Bone Marrow Transplantation (2013) **48,** 74–79
© 2013 Macmillan Publishers Limited All rights reserved 0268-3369/13
www.nature.com/bmt

ORIGINAL ARTICLE

Stenotrophomonas maltophilia infection in hematopoietic SCT recipients: high mortality due to pulmonary hemorrhage

K Tada[1], S Kurosawa[1], N Hiramoto[1], K Okinaka[1], N Ueno[1], Y Asakura[1], S-W Kim[1], T Yamashita[1], S-I Mori[1], Y Heike[1], AM Maeshima[2], R Tanosaki[2], K Tobinai[1] and T Fukuda[1]

To clarify the clinical features and outcome of *Stenotrophomonas maltophilia* infection among hematopoietic SCT (HCT) recipients, we retrospectively reviewed the records of 1085 consecutive HCT recipients and identified 42 episodes in 31 HCT recipients with *S. maltophilia* infection. We compared these recipients with 30 non-HCT patients with *S. maltophilia* infection. The mortality rate in HCT recipients was significantly higher than that in non-HCT patients (relative risk 5.7, $P = 0.04$), and we identified seven patients with pulmonary hemorrhage due to *S. maltophilia*, exclusively in the HCT cohort. Six of these latter seven patients died within 1 day from the onset of hemorrhage and the isolate was identified after death in most cases; one patient, who received empiric therapy for *S. maltophilia* and granulocyte transfusion, survived for more than 2 weeks. The patients with pulmonary hemorrhage had a more severe and longer duration of neutropenia, persistent fever despite of the use of broad-spectrum antibiotics, complication by pneumonia and higher C-reactive protein levels than those without pulmonary hemorrhage. In conclusion, *S. maltophilia* was associated with fulminant and fatal pulmonary hemorrhage in HCT recipients. Empiric therapy with antibiotics before the onset of pulmonary hemorrhage may be effective in HCT recipients who carry the conditions identified.

Bone Marrow Transplantation (2013) **48,** 74–79; doi:10.1038/bmt.2012.87; published online 28 May 2012

Keywords: *Stenotrophomonas maltophilia*; SCT; pulmonary hemorrhage

INTRODUCTION

Stenotrophomonas maltophilia is a non-fermentative, Gram-negative bacillus that is ubiquitous in the natural and hospital environment,[1–4] and exhibits intrinsic resistance to many antibiotics including β-lactams, carbapenems and aminoglycosides.[5,6] Although *S. maltophilia* is not usually highly virulent, it is a significant pathogen in immune-compromised patients, and the incidence of *S. maltophilia* infection is increasing.[7]

Previously, in a heterogeneous group that included recipients of hematopoietic SCT (HCT) and non-HCT patients with solid tumor or hematological malignancy, risk factors for acquiring *S. maltophilia* infection were reported to be prolonged neutropenia, exposure to broad-spectrum antibiotics, mucositis, indwelling medical devices such as an intravascular catheter or ventilation tubes and long hospital stays.[8–12] In a similarly heterogeneous group, risk factors for mortality of *S. maltophilia* infection were reported to be neutropenia, hematological malignancy, immunosuppressive therapy, shock status at infection onset and intensive-care unit stays.[13–17] However, only limited information is available on HCT recipients.[12,18,19] Many of the previously reported risk factors are commonly seen in HCT recipients because of their severe immunosuppressive status and mucositis due to preparative conditioning and immunosuppressive therapy for GVHD prophylaxis.

Hence, *S. maltophilia* infection in HCT recipients may have a different spectrum and greater severity compared with that in patients with solid tumor or non-HCT setting hematological malignancy. To clarify the clinical features and outcome of *S. maltophilia* infection with a particular focus on HCT recipients, we retrospectively analyzed clinical data on patients who had *S. maltophilia* infection.

PATIENTS AND METHODS

Patients

We retrospectively reviewed the medical and microbiological records of all the HCT recipients at the National Cancer Center Hospital (Tokyo, Japan) between January 2001 and December 2010, and identified episodes of *S. maltophilia* blood stream infection (BSI) among the HCT recipients. We also reviewed the medical and microbiological records of all patients whose blood cultures were positive for *S. maltophilia* at our institution in the same period and identified episodes of *S. maltophilia* BSI among the non-HCT patients. We then compared the clinical features and outcomes in the HCT cohort with those in the non-HCT control cohort.

Definitions

An episode of *S. maltophilia* BSI was defined as one or more positive blood cultures for *S. maltophilia* with clinical signs of infection. When *S. maltophilia* was again detected in the same patient at an interval of 8 or more days after the first BSI episode had improved, the detection of the isolate was regarded as a different episode of BSI, as previously reported.[13,20] The severity of illness was assessed by the bacteremia score according to the University of Pittsburgh (PITT score).[21,22] The D-index and cumulative D-index, which were calculated as the area over the neutrophil curve that is based on a graph plotting the absolute neutrophil counts during neutropenia,[23] were also investigated to evaluate the impact of both duration and severity of neutropenia on *S. maltophilia* infection.

Infection control in HCT recipients and microbiological investigations

Among the HCT recipients, prophylaxis for infection consisted of trimethoprim-sulfamethoxazole (ST), ciprofloxacin, fluconazole and acyclovir. As therapy for febrile neutropenia, cefepim was first administered in

[1]Department of Hematology and Hematopoietic Stem Cell Transplantation, National Cancer Center Hospital, Tokyo, Japan and [2]Department of Pathology and Clinical Laboratories, National Cancer Center Hospital, Tokyo, Japan. Correspondence: Dr T Fukuda, Department of Hematology and Hematopoietic Stem Cell Transplantation, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan.
E-mail: tafukuda@ncc.go.jp
Received 29 December 2011; revised 3 April 2012; accepted 9 April 2012; published online 28 May 2012

most cases, and in cases that did not improve within 2–3 days, either it was switched to carbapenem or vancomycin was added.

