte engraftment. Complete donor-type chimerism was defined when donor cells consisted of more than 90% of analyzed cells. PIR was characterized by the presence of at least three of the following symptoms with no direct consequences of infection or adverse effects of medication six or more days before engraftment, as described previously (*4*, *5*): a high fever (>38.5°C), skin eruptions, body weight gain greater than 5% of baseline, or peripheral edema. Those who had all four symptoms and at least two of the following criteria indicating severe organ damage were classified as severe type; (1) SpO_2 less than 92% or pleural/pericardial effusions present; (2) serum creatinine level more than or equal to 3 times of baseline; (3) total bilirubin level more than 3 mg/dL or asparatate aminotransferase/alanine aminotransferase levels more than three times of upper limit of normal; and (4) development of hemophagocytosis in BM.

The main parameters analyzed between groups were as follows: (1) cumulative incidences of neutrophil or platelet engraftment; (2) cumulative incidences of NRM and relapse; (3) incidences of PIR, acute and chronic GVHD; and (4) overall and progression-free survival (OS and PFS). The analysis was performed as of April, 2010. OS was calculated from the day of transplantation until death from any cause or last follow-up. PFS was calculated from the day of transplantation until relapse, second transplantation due to engraftment failure, or death from any cause or last follow-up. NRM was defined as death in the absence of disease progression. Deaths occurring after disease progression were categorized as relapse regardless of the cause of death. Infection was considered the cause of death when bacterial, viral, or fungal infection was determined to be the proximate cause of death in patients who had not relapsed. Patients underwent BM aspiration at the time of engraftment or if clinically indicated. Relapse for acute myeloid leukemia, acute lymphoblastic leukemia, MDS, or chronic myeloid leukemia was determined by flow cytometric, morphologic, or cytogenetic evidence of malignant or dysplastic cells with clonal markers similar to those observed before transplantation. Relapse for ML was defined as progressive adenopathy or BM involvement. Acute and chronic GVHD were defined and graded by standard criteria (*29*). Relapse and NRM rates were estimated using cumulative incidence analysis and were considered competing risks (*30*). Similarly, in the analysis of PIR rates, death due to other causes or relapse leading to early withdrawal of immune suppression were considered competing risks.

Statistical Methods

Chi-square test was used to compare patient characteristics between two groups in matched-pair analysis. For continuous variables, Mann-Whitney nonparametric test was used. The probabilities of OS and PFS were estimated and plotted using the Kaplan-Meier method (*31*). Cumulative incidence curves were drawn using Gray's method (*32*). The level of significance in all cases was set at P less than 0.05. The effect of various categoric variables on survival probabilities was studied with the log-rank test. A Cox proportional hazard model with limited variables because of small sample was used to determine the significance of multiple variables in determining these outcomes. All analyses were carried out using StatView statistical software for Kaplan-Meier curve, and S-PLUS software (Mathsoft, Seattle, WA) for cumulative incidence curve.

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Brief report

Successful sustained engraftment after reduced-intensity umbilical cord blood transplantation for adult patients with severe aplastic anemia

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We retrospectively analyzed 12 consecutive adult severe aplastic anemia patients who received unrelated umbilical cord blood transplantation after a reduced-intensity conditioning regimen (RI-UCBT). The conditioning regimen consisted of 125 mg/m^2 fludarabine, 80 mg/m^2 melphalan, and 4 Gy of total body irradiation. The median infused total nucleated cell number and CD34$^+$ cell number were 2.50 × 10^7/kg and 0.76 × 10^5/kg, respectively. Eleven of the 12 patients achieved primary neutrophil and platelet engraftment. All patients who achieved engraftment had complete hematologic recovery with complete donor chimerism, except for one patient who developed late graft failure 3 years after RI-UCBT. Two of the 12 patients died of idiopathic pneumonia syndrome, and the remaining 10 patients are alive, having survived for a median of 36 months. Our encouraging results indicate that RI-UCBT may become a viable therapeutic option for adult severe aplastic anemia patients who lack suitable human leukocyte antigen-matched donors and fail immunosuppressive therapy. (*Blood*. 2011;117(11):3240-3242)

Introduction

Bone marrow transplantation from a human leukocyte antigen (HLA)-matched sibling is recommended as first-line therapy for younger patients with severe aplastic anemia (SAA).[1,2] However, many patients lack HLA-matched sibling donors. Bone marrow transplantation from an HLA-matched unrelated donor has been an alternative therapeutic option for patients who fail one or more courses of immunosuppressive therapy, but high rates of graft failure (GF), graft-versus-host disease (GVHD), and infection still remain to be solved.[3] The number of unrelated umbilical cord blood transplantations (UCBTs) has been increasing.[4] However, little information has been available on whether UCBT is feasible for SAA patients. We reported successful urgent UCBT using reduced-intensity (RI) conditioning for a 70-year-old SAA patient in 2003.[5] Here we present successful sustained engraftment of 11 consecutive patients with SAA who received RI-UCBT with the same RI conditioning regimen after the first report.

Methods

This study included 12 consecutive adult patients with acquired SAA who underwent RI-UCBT at our institute from September 2002 through January 2009. The patients' characteristics and umbilical cord blood (UCB) units are summarized in Table 1. Their median age was 49 years (range, 20-70 years). Four cases of severe, 6 of very severe, and 2 of fulminant type were included according to criteria as previously reported.[2,6] Fulminant type was defined as no neutrophils in the peripheral blood at diagnosis despite administration of granulocyte-colony stimulating factor. Ten patients, except for the 2 patients with fulminant type, had failed at least one course of immunosuppressive therapy. All patients gave their written informed consent in accordance with the Declaration of Helsinki, and the study was approved by the Toramon Hospital Institutional Review Board. UCB units were obtained from the Japanese Cord Blood Bank Network, and single UCB unit was infused in all the studied patients. All UCB units were serologically typed for HLA-A, -B, and -DR antigen before selection and were tested by high-resolution DNA typing before transplantation. The degree of mismatch is expressed using antigen level at HLA-A and -B, and allele level at DRB1. ABO incompatibility was not incorporated as one of the factors used in CB unit selection. The median total nucleated cell number and CD34$^+$ cell number at cryopreservation were 2.50 × 10^7/kg (range, 1.83-4.39 × 10^7/kg) and 0.76 × 10^5/kg (range, 0.27-1.52 × 10^5/kg), respectively. Anti-HLA antibodies were screened before transplantation 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 25 mg/m^2 fludarabine daily for 5 days, 40 mg/m^2 melphalan daily for 2 days, and 4 Gy of total body irradiation 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 was estimated using the Kaplan-Meier method.