Two sets of blood culture samples from a double-lumen intravascular catheter and another from peripheral blood were routinely taken at the initial episode of fever. If fever persisted, one set of the blood culture samples was taken daily from either the lumen of the intravascular catheter or a peripheral vessel alternately. Blood culture samples were processed using a BACTEC 9240 (before 2008) or BACTEC FX (after 2009) system (Becton Dickinson Microbiology Systems, Sparks, MD, USA). Susceptibility to antibiotics was tested by the broth microdilution method according to the guidelines of the National Committee for Clinical Laboratory Standards.

Statistical analysis

The end point was defined as death within 4 weeks from the onset of a positive blood culture for *S. maltophilia*. Categorical variables were analyzed using a Chi-squared test or Fisher's exact test as appropriate. Continuous variables were compared using the Mann–Whitney *U*-test. To investigate risk factors for death within 4 weeks in all cases including both HCT and non-HCT cases, a multivariate logistic regression analysis was performed. The following factors were used as covariates: age (<45 vs $\geqslant 45$ y), severe neutropenia at BSI onset, PITT score ($\leqslant 1$ vs >1), complication by pneumonia and therapy for underlying disease (HCT vs non-HCT). The statistical analysis was performed with the SPSS 11.0 statistical software package (SPSS Inc, Tokyo, Japan).

RESULTS

Patient characteristics

In the study period between 2001 and 2010, a total of 1085 HCT (847 allogeneic HCT and 238 autologous HCT) procedures were performed in our institution. A total of 42 episodes (35 episodes in allogeneic HCT recipients and 7 episodes in autologous HCT recipients) of *S. maltophilia* BSI were identified in 31 HCT recipients (2.9%). There was no obvious outbreak of *S. maltophilia* infection in the study period.

The patient characteristics are shown in Table 1. Broad-spectrum cefem or carbapenem was administered in 60% of the episodes at BSI onset. With regard to the therapy for *S. maltophilia* infection, the intravascular catheter was removed in 10 (24%) episodes in which catheter-related BSI was suspected. When *S. maltophilia* BSI was diagnosed, ST or fluoroquinolone was started based on the susceptibility test in 19 episodes, whereas no antibiotics were additionally administered in 7 episodes where *S. maltophilia* was only detected in blood culture after death.

Clinical outcome

In all, 14 patients died within 4 weeks from the onset of a positive blood culture for *S. maltophilia*. We divided causes of death into two patterns; eight patients were judged to have died from a single cause due to *S. maltophilia* infection, whereas six appeared to have died of complex causes, which consisted of *S. maltophilia* infection and some other cause (underlying disease progression in two, GVHD in two, other infection in one and suffocation due to vomiting in one). Pulmonary hemorrhage accounted for half of the 14 deaths. Among the 847 allogeneic HCT recipients, 18 patients (2.1%) developed pulmonary hemorrhage (infection of *S. maltophilia*: $n=7$, Aspergillus species: $n=3$, *Pseudomonas aeruginosa*: $n=1$, Staphylococcus species: $n=1$, cytomegalovirus: $n=1$, idiopathic pneumonia syndrome/diffuse alveolar hemorrhage: $n=2$, disseminated intravascular coagulation: $n=1$, tumor invasion: $n=1$ and unknown cause: $n=1$).

Seven cases with pulmonary hemorrhage

The details of the seven patients with pulmonary hemorrhage are shown in Table 2. All the episodes of pulmonary hemorrhage occurred during a period of profound neutropenia (neutrophil count 0/μL).

Table 1. Patients and clinical characteristics of HCT recipients who developed *S. maltophilia* BSI

	Total 31 patients (42 episodes)	*%*
Age, median, range	44 years, 4–67 (43 years, 4–67)	
Sex (male/female)	25/6 (34/7)	81/19 (81/19)
Underlying disease		
Leukemia	19 (24)	61 (57)
Lymphoma	7 (9)	23 (21)
MDS/myelofibrosis	3 (3)	10 (7)
Solid tumor	2 (6)	6 (14)
Type of HCT		
Allogeneic	28 (35)	90 (83)
Autologous	3 (7)	10 (17)
Conditioning for allo-HCT		
Myeloablative	15 (19)	54 (54)
Reduced intensity	13 (16)	46 (46)
Donor and source		
Related PB	8 (9)	26 (21)
Related BM	1 (1)	3 (2)
Unrelated BM	14 (18)	45 (43)
Cord blood	5 (7)	16 (17)
Autologous PB	3 (7)	10 (17)
Immunosuppressive agents		
CSP ± steroid	13 (16)	42 (38)
TAC ± steroid	9 (11)	29 (26)
Steroid	4 (4)	13 (10)
None	5 (11)	16 (26)
CV indwelling	30 (41)	97 (98)
Antibiotics at BSI onset		
Carbapenem ± vancomycin	13 (18)	42 (43)
Broad cefem or penicillin	6 (7)	19 (17)
Ciprofloxacin (prophylaxis)	8 (9)	26 (21)
ST (prophylaxis)	2 (4)	6 (10)
Other	1 (1)	3 (2)
None	1 (3)	1 (7)
Therapy for infection		
CV removal	7 (10)	23 (24)
Granulocyte Transfusion	2 (2)	6 (5)
ST	5 (5)	16 (12)
ST + quinolone	3 (3)	10 (7)
ST + ceftazidime	1 (2)	3 (5)
Quinolone ± minocyclin	7 (9)	23 (21)
Ceftazidime	3 (4)	10 (7)
None	9 (9)	29 (21)

Abbreviations: BSI = blood stream infection; CSP = cyclosporine; CV = central venous catheter; HCT = hematopoietic SCT; MDS = myelodysplastic syndrome; PB = peripheral blood; ST = trimethoprim-sulfamethoxazole; TAC = tacrolimus.

As initial symptoms, all patients showed persistent fever that did not respond to broad-spectrum antibiotics. Other symptoms included chest pain ($n=4$), back pain ($n=2$), hemosputum ($n=2$) or dyspnea ($n=1$). Imaging test findings of chest X-ray ($n=5$) or computed tomography ($n=5$) in all patients showed consolidation that was consistent with symptoms such as chest pain. Six patients (cases 1–3 and 5–7) developed massive hemoptysis within 1–4 days after the initial chest symptoms. At the onset of massive hemoptysis, all the patients developed respiratory and circulatory failure due to bleeding and sepsis. Six patients, but not case 7, died within 1 day after hemoptysis.

© 2013 Macmillan Publishers Limited

Table 2. Details of patients with pulmonary hemorrhage due to *S. maltophilia*

Case	Age/ gender	Underlying disease	Type of HCT	Conditioning	Onset day[a] of hemorrhage	Day[a] of death	Day[a] of identification of S maltophilia		Treatment for S maltophilia infection
							Sputum	Blood	
1	27/M	AML	CBT	CA + CY + TBI 12 Gy	10	10	After death	After death	None
2	37/M	Myelofibrosis	U-BMT	BU + CY	11	11	After death	After death	None
3	54/M	AML	U-BMT	CY + TBI 12 Gy	15	16	12	After death	None
4	58/M	AML	CBT (second allogeneic HCT)	Flu + BU + TBI 2 Gy	18	18	9	After death	CAZ
5	43/M	AML	CBT (second allogeneic HCT)	Flu + Mel	6	7	Previously known	After death	ST + PZFX + CAZ
6	24/M	AML	R-PBSCT	BU + CY	445 (11)[b]	446 (12)[b]	NA	445 (11)[b]	ST
7	51/M	AML	U-BMT	BU + CY	39	55	42	40	ST + PZFX granulocyte transfusion

Abbreviations: CA = cytarabine; CAZ = ceftazidime; CBT = cord blood transplantation; Flu = fludarabine; HCT = hematopoietic SCT; Mel = melpharan; NA = not assessment; PZFX = pazufloxacin; R-PBSCT = related PBSCT; ST = trimethoprim-sulfamethoxazole; U-BMT = unrelated BMT. [a]Day after allogeneic HCT. [b]Day after chemotherapy using idarubicin and cytarabine for relapse after allogeneic HCT.

Because of the significantly rapid clinical course, *S. maltophilia* was detected in blood culture after death or 1 day before death in six patients (cases 1–6) and, similarly, *S. maltophilia* was detected in sputum culture after death in two patients (case 1 and 2).

Therapy for *S. maltophilia* was not initiated in three cases (cases 1–3) because the isolate was identified after death. In case 7, the administration of ST and pazufloxacin was started before the onset of hemoptysis and the identification of isolate because pulmonary hemorrhage due to *S. maltophilia* was suspected based on a typical clinical course and imaging test findings. Granulocyte transfusion was also started 38 h after the onset of hemoptysis and 28 h after the detection of *S. maltophilia* in blood culture. This patient survived for 16 and 15 days after the onset of hemoptysis and the identification of BSI, respectively. However, he eventually died due to *S. maltophilia* infection associated with primary graft failure. In case 5, the administration of ST and pazufloxacin was started before hemoptysis because he had a past history of *S. maltophilia* infection. The isolates exhibited resistance to the antibiotics used and he died within 1 day after hemoptysis. Bronchial arterial embolization was performed for pulmonary hemorrhage in two patients (case 1 and 5), however, neither were rescued.

Pulmonary hemorrhage due to *S. maltophilia* was diagnosed by the detection of *S. maltophilia* in blood and sputum cultures, histopathological findings of pulmonary hemorrhage and the presence of massive infiltration of Gram-negative rods in lungs by autopsy in three cases (cases 2, 3 and 5) (Figure 1). Cases 1 and 7 were diagnosed by the detection of *S. maltophilia* in blood and sputum cultures and the confirmation of pulmonary hemorrhage using bronchoscopy. Case 4 was diagnosed by the detection of *S. maltophilia* in blood and sputum cultures and clinical symptoms such as hemoptysis. Case 6 was diagnosed by the detection of *S. maltophilia* in blood culture and pulmonary hemorrhage confirmed by bronchoscopy.

Although the histopathological findings at autopsy in case 2 demonstrated one focal small nodule of aspergillosis in the right upper lobe, pulmonary hemorrhage was mainly found in the bilateral lower lobes, and therefore was mainly assumed to be due to *S. maltophilia* infection. There was no histopathological evidence of fungal infection in the other autopsy cases. Serum galactomannan Ag and β-D-glucan were tested at around the onset of pulmonary hemorrhage and results were negative in six cases, but not in case 2. The clinical characteristics and outcome in patients with and without pulmonary hemorrhage are compared in Table 3. Patients with pulmonary hemorrhage were associated with severe and longer duration of neutropenia, higher C-reactive protein levels at BSI onset, a higher D- and cumulative D-index, a higher incidence of complication by pneumonia and higher mortality than those without pulmonary hemorrhage.

Comparison of HCT recipients with non-HCT patients

The clinical characteristics and outcomes of HCT recipients (42 episodes) were compared with those of 30 non-HCT patients (15 episodes with hematological malignancy and 15 with solid tumor) who developed *S. maltophilia* infection (Table 3). HCT recipients were more likely to be associated with severe neutropenia (<100/μL), use of immunosuppressive agents and a higher mortality within 4 weeks after *S. maltophilia* BSI than non-HCT patients.