Results and discussion

Patients' outcomes are summarized in Table 2. Eleven of the 12 patients achieved primary neutrophil and platelet engraftment. The median times to achieve neutrophil engraftment and platelet count more than 20 × 10^9/L were 18 days (range, 12-28 days) and

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Table 1. Characteristics of patient, grafts, and GVHD prophylaxis

Case no.	Age, y	Previous treatment	Interval from diagnosis to UCBT, mo	Previous transfusion times (RBCs/platelet)	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	NT	A/A	4.00	1.23	CSA
2	20	ATG + CSA	78	> 20/> 20	VSAA	4/6	NT	B/O	2.65	1.07	CSA
3	22	ATG + CSA, PSL	157	> 20/> 20	SAA	4/6	NT	A/O	2.26	0.27	Tac
4	26	ATG + CSA	3	> 20/> 20	VSAA	5/6	NT	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	NT	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	NT	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

RBC indicates red blood cell; CB, cord blood; R, recipient; D, donor; TNC, total nucleated cells; CSA, cyclosporine-A; ATG, antithymocyte globulin; PSL, prednisone; VSAA, very severe aplastic anemia; NT, not tested; Tac, tacrolimus; and MMF, mycophenolate mofetil.

42 days (range, 26-64 days), respectively. All patients who achieved engraftment had complete hematologic 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 transplantation was not detected (Tables 1-2). 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 after transplantation, 3 developed limited type of chronic GVHD. No patients developed grade III-IV acute GVHD and extensive type of chronic GVHD. Two of the 12 patients died of 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 overall survival at 3 years was 83.3% (Figure 1). The surviving patients had high Karnofsky performance status score with a median of 90% (range, 60%-100%).

The present study demonstrated that our RI conditioning regimen allows a sufficient sustained engraftment of UCB in adult SAA patients. The RI conditioning regimen was originally developed in our institute for UCBT for various hematologic malignancies.[9] Eleven of the 12 patients achieved primary engraftment, which compares favorably with previously reported engraftment rates of UCBT for SAA.[11-16] Our RI conditioning regimen would be more potent than the others to overcome immunologic 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 (total nucleated cell, ≥ 2.5 vs $< 2.5 \times 10^7$/kg; CD34$^+$, ≥ 0.8 vs $< 0.8 \times 10^5$/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 posttransplantation immunosuppression, may be important to achieve better engraftment after UCBT for SAA patients. Interestingly, all 6 patients who were screened for HLA antibodies before transplantation 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 hematologic malignancies, the

Table 2. Outcomes of 12 patients after reduced-intensity unrelated cord blood transplantation

Case no.	Days to ANC > 0.5 × 10^9/L	Days to PC > 20 × 10^9/L	% Donor chimerism (days tested, methods)	aGVHD	cGVHD	Discontinuation of IS (mo)	Complications	Survival (mo)
1	12	52	100 (14, FISH)	Grade II (skin)	No	Yes (3)	Possible IPA	Alive (91)
2	20	64	> 90 (49, PCR-STR)	Grade II (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 second RI-UCBT	Alive (69)
5	16	26	96.6 (14, FISH)	Grade I (skin)	Limited	Yes (14)	Norwalk virus colitis, EBV-PTLD	Alive (39)
6	28	64	99.6 (11, FISH)	No	NE	No	IPS	Dead; IPS (3)
7	No	No	48.8 (10, FISH), 4.3 (15, FISH)	NE	NE	NE	Primary GF, rescued by second RI-UCBT	Alive (32)
8	18	28	99.2 (13, FISH)	Grade II (skin, gut)	No	Yes (7)	CMV colitis, EBV-PTLD	Alive (28)
9	28	43	> 90 (14, PCR-STR)	Grade I (skin)	NE	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)	Grade II (skin, gut)	No	No	No	Alive (22)
12	13	28	100 (14, FISH)	Grade II (gut)	No	No	No	Alive (14)

ANC indicates absolute neutrophil count; PC, platelet count; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; IS, immunosuppressant; FISH, fluorescence in situ hybridization; PCR-STR, PCR of short tandem repeat; NE, not evaluable; IPA, invasive pulmonary aspergillosis; EBV-PTLD, Epstein-Barr virus–associated posttransplantation lymphoproliferative disorder; and IPS, idiopathic pneumonia syndrome.

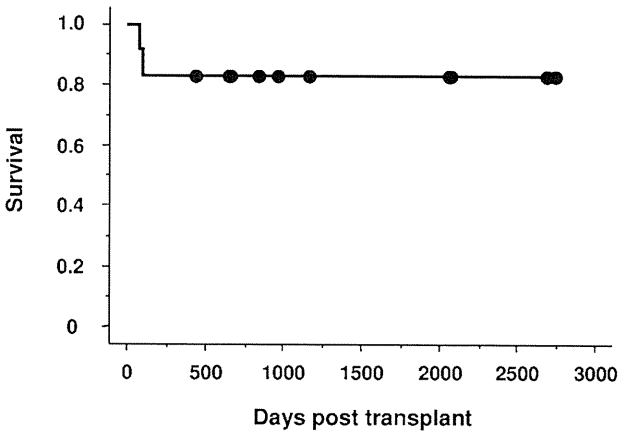

Figure 1. Survival of 12 patients with SAA undergoing unrelated cord blood transplantation.

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 antibody-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 quality of life, and 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; 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 immunosuppressive therapy. Although our results should be interpreted with caution because of the small number of patients and still short follow-up duration, we think that RI-UCBT with the conditioning regimen presented here deserves further evaluation in a prospective trial, hopefully in a multicenter setting.