Although there was no significant difference in the proportion of patients with *S. maltophilia* pneumonia between the HCT recipients and non-HCT patients, pulmonary hemorrhage was seen only in HCT recipients. By a multivariate analysis in all the 72 episodes, including both HCT ($n = 42$) and non-HCT cases ($n = 30$), the independent risk factors for mortality within 4 weeks after *S. maltophilia* BSI were HCT recipient (relative risk 5.7, 95% confidence interval 1.1–30.1, $P = 0.04$) and complication by pneumonia (relative risk 10.7, 95% confidence interval 2.6–44.2, $P = 0.001$).

Susceptibility of strains of *S. maltophilia* from HCT recipients

A total of 41 strains of *S. maltophilia* isolated from HCT recipients were tested with regard to their susceptibility to antibiotics. The percentages of isolates that were susceptible to ST (81%), minocycline (93%) and levofloxacin (68%) were relatively high, whereas fewer isolates were susceptible to ceftazidime (26%), amikacin (21%), cefepime (5%) and imipenem (3%). There were no significant differences in susceptibility to each antibiotic between isolates from HCT recipients and those from non-HCT patients.

© 2013 Macmillan Publishers Limited

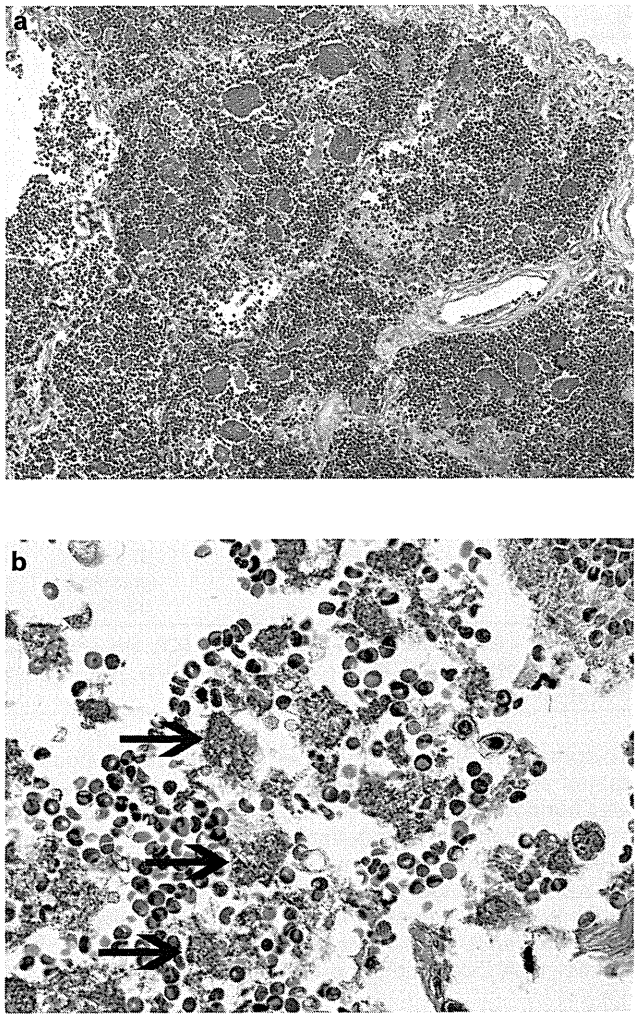

Figure 1. Lung section from case 5 (autopsy). (**a**) Massive intraalveolar hemorrhage and disseminated foci of basophilic bacteria (hematoxylin and eosin stain, × 40). (**b**) Higher magnification of hematoxylin and eosin staining showed a striking number of basophilic bacilli (arrow) without infiltration of the alveoli neutrophils and lymphocytes (× 400).

DISCUSSION

This is a retrospective study reporting the clinical characteristics and outcomes of *S. maltophilia* infection with a particular focus on HCT recipients. Our data revealed that HCT recipients who had *S. maltophilia* infection were more likely to be associated with pulmonary hemorrhage, which was the main cause of death in the cohort, and had a higher mortality within 4 weeks after *S. maltophilia* BSI than non-HCT patients.

Previous studies of *S. maltophilia* infection in HCT recipients included many patients with catheter-related BSI and emphasized the removal of an intravascular catheter and the administration of appropriate antibiotics according to the results of a susceptibility test.[13,18,19] Our results were consistent with those of previous studies because 28 out of 42 episodes (67%) of *S. maltophilia* infection in this study were successfully treated. However, in previous reports that included *S. maltophilia* infection in HCT recipients, there was no information on pulmonary hemorrhage due to *S. maltophilia*.[13,18,19] Additionally, in previous large studies of post-transplant pulmonary hemorrhage, *S. maltophilia* had not been detected as a cause of pulmonary hemorrhage.[24–26] There have been only a few reported cases in a non-HCT setting that were associated with pulmonary hemorrhage due to *S. maltophilia* after intensive chemotherapy against hematological malignancy.[27–30] Hence, this is the first report to comprehensively describe the overall picture of pulmonary hemorrhage due to *S. maltophilia* with a particular focus on HCT recipients, including the histopathological findings of autopsy, incidence, typical clinical course, risk factors and outcome.

By reviewing the medical records in detail, we identified the clinical characteristics of post-transplant pulmonary hemorrhage due to *S. maltophilia*. Risk factors for pulmonary hemorrhage due to *S. maltophilia* were HCT recipient, prolonged days of neutropenia, high C-reactive protein level at BSI onset and complication by pneumonia. In addition to the duration of neutropenia, the D- and cumulative D-index[23] were also considered to be factors that predicted pulmonary hemorrhage, whereas a high PITT score at the onset of BSI was not associated with pulmonary hemorrhage.

Typical findings that were recognized before pulmonary hemorrhage were persistent fever despite of the use of broad-spectrum antibiotics, chest symptoms, such as chest pain, and apparent consolidation in imaging test. In many cases, it was impossible to start antibiotic therapy based on the identification of *S. maltophilia* infection in blood or sputum culture because most patients developed a very aggressive clinical course and died before the *S. maltophilia* infection was detected. Hence, *S. maltophilia* infection should be predicted in the HCT recipients based on the presence of risk factors for pulmonary hemorrhage due to *S. maltophilia*, and treatment for *S. maltophilia* infection should be considered before hemoptysis occurs.

In one of our cases (case 7 in Table 2), *S. maltophilia* infection was suspected based on typical findings and risk factors for pulmonary hemorrhage due to *S. maltophilia*, and empiric therapy that consisted of ST and pazufloxacin was started before hemoptysis and the detection of isolate. Granulocyte transfusion was also started, which resulted in a long survival after pulmonary hemorrhage was observed. This case suggests that empiric therapy for *S. maltophilia* infection might be useful if typical findings appear in HCT recipients who have risk factors for pulmonary hemorrhage due to *S. maltophilia*.

Current treatment recommendations for antibiotics against *S. maltophilia* are based on historical evidence, case series, case reports and *in vitro* susceptibility tests because of the lack of controlled trials.[1,6] In general, ST has been shown to have the most potent and reliable *in vitro* activity against *S. maltophilia*, and alternate agents are new fluoroquinolone, tigecycline and ticarcillin–clavulanate. The isolates from both HCT recipients and non-HCT patients in our study were confirmed to have a high *in vitro* susceptibility to ST and new fluoroquinolone, however, tigecycline and ticarcillin–clavulanate were not tested because these drugs have not yet been approved in our country. Hence, ST alone or in combination with other susceptible agents is considered to be the treatment of choice for suspected or culture-proven *S. maltophilia* infection in HCT recipients. However, the myelotoxicity of ST might be a concern in the setting of HCT before engraftment.

The mechanism of *S. maltophilia*-induced pulmonary hemorrhage remains uncertain. *In vitro* data demonstrated that *S. maltophilia* produces proteases, which can break down the protein components of collagen, fibronectin and fibrinogen,[31,32] and this may contribute to local tissue damage and hemorrhage.[1] Because our present histopathological findings at autopsy demonstrated alveolar hemorrhage and the massive infiltration of Gram-negative rods in lungs without invasion by neutrophils or lymphocytes, *S. maltophilia* itself might damage lung tissue, which leads to pulmonary hemorrhage. In an HCT recipient with a highly immunosuppressive background, it is speculated that *S. maltophilia* infects and proliferates in lung tissue, which is fragile due to chemotherapy or TBI as a preparative conditioning, and thus leads to pulmonary hemorrhage with a coexisting tendency for

© 2013 Macmillan Publishers Limited

Table 3. Comparison of clinical characteristics and outcomes

	HCT cohort without pulmonary hemorrhage n = 35 (%)	*HCT cohort with pulmonary hemorrhage n = 7 (%)*	P	*HCT cohort n = 42 (%)*	*Non-HCT cohort n = 30 (%)*	P
Age, median, range	44, 4–67	43, 24–58	0.7	44, 4–67	49, 4–78	0.1
CRP (mg/dL) at BSI onset, median, range	3.0, 0.2–30.3	25.4, 5–31.2	0.001	4.9, 0.2–31.2	5.3, 0.5–23.4	0.9
Neutropenia (<500/μL) at BSI onset	20 (57)	7 (100)	0.04	27 (64)	13 (43)	0.08
Profound neutropenia (<100/μL) at BSI onset	19 (54)	7 (100)	0.03	26 (62)	10 (33)	0.02
Total days of neutropenia[a], median, range	4, 0–143	25, 6–143	0.02	7, 0–143	0, 0–92	0.09
Total days of profound neutropenia[a], median, range	2, 0–133	12, 4–133	0.02	4, 0–133	0, 0-84	0.05
D-index, median, range	1550, 0-70070	10400, 2600-70070	0.02	2950, 0-70070	0, 0-44400	0.09
Cumulative D-index, median, range	125, 0-46570	8400, 2600-64570	0.006	1550, 0-64570	0, 0-30200	0.08
PITT score >1	3 (9)	7 (100)	0.2	10 (17)	7 (23)	0.8
Coinfection	6 (17)	2 (29)	0.4	8 (19)	5 (17)	0.9
Use of immunosuppressive agents	24 (69)	7 (100)	0.2	31 (74)	2 (7)	<0.001
Pneumonia	10 (29)	7 (100)	0.001	17 (40)	9 (30)	0.4
Pulmonary hemorrhage	–	–	–	7 (17)	0 (0)	0.02
Death within 4 weeks	7 (20)	7 (100)	<0.001	14 (33)	3 (10)	0.02

Abbreviations: BSI = blood stream infection; CRP = C-reactive protein; HCT = hematopoietic SCT. [a]Day from onset of neutropenia (<500/μL) or profound neutropenia (<100/μL) to recovery of neutropenia or profound neutropenia. If a patient died without recovery of neutropenia, days of neutropenia or profound neutropenia were counted until the day of mortality.

bleeding due to a low platelet count and coagulation disorder. Further molecular microbiological studies are warranted to clarify the mechanism of *S. maltophilia*-induced pulmonary hemorrhage.