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Authorship

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

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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

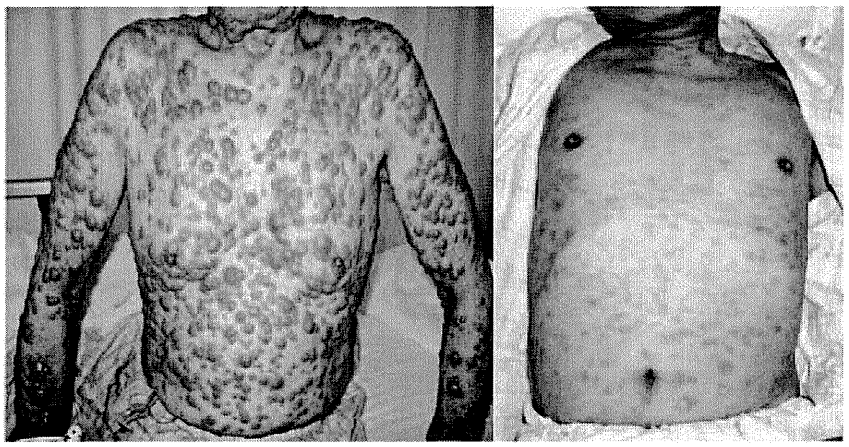

Figure 1 Skin tumors covering whole body of the patient just before RI-UCBT (**a**). Skin tumors of the patient had disappeared in 90 days after RI-UCBT (**b**).

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-based optimal dose prediction of donor source-dependent response to mycophenolate mofetil in unrelated hematopoietic cell transplantation

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Abstract Mycophenolate mofetil (MMF) has been widely used for prophylaxis against graft-versus-host disease (GVHD) following allogeneic hematopoietic stem cell transplantation (allo-SCT). However, no clear advantage over methotrexate has been reported, other than reduced incidence of mucositis. We speculated that the wide inter-individual variation of plasma mycophenolic acid (MPA) levels veiled the benefits of MMF. Data from 36 unrelated allogeneic bone marrow (allo-BMT) and cord blood transplantation (CBT) were analyzed retrospectively based on MPA area under the curve (AUC_{0-24h}). In allo-BMT, high AUC_{0-24h} (>30 μg h/ml) resulted in no incidence of grade II–IV acute/extensive chronic GVHD and tended to show higher overall and disease-free survival, lower relapse rates, and non-relapse mortality. In CBT, AUC_{0-24h} less than 30 μg h/ml was sufficient for low incidence of acute/chronic GVHD and high survival. Strong correlation between AUC_{0-24h} and C_{2h}, plasma MPA concentration at 2 h after administration was observed. Single point assessment of C_{2h} was shown to provide a useful surrogate of AUC_{0-24h} to predict GVHD incidence. The results of this study suggest that individualized MMF dosing in a donor source-dependent fashion may be important for maximizing the benefit of MMF in allo-SCT.

Keywords MMF · GVHD prophylaxis · Drug monitoring · Unrelated donor

1 Introduction

Graft-versus-host disease (GVHD) is a major cause of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (allo-SCT). The combination of cyclosporine (CsA) or tacrolimus (FK506) with short-course methotrexate (MTX) after allo-SCT has been the gold standard for GVHD prophylaxis for more than 20 years [1, 2]. Common adverse effects of MTX in allo-SCT are mucositis and delayed neutrophil engraftment, both of which can be sometimes hardly of negligible clinical concern.

Mycophenolate mofetil (MMF) has recently been used as a new immunosuppressive agent for solid organ transplantation [3] and, subsequently, it is an alternative of MTX in allo-SCT for acute GVHD prophylaxis [4–14]. It is now well known that MMF does not cause mucositis and facilitates engraftment possibly attributed to its non-cytotoxicity, which avoids the major problems associated with MTX [7, 13, 15, 16].

On the basis of real-time pharmacokinetic monitoring, we have recently demonstrated that every 8-h administration of MMF after allo-SCT may maintain higher plasma levels of mycophenolic acid (MPA), the active metabolite of MMF, than that with every 12 h even with the same daily dose [15]. However, it is unclear whether higher plasma MPA levels actually result in more favorable outcome.

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Few studies have proposed that there is a slight difference or none at all between CsA/MTX and CsA/MMF in terms of acute and chronic GVHD, relapse rate, and overall survival (OS) [8, 10, 12, 14, 17]. Pharmacokinetic studies in organ transplantation, as well as in allo-SCT, have demonstrated the wide inter-individual variations in plasma MPA and its pharmacokinetic parameter, the area under the curve (AUC) [15]. Therefore, it is possible that a certain population, with higher AUC for instance, in the same daily dose of MMF might display better clinical outcomes compared to that with MTX. To fully utilize the benefits of MMF, it is essential to isolate such a favorable population and to individualize treatment of other populations.

This study is a retrospective evaluation of the potential efficacy of the individualization depending on the levels of plasma MPA-AUC for hours 0–24 (AUC_{0-24h}) in unrelated allogeneic bone marrow (allo-BMT) and cord blood transplantation (CBT).

2 Materials and methods

2.1 Patient characteristics on analysis of transplant outcome of unrelated allo-SCT in association with MPA-AUC

Between May 2005 and January 2009, a total of 50 allo-SCT patients [8 related peripheral blood stem cell transplantation, 2 related BMT, 17 unrelated BMT, and 23 unrelated CBT (single unit)] were enrolled in the MPA pharmacokinetic studies at the Kobe University Hospital. Eligibility requirements included age younger than 69 years and Eastern Cooperative Oncology Group (ECOG) performance status of two or lower. MMF was preferentially administered to patients based on one or more of the following criteria: those who underwent unrelated CBT, those who were expected to have severe infection from oral mucositis as a result of MTX use or MRSA carrier patients, or those who had high-risk disease features, including acute leukemia beyond first remission, high-risk myelodysplastic syndrome (refractory anemia with excess blasts), chronic myelogenous leukemia with accelerated phase, and chemotherapy-resistant hematological malignancies. Excluded from these protocols were patients who had serum creatinine of greater than 2.0 mg/dl, abnormal liver function with total bilirubin of greater than 2.0 mg/dl, or cardiac ejection fraction of less than 50%. Patients were also excluded if serology was positive for human immunodeficiency virus, or if uncontrolled infections were present. The Institutional Review Board at Kobe University Hospital approved the study protocols. Written informed consent was obtained from all patients.

Among these patients, all unrelated BMT and CBT cases, except inappropriate cases for the analyses of survival rates (one graft failure rescued by subsequent transplant, one flared acute GVHD due to uncontrollable compliance to FK506 in unrelated BMT, two graft failures rescued by subsequent transplant, or the spontaneous recovery of autologous hematopoiesis in CBT), relapse rate (one aplastic anemia), and incidences of GVHD (one early death due to sinusoidal obstructive syndrome in unrelated BMT and one early death before the engraftment due to infection in unrelated CBT, in addition to the excluded patients for the analysis of survival) were evaluated for the transplant outcome [acute and chronic GVHD, OS, disease-free survival (DFS), relapse rate, and non-relapse mortality] in association with the level of MPA-AUC_{0-24h} and day 16 C_{2h} (peak plasma MPA concentration at 2 h after MMF administration). The characteristics of these patients are summarized in Table 1.

2.2 Allogeneic donors

Bone marrow from unrelated donors were 5/6 or 6/6 HLA-matched by DNA typing. Cord blood transplants were 3/6–6/6 HLA-matched and contained minimal cell count of 1.8×10^7 nucleated cells/kg recipient body weight before freezing.

2.3 Conditioning regimens and post-grafting immunosuppression

Conditioning regimens were diverse, wherein 23 of 36 patients received cyclophosphamide-based myeloablative conditioning regimens and the remaining 13 patients received fludarabine-based reduced-intensity conditioning regimens (Table 1). No one received a conditioning regimen containing T-cell depleting agents such as anti-thymocyte globulin. Thirty-one of 36 patients received total body irradiation (TBI). For immunosuppressive therapy, all patients received FK506 plus MMF except for one patient who received CsA until the onset of acute GVHD at day 20. FK506 at a daily dose of 0.03 mg/kg was administered by continuous intravenous infusion from day −1, and then shifted to oral formulation when tolerated. Doses were adjusted to maintain FK506 trough concentrations between 5 and 15 ng/ml. MMF was given orally 4–6 h after allo-SCT on day 0; then, on the succeeding days, it was given every 12 h (15–25 mg/kg/dose including dose-escalating cases up to a total daily dose of 3 g/day) or 8 h (fixed 1000 mg/dose, 3 g/day). MMF was generally discontinued on day 30, but in some patients it was continued beyond day 30 and tapered on an individual basis depending on the risk of relapse and the appearance of GVHD [18].

Table 1 Patient characteristics, toxicities, and causes of death

	AUC < 30 ($N = 15$)	AUC > 30 ($N = 21$)
Median age, years (range)	43 (33–60)	50 (20–66)
Gender (male:female)	10:5	10:11
Diagnosis		
AML	3	2
ALL	4	7
CML	1	1
MDS	6	8
NHL	1	2
AA	0	1
Risk category		
BMT		
Standard	4	2
High	6	3
CBT		
Standard	3	9
High	2	7
Conditioning regimen (no. of TBI containing)		
Myeloablative	12 (11)	11 (10)
Non-myeloablative	3 (2)	10 (8)
Engraftment (median)		
BMT	Day 11	Day 11
CBT	Day 20	Day 21
Toxicities		
BMT		
Diarrhea	6/10 (60%)	2/5 (40%)
CMV-Ag	7/10 (70%)	3/5 (60%)
CBT		
Diarrhea	4/5 (80%)	9/16 (56%)
CMV-Ag	2/5 (40%)	12/16 (75%)
Causes of death		
BMT		
Relapse	2	0
GVHD	2	0
IP	2	0
Viral infection	1	0
SOS	0	1
CBT		
Relapse	0	8
Bacterial infection	0	1
Viral infection	1	2
SOS	1	0
Others	0	1

AUC area under curve 0–24 h, *AML* acute myelogenous leukemia, *ALL* acute lymphoblastic leukemia, *CML* chronic myelogenous leukemia, *MDS* myelodysplastic syndrome, *NHL* non-Hodgkin lymphoma, *AA* aplastic anemia, *IP* interstitial pneumonitis, *SOS* sinusoidal obstructive syndrome, *CMV-Ag* cytomegalovirus antigenemia, *Engraftment* neutrophil engraftment

2.4 Pharmacokinetic analysis

Blood samples were collected at 0, 1, 2, 4, 8, and/or 12 h after the morning dose of MMF on days 2, 9, and 16 after allo-SCT in ethylenediaminetetraacetic acid (EDTA) tubes. The samples were centrifuged for 10 min at 4°C, and the plasma was stored at −80°C.

Total MPA levels were quantified by reverse-phase high-performance liquid chromatography (HPLC) with UV detection (Shimadzu, Kyoto, Japan). Non-compartmental analyses of total MPA concentration–time data were conducted to estimate the AUC_{0-12h} (twice daily) and AUC_{0-8h} (thrice daily) exactly as previously described [15]. The AUC_{0-24h} of the group given twice daily were calculated by 2/3 × (the sum of AUC_{0-12h} on days 2, 9, and 16), and those of the group given thrice daily were calculated by 3/3 × (the sum of AUC_{0-8h} on days 2, 9, and 16).

A linear correlation of C_{2h} on days 2, 9, and 16 after allo-SCT and AUC_{0-24h} was assessed for the available data from all 50 patients. Similar analysis was performed with the data of C_{2h} on day 16 and utilized for the evaluation of clinical outcome. As for clinical outcome, C_{2h} on day 2 or day 9 in two cases was substituted for C_{2h} on day 16 because of non-available data for various reasons.