Our results also showed that most patients who died without pulmonary hemorrhage had complex causes of death, such as underlying disease progression or uncontrolled GVHD in addition to *S. maltophilia* infection. This might be due to the fact that infections due to *S. maltophilia* occur often in patients in poor condition.

Our study has some limitations; it includes a relatively small number of patients in a single institution and uses a retrospective study design. However, this is the largest study to focus on *S. maltophilia* infection in HCT recipients and is the first study to report the significance of pulmonary hemorrhage as a cause of death.

In conclusion, we showed that *S. maltophilia* infection in HCT recipients is associated with higher mortality than that in non-HCT patients, and causes fulminant and fatal pulmonary hemorrhage, which is a main cause of death in HCT recipients with *S. maltophilia* infection. We also showed that patients with pulmonary hemorrhage were associated with persistent fever despite of the use of broad-spectrum antibiotics, complication by pneumonia, severe and significantly longer duration of neutropenia and higher C-reactive protein levels at the onset of BSI than those without pulmonary hemorrhage. Empiric therapy before the onset of pulmonary hemorrhage may be effective in HCT recipients who exhibit these identified conditions because most patients with pulmonary hemorrhage due to *S. maltophilia* die within a short period without the detection of infection. Multicenter prospective or retrospective studies that focus on HCT recipients are warranted to evaluate the optimum therapeutic strategy against this fatal and intrinsic multidrug-resistant microbe.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1 Looney WJ, Narita M, Muhlemann K. *Stenotrophomonas maltophilia*: an emerging opportunist human pathogen. *Lancet Infect Dis* 2009; **9**: 312–323.
2 Hoefel D, Monis PT, Grooby WL, Andrews S, Saint CP. Profiling bacterial survival through a water treatment process and subsequent distribution system. *J Appl Microbiol* 2005; **99**: 175–186.
3 Spencer RC. The emergence of epidemic, multiple-antibiotic-resistant *Stenotrophomonas (Xanthomonas) maltophilia* and *Burkholderia (Pseudomonas) cepacia*. *J Hosp Infect* 1995; **30**(Suppl): 453–464.
4 Cervia JS, Ortolano GA, Canonica FP. Hospital tap water as a source of *Stenotrophomonas maltophilia* infection. *Clin Infect Dis* 2008; **46**: 1485–1487.
5 Khardori N, Reuben A, Rosenbaum B, Rolston K, Bodey GP. In vitro susceptibility of *Xanthomonas (Pseudomonas) maltophilia* to newer antimicrobial agents. *Antimicrob Agents Chemother* 1990; **34**: 1609–1610.
6 Nicodemo AC, Paez JI. Antimicrobial therapy for *Stenotrophomonas maltophilia* infections. *Eur J Clin Microbiol Infect Dis* 2007; **26**: 229–237.
7 Safdar A, Rolston KV. *Stenotrophomonas maltophilia*: changing spectrum of a serious bacterial pathogen in patients with cancer. *Clin Infect Dis* 2007; **45**: 1602–1609.
8 Boktour M, Hanna H, Ansari S, Bahna B, Hachem R, Tarrand J *et al.* Central venous catheter and *Stenotrophomonas maltophilia* bacteremia in cancer patients. *Cancer* 2006; **106**: 1967–1973.
9 Metan G, Hayran M, Hascelik G, Uzun O. Which patient is a candidate for empirical therapy against *Stenotrophomonas maltophilia* bacteraemia? An analysis of associated risk factors in a tertiary care hospital. *Scand J Infect Dis* 2006; **38**: 527–531.
10 Trouillet JL, Chastre J, Vuagnat A, Joly-Guillou ML, Combaux D, Dombret MC *et al.* Ventilator-associated pneumonia caused by potentially drug-resistant bacteria. *Am J Respir Crit Care Med* 1998; **157**: 531–539.
11 Martino R, Martinez C, Pericas R, Salazar R, Solá C, Brunet S *et al.* Bacteremia due to glucose non-fermenting gram-negative bacilli in patients with hematological neoplasias and solid tumors. *Eur J Clin Microbiol Infect Dis* 1996; **15**: 610–615.
12 Labarca JA, Leber AL, Kern VL, Territo MC, Brankovic LE, Bruckner DA *et al.* Outbreak of *Stenotrophomonas maltophilia* bacteremia in allogenic bone marrow transplant patients: role of severe neutropenia and mucositis. *Clin Infect Dis* 2000; **30**: 195–197.
13 Araoka H, Baba M, Yoneyama A. Risk factors for mortality among patients with *Stenotrophomonas maltophilia* bacteremia in Tokyo, Japan, 1996-2009. *Eur J Clin Microbiol Infect Dis* 2010; **29**: 605–608.
14 Micozzi A, Venditti M, Monaco M, Friedrich A, Taglietti F, Santilli S *et al.* Bacteremia due to *Stenotrophomonas maltophilia* in patients with hematologic malignancies. *Clin Infect Dis* 2000; **31**: 705–711.