2.5 Evaluation of toxicities, GVHD, and engraftment

MMF has been reported to be associated with gastrointestinal toxicity. Diarrhea of grade III or more, according to the National Cancer Institute Common Toxicity Criteria for Adverse Events version 3.0, was assessed, within 30 days after allo-SCT for descriptive purposes. All patients were also monitored for cytomegalovirus (CMV) reactivation on a weekly basis through measurement of CMV pp65 antigen. Preemptive therapy with ganciclovir was given when one or more antigen-positive cells per slide were observed. Acute and chronic GVHD were graded according to the consensus grading criteria [19, 20]. Chronic GVHD was assessed among patients who survived more than 100 days post-transplant. Neutrophil engraftment was defined as occurring on the first of two consecutive days after allo-SCT with neutrophil counts of more than 0.5×10^9/l.

2.6 Statistical analysis

The Mann–Whitney's *U* test was used to compare the AUC_{0-24h} between the groups of twice and thrice daily MMF administration schedule. The data for survival, relapse, non-relapse mortality and GVHD measured from the date of transplantation were examined using the Kaplan–Meier method and then compared using the log rank test. Correlation of AUC_{0-24h} and C_{2h} was assessed by

Spearman's correlation coefficient test. Significance was set at $p < 0.05$.

3 Results

3.1 Pharmacokinetics of MPA

The concentration–time profiles for plasma total MPA levels of the patients who received 3 g/day MMF twice or thrice daily are shown in Fig. 1. The patients who received 3 g/day divided twice daily were those enrolled in the dose-escalating study as described in Sect. 2. According to the pharmacokinetics on days 2 and 9 in each individual, the daily dose had been escalated to 3 g/day until day 14 [15]. Therefore, the analysis was performed using the data on day 16 post-transplant to compare the two groups, twice and thrice daily with the same daily dose. There was a markedly wide inter-individual variation of MPA levels (Fig. 1a, b). However, the patients with relatively high MPA level at 0 or 2 h (peak value) maintained high concentration along with the time, and the ones with low levels persisted through the monitoring period. The low intra-patient variability of plasma MPA levels was confirmed exactly in allo-SCT, as previously reported in the organ transplantation [21]. As shown in Fig. 1c, the AUC_{0-24h} in the thrice-daily group showed a trend toward higher values than the twice-daily group [mean ± SEM: 27.52 ± 3.30 μg h/ml ($n = 10$) in twice vs. 44.80 ± 4.88 μg h/ml ($n = 31$) in thrice, $p = 0.050$]. Because the median AUC_{0-24h} of all patients in both groups was 30.4 μg h/ml ($n = 41$), this study analyzed the incidence of GVHD, survival rate, relapse rate and non-relapse mortality in association with AUC_{0-24h} higher or lower than 30 μg h/ml.

3.2 Correlation between AUC_{0-24h} and C_{2h}

The best correlation between AUC_{0-24h} and C_{2h} of MPA on days 2, 9, and 16 was observed ($r^2 = 0.657$, $p < 0.0001$, Fig. 2a), suggesting that the value of C_{2h}, which can be obtained by a single point assessment, may be a useful surrogate of AUC_{0-24h}. According to the linear correlation, C_{2h} 2.5 μg/ml represented AUC_{0-24h} 30 μg h/ml (Fig. 2a). As a single assessment point of C_{2h}, we picked up day 16 because it is about the date of engraftment and the initiation of acute GVHD after allo-SCT. Indeed, strong correlation between AUC_{0-24h} and day 16 C_{2h} was observed ($r^2 = 0.641$, $p < 0.0001$), and again day 16 C_{2h} 2.5 μg/ml represented AUC_{0-24h} 30 μg h/ml (Fig. 2b). Thus, we analyzed clinical outcome depending not only on AUC_{0-24h}, but also on day 16 C_{2h} as a candidate of simplified surrogate of AUC_{0-24h}.

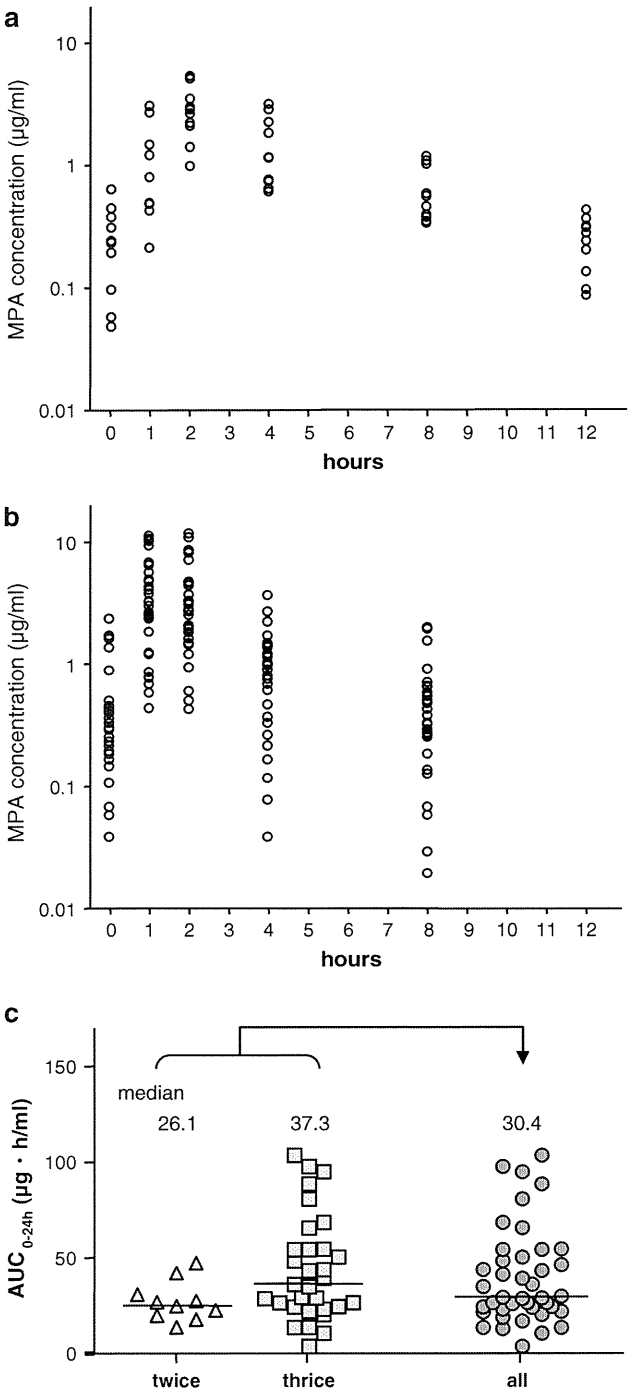

Fig. 1 Pharmacokinetic results of MPA. Concentration–time profiles of total MPA on day 16 post-transplant in the groups given **a** 3 g/day twice a day (every 12 h, $n = 10$) and **b** 3 g/day three times a day (every 8 h, $n = 31$). **c** AUC_{0-24h} in each group. *Bar* median

3.3 Acute and chronic GVHD

Grades II–IV acute GVHD occurred in 10 out of 34 evaluable patients (29.4%) with onset between 21 and 57 days in unrelated allo-SCT. Subjects with $AUC_{0-24h} >$ 30 μg h/ml had a significantly lower cumulative incidence

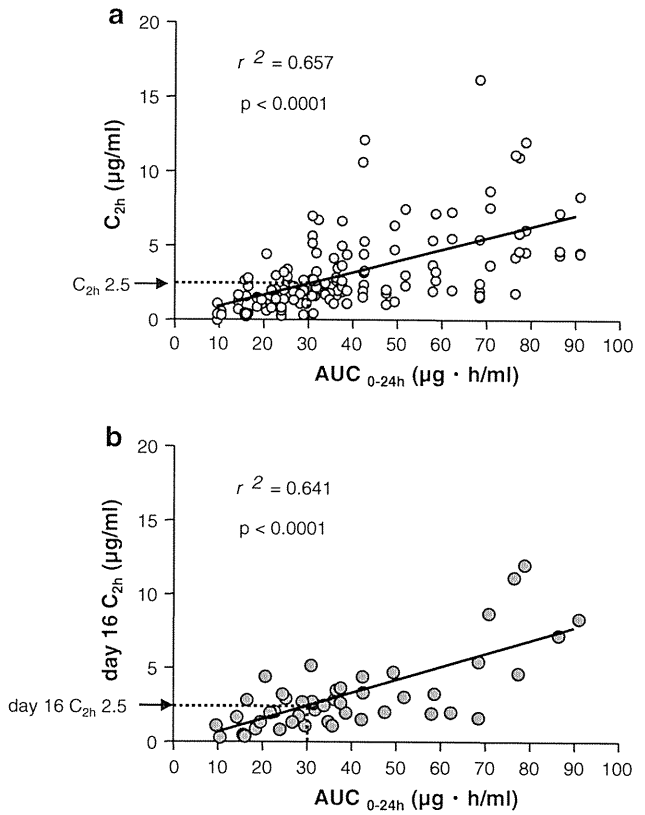

Fig. 2 Correlation between MPA-ACU_{0-24h} and MPA concentration of 2 h (C_{2h}). Data of C_{2h} on **a** days 2, 9, and 16, and **b** day 16 only

than those with AUC_{0-24h} < 30 μg h/ml (15.8 vs. 46.7%, respectively, $p < 0.05$, Fig. 3a). This difference mainly originated from unrelated allo-BMT (0 vs. 60%, respectively, $p = 0.07$, Fig. 3b). In CBT, cumulative incidence of acute GVHD was low (20%) independent of AUC_{0-24h} level (Fig. 3c). These results were reproducible by the analysis of day 16 C_{2h} with more clear significance (Fig. 3a–c).

Extensive chronic GVHD occurred in 4 (13.3%) out of 30 evaluable patients in unrelated allo-SCT. Subjects with AUC_{0-24h} > 30 μg h/ml had a significantly lower cumulative incidence than those with AUC_{0-24h} < 30 μg h/ml (0 vs. 30.8%, respectively, $p < 0.05$, Fig. 4a). This difference mainly originated from unrelated allo-BMT (0 vs. 44.4%, respectively, $p = 0.12$, Fig. 4b). In CBT, no extensive chronic GVHD occurred regardless of AUC_{0-24h} level (Fig. 4c). These results were reproducible by the analysis of day 16 C_{2h} (Fig. 4a–c).

3.4 OS, DFS, relapse rate, and non-relapse mortality

In unrelated allo-BMT, there was a trend toward better OS and DFS at 3 years in the subjects with AUC_{0-24h} > 30 μg h/ml compared to those with AUC_{0-24h} < 30 μg h/ml (80%; 95% CI 44.9–100 vs. 40%; 95% CI 9.6–70.4%, respectively) with a median follow-up of 646 days (range 22–1633 days), although none of these data reached statistical significance ($p = 0.243$, Table 2). Relapse rate was low in both groups (0% in AUC_{0-24h} > 30 μg h/ml and 22.2% in AUC_{0-24h} < 30 μg h/ml). Non-relapse mortality in the subjects with AUC_{0-24h} > 30 μg h/ml tended to be lower compared to those with AUC_{0-24h} < 30 μg h/ml. Similar results were obtained from the analysis of day 16 C_{2h}.

Surprisingly, these results appeared to be completely adverse in CBT. The subjects with AUC_{0-24h} > 30 μg h/ml showed remarkably lower OS (30%; 95% CI 6.9–53.1%, $p = 0.054$) and DFS (18.8%; 95% CI 0–37.9%, $p < 0.05$) due mainly to high incidence of relapse (72%, $p < 0.05$, Table 2), whereas the group with AUC_{0-24h} < 30 μg h/ml, although the number of patients was small, displayed continuous high OS and DFS (80%; 95% CI 44.9–100%) with no relapse. Day 16 C_{2h}, however, failed to extract this high-risk population of relapse-related mortality. Non-relapse mortality was low in CBT regardless of AUC_{0-24h} or day 16 C_{2h}. The causes of death are summarized in Table 1.