© 2013 Macmillan Publishers Limited

15 Muder RR, Harris AP, Muller S, Edmond M, Chow JW, Papadakis K *et al.* Bacteremia due to Stenotrophomonas (Xanthomonas) maltophilia: a prospective, multicenter study of 91 episodes. *Clin Infect Dis* 1996; **22**: 508–512.
16 Paez JI, Tengan FM, Barone AA, Levin AS, Costa SF. Factors associated with mortality in patients with bloodstream infection and pneumonia due to *Stenotrophomonas maltophilia*. *Eur J Clin Microbiol Infect Dis* 2008; **27**: 901–906.
17 Paez JI, Costa SF. Risk factors associated with mortality of infections caused by *Stenotrophomonas maltophilia*: a systematic review. *J Hosp Infect* 2008; **70**: 101–108.
18 Chaplow R, Palmer B, Heyderman R, Moppett J, Marks DI. *Stenotrophomonas maltophilia* bacteraemia in 40 haematology patients: risk factors, therapy and outcome. *Bone Marrow Transplant* 2010; **45**: 1109–1110.
19 Yeshurun M, Gafter-Gvili A, Thaler M, Keller N, Nagler A, Shimoni A. Clinical characteristics of *Stenotrophomonas maltophilia* infection in hematopoietic stem cell transplantation recipients: a single center experience. *Infection* 2010; **38**: 211–215.
20 Friedman ND, Korman TM, Fairley CK, Franklin JC, Spelman DW. Bacteraemia due to *Stenotrophomonas maltophilia*: an analysis of 45 episodes. *J Infect* 2002; **45**: 47–53.
21 Korvick JA, Bryan CS, Farber B, Beam Jr TR, Schenfeld L, Muder RR *et al.* Prospective observational study of Klebsiella bacteremia in 230 patients: outcome for antibiotic combinations versus monotherapy. *Antimicrob Agents Chemother* 1992; **36**: 2639–2644.
22 Chow JW, Yu VL. Combination antibiotic therapy versus monotherapy for gram-negative bacteraemia: a commentary. *Int J Antimicrob Agents* 1999; **11**: 7–12.
23 Portugal RD, Garnica M, Nucci M. Index to predict invasive mold infection in high-risk neutropenic patients based on the area over the neutrophil curve. *J Clin Oncol* 2009; **27**: 3849–3854.
24 Majhail NS, Parks K, Defor TE, Weisdorf DJ. Diffuse alveolar hemorrhage and infection-associated alveolar hemorrhage following hematopoietic stem cell transplantation: related and high-risk clinical syndromes. *Biol Blood Marrow Transplant* 2006; **12**: 1038–1046.
25 Wanko SO, Broadwater G, Folz RJ, Chao NJ. Diffuse alveolar hemorrhage: retrospective review of clinical outcome in allogeneic transplant recipients treated with aminocaproic acid. *Biol Blood Marrow Transplant* 2006; **12**: 949–953.
26 Gupta S, Jain A, Warneke CL, Gupta A, Shannon VR, Morice RC *et al.* Outcome of alveolar hemorrhage in hematopoietic stem cell transplant recipients. *Bone Marrow Transplant* 2007; **40**: 71–78.
27 Elsner HA, Duhrsen U, Hollwitz B, Kaulfers PM, Hossfeld DK. Fatal pulmonary hemorrhage in patients with acute leukemia and fulminant pneumonia caused by *Stenotrophomonas maltophilia*. *Ann Hematol* 1997; **74**: 155–161.
28 Rousseau A, Morcos M, Amrouche L, Foïs E, Casetta A, Rio B *et al.* Lethal pulmonary hemorrhage caused by a fulminant Stenotrophomonas maltophilia respiratory infection in an acute myeloid leukemia patient. *Leuk Lymphoma* 2004; **45**: 1293–1296.
29 Ortin X, Jaen-Martinez J, Rodriguez-Luaces M, Alvaro T, Font L. Fatal pulmonary hemorrhage in a patient with myelodysplastic syndrome and fulminant pneumonia caused by *Stenotrophomonas maltophilia*. *Infection* 2007; **35**: 201–202.
30 Takahashi N, Yoshioka T, Kameoka Y, Tagawa H, Fujishima N, Saitoh H *et al.* Fatal hemorrhagic pneumonia caused by Stenotrophomanas maltophilia in a patient with non-Hodgkin lymphoma. *J Infect Chemother* 2011; **17**: 858–862.
31 O'Brien M, Davis GH. Enzymatic profile of *Pseudomonas maltophilia*. *J Clin Microbiol* 1982; **16**: 417–421.
32 Windhorst S, Frank E, Georgieva DN, Genov N, Buck F, Borowski P *et al.* The major extracellular protease of the nosocomial pathogen *Stenotrophomonas maltophilia*: characterization of the protein and molecular cloning of the gene. *J Biol Chem* 2002; **277**: 11042–11049.

© 2013 Macmillan Publishers Limited

Prognosis of acute myeloid leukemia harboring monosomal karyotype in patients treated with or without allogeneic hematopoietic cell transplantation after achieving complete remission

Masamitsu Yanada,[1] Saiko Kurosawa,[2] Takuhiro Yamaguchi,[3] Takuya Yamashita,[2] Yukiyoshi Moriuchi,[4] Hiroatsu Ago,[5] Jin Takeuchi,[6] Hirohisa Nakamae,[7] Jun Taguchi,[8] Toru Sakura,[9] Yasushi Takamatsu,[10] Fusako Waki,[11] Hiroki Yokoyama,[12] Masato Watanabe,[13] Nobuhiko Emi,[1] and Takahiro Fukuda[2]

[1]Department of Hematology, Fujita Health University, Aichi; [2]Hematology and Stem Cell Transplantation Division, National Cancer Center Hospital, Tokyo; [3]Division of Biostatistics, Tohoku University Graduate School of Medicine, Miyagi; [4]Department of Hematology, Sasebo City General Hospital, Nagasaki; [5]Department of Hematology and Oncology, Shimane Prefectural Central Hospital, Shimane; [6]Department of Hematology and Rheumatology, Nihon University School of Medicine, Tokyo; [7]Hematology, Osaka City University, Osaka; [8]Department of Hematology and Molecular Medicine Unit, Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki; [9]Department of Hematology, Saiseikai Maebashi Hospital, Gunma; [10]Division of Medical Oncology, Hematology and Infectious Disease, Department of Medicine, Fukuoka University Hospital, Fukuoka; [11]Division of Hematology, Department of Internal Medicine, Faculty of Medicine, Kagawa University, Kagawa; [12]Division of Clinical Oncology and Hematology, Jikei University School of Medicine, Tokyo; and [13]Division of Hematology, Yamada Hospital, Gifu, Japan