3.5 Toxicities and neutrophil engraftment

No patient developed grade III or IV oral mucositis in all subjects given MMF. Major gastrointestinal toxicity was diarrhea. The incidence of grade III or more diarrhea was 58.3% (21/36), but there was no significant difference between the groups with high and low AUC_{0-24h} (Table 1). It might be that the incidence of grade III or more diarrhea was proportionately higher (60% in BMT and 80% in CBT) in the subjects with AUC_{0-24h} < 30 μg h/ml. It was supposedly due to myeloablative conditioning regimen, which was employed to more patients (12/15, 80%) with AUC_{0-24h} < 30 μg h/ml compared to those with AUC_{0-24h} > 30 μg h/ml (11/21, 52%). For most cases, diarrhea was transient and curable without the withdrawal of MMF.

Although relatively high incidence of CMV reactivation was observed in both high and low AUC groups (Table 1), most cases were controllable with preemptive therapy. Death of CMV pneumonia occurred in two (13.3%) of the subjects with AUC_{0-24h} < 30 μg h/ml and in two (9.5%) of those with AUC_{0-24h} > 30 μg h/ml (Table 1). Higher AUC_{0-24h} did not facilitate neutrophil engraftment (Table 1).

4 Discussion

This study discovered a certain population in which MMF would be highly beneficial in unrelated allo-SCT because of wide inter-patient variability of plasma MPA levels even with the same MMF exposure. The benefit is predictable by the pharmacokinetic parameters during the early days after

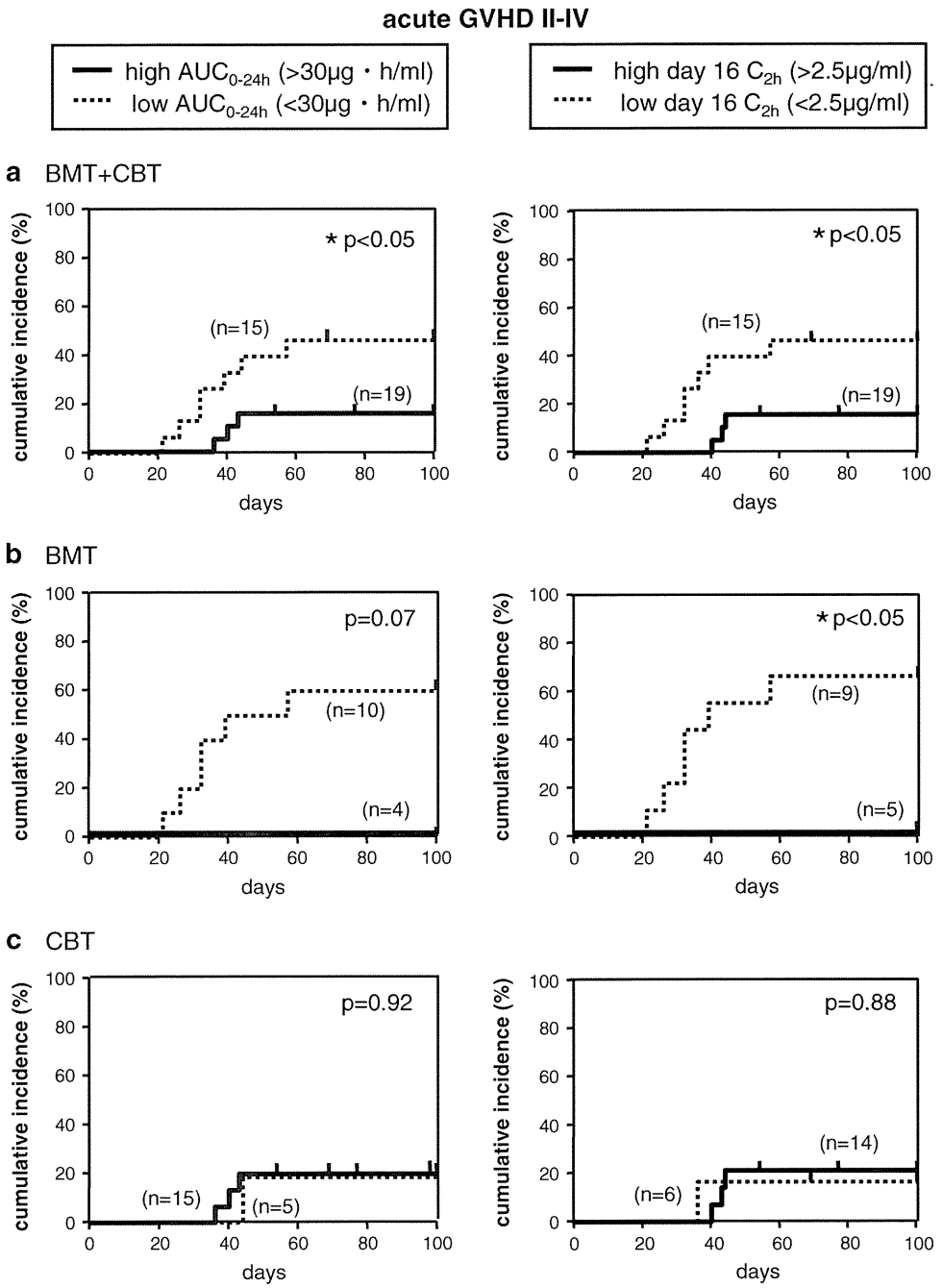

Fig. 3 Cumulative incidence of acute GVHD. Cumulative incidence of grade II–IV acute GVHD in comparison between patients with AUC_{0-24h} < 30 μg h/ml and AUC_{0-24h} > 30 μg h/ml (*left column*), day 16 C_{2h} < 2.5 μg/ml, and day 16 C_{2h} > 2.5 μg/ml (*right column*). **a** Total unrelated allo-SCT patients, **b** unrelated allo-BMT, and **c** unrelated CBT

transplantation. Thus, we propose that the MMF dosing strategies in unrelated allo-SCT should be changed depending on donor source and individual monitoring of AUC_{0-24h} or C_{2h}.

4.1 Unrelated BMT

The incidence of acute GVHD in patients with AUC_{0-24h} < 30 μg h/ml (Fig. 3b) was similar to or even higher than the data of Japan Marrow Donor Program 2007 (40 ± 2%, 3944 HLA-matched unrelated BMTs, wherein MTX was used in the vast majority of these patients), whereas no GVHD was observed in patients with AUC_{0-24h} > 30 μg h/ml (Fig. 3b). In unrelated BMT, MPA-AUC_{0-24h} should be maintained at more than 30 μg h/ml to surpass MTX for acute GVHD prophylaxis. Most acute GVHD progressed to chronic GVHD (Fig. 4b), which implies the importance of suppression of prior acute GVHD by high AUC_{0-24h} to avoid subsequent chronic GVHD. Importantly, the high AUC_{0-24h} did not increase the risk of relapse (Table 2) or one of the possible adverse effects of MMF, which is CMV infection. Moreover, the greater AUC did not increase the incidence or grade of diarrhea or delay neutrophil engraftment. Thus, the

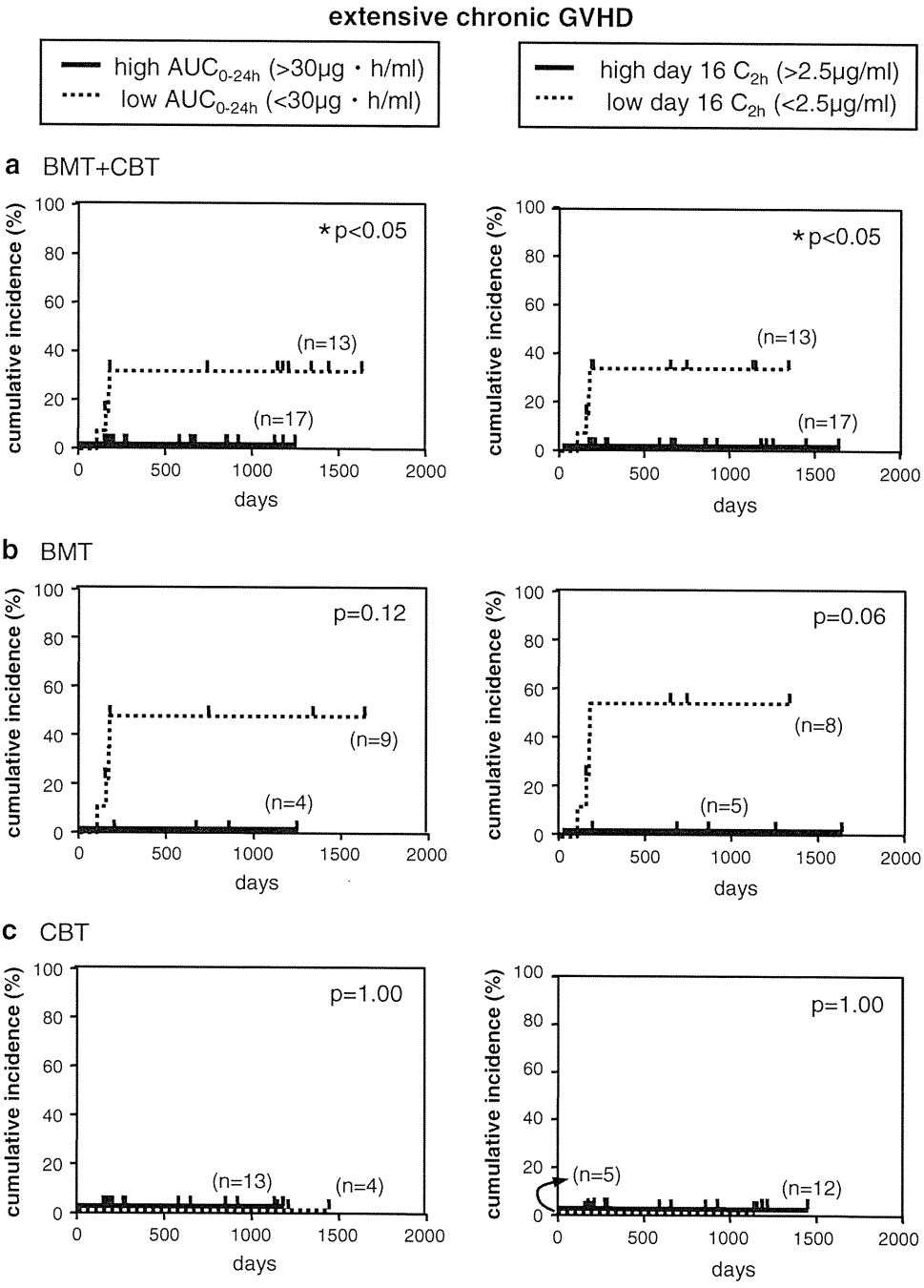

Fig. 4 Cumulative incidence of chronic GVHD. Cumulative incidence of extensive chronic GVHD in comparison between patients with $AUC_{0-24h} < 30$ μg h/ml and $AUC_{0-24h} > 30$ μg h/ml (*left column*), day 16 $C_{2h} < 2.5$ μg/ml, and day 16 $C_{2h} > 2.5$ μg/ml (*right column*). **a** Total unrelated allo-SCT patients, **b** unrelated allo-BMT, and **c** unrelated CBT

diarrhea could not be mainly caused by administration of MMF. It was difficult to interpret why the incidence of relapse was lower in patients with higher AUC after unrelated BMT (Table 2). Indeed, no grade II–IV acute GVHD and chronic extensive GVHD occurred in this group except for one early death. Nevertheless, grade I skin GVHD and limited chronic GVHD occurred in most of the patients. Therefore, higher AUC in BMT might suppress severe GVHD, but not immunological effect per se (i.e., graft-versus-leukemia effect: GVL). There was a trend toward higher survival in patients given a greater dose of AUC (Table 2). On the other hand, the major causes of death in patients given low AUC_{0-24h} other than relapse were GVHD and interstitial pneumonitis, both of which were thought to be due to immunological reaction and might be avoidable with higher AUC_{0-24h}. Collectively, MMF is particularly useful in unrelated allo-BMT patients if AUC_{0-24h} is higher than 30 μg h/ml. In lower AUC_{0-24h} patients, it would be a reasonable strategy to increase AUC_{0-24h} to expect low risk of acute and chronic GVHD resulting in high incidence of survival with no increased risk of relapse. Alternatively, additional immunosuppressant(s),