ABSTRACT

To evaluate the prognostic impact of monosomal karyotype on post-remission outcome in acute myeloid leukemia, we retrospectively analyzed 2,099 patients who had achieved complete remission. Monosomal karyotype was noted in 73 patients (4%). Of these, the probability of overall survival from first complete remission was 14% at four years, which was significantly lower than that reported in patients without monosomal karyotype, primarily due to a high relapse rate (86%). Monosomal karyotype remained significantly associated with worse overall survival among patients with unfavorable cytogenetics or complex karyotype, and even in patients who underwent allogeneic hematopoietic cell transplantation during first complete remission. These findings confirm that monosomal karyotype has a significantly adverse effect on post-remission outcome in patients with acute myeloid leukemia treated with and without allogeneic hematopoietic cell transplantation in first complete remission, emphasizing the need for the development of alternative therapies for this patient population.

Key words: acute myeloid leukemia, monosomal karyotype, cytogenetics, post-remission therapy, allogeneic hematopoietic cell transplantation.

Citation: Yanada M, Kurosawa S, Yamaguchi T, Yamashita T, Moriuchi Y, Ago H, Takeuchi J, Nakamae H, Taguchi J, Sakura T, Takamatsu Y, Waki F, Yokoyama H, Watanabe M, Emi N, and Fukuda T. Prognosis of acute myeloid leukemia harboring monosomal karyotype in patients treated with or without allogeneic hematopoietic cell transplantation after achieving complete remission. Haematologica 2012;97(6):915-918. doi:10.3324/haematol.2011.058289

©2012 Ferrata Storti Foundation. This is an open-access paper.

Introduction

Acute myeloid leukemia (AML) is a heterogeneous disease that includes subsets with distinct biological, clinical and prognostic features. It has been well established that cytogenetic abnormalities at diagnosis are associated with the biology of the disease and have important prognostic implications.[1-3] The coexistence of multiple cytogenetic abnormalities designated as complex karyotype (CK) has been recognized as a factor that predicts an extremely unfavorable outcome in AML.[4-7] However, the prognostic significance of CK has recently been challenged by Breems *et al.* who showed that the monosomal karyotype (MK), defined as 2 or more distinct autosomal monosomies or a single autosomal monosomy in the presence of other structural abnormalities, adversely affects the prognosis, and that the overlap of MK with CK is the main contributor to the unfavorable impact of CK.[8] According to Breems *et al.* and reports published subsequently by other groups,[7-10] patients with MK$^+$ AML show low complete remission (CR) rates ranging from 18% to 48% and overall survival (OS) rates of less than 10%. On the other hand, it has been suggested that such a poor outcome may be improved by allogeneic hematopoietic cell transplantation (HCT).[11]

To further clarify the prognosis of patients with MK$^+$ AML, especially regarding outcome after allogeneic HCT during first CR (CR1), we performed a retrospective analysis by using a dataset that included more than 2,000 AML patients in CR. Since failure to achieve CR is obviously associated with a dismal prognosis regardless of the presence or absence

The online version of this article has a Supplementary Appendix.

Acknowledgments: this work was supported by grants from the Japanese Ministry of Health, Labour and Welfare and the Advanced Clinical Research Organization.

Manuscript received on November 8, 2011. Revised version arrived on December 13, 2011. Manuscript accepted on December 15, 2011.

Correspondence: Saiko Kurosawa, MD, Hematology and Stem Cell Transplantation Division, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo, 104-0045, Japan. Phone international: +81.3.35422511; Fax international: +81.3.35423815; E-mail: skurosaw@ncc.go.jp

of MK, the present analysis focused on patients who achieved CR with one or two courses of chemotherapy.

Design and Methods

Patients

For this study, we used a Japanese nationwide database of adult AML patients. Eligible patients were required to be between 16 and 70 years of age, to be diagnosed with AML from 1999 to 2006 according to the World Health Organization (WHO) classification,[12] and to have achieved CR with one or two courses of chemotherapy. We excluded patients with acute promyelocytic leukemia (n=386) and those without pre-treatment cytogenetic results (n=36); this left 2,099 patients available for analysis. This study was approved by the Institutional Review Board at the National Cancer Center Hospital.

Cytogenetic analysis

Cytogenetic analysis was performed on metaphases from samples of bone marrow or blood obtained prior to induction therapy by using standard banding techniques. Karyotypes were determined according to the International System for Human Cytogenetic Nomenclature.[13] An abnormality was considered to be clonal when at least 2 metaphases had the same aberration in the case of either a structural abnormality or an additional chromosome. If there was a monosomy, it had to be present in at least 3 metaphases to be considered significant. Cytogenetics was classified as favorable, intermediate, unfavorable or unknown risk according to the Southwest Oncology Group (SWOG) criteria.[5] Apart from the SWOG classification, the MK status was assessed retrospectively for this study according to the definition proposed by Breems *et al.*[8] Accordingly, patients were divided into 4 cytogenetic subgroups: core binding factor AML (CBF AML), cytogenetically normal AML (CN AML), cytogenetically abnormal non-CBF AML without MK (MK$^-$ AML), and cytogenetically abnormal non-CBF AML with MK (MK$^+$ AML).

Statistical analysis

A Kaplan-Meier survival analysis was performed to estimate the probabilities of OS and relapse-free survival (RFS). OS was defined as the time from the achievement of first CR (CR1) to death or last visit, and RFS as the time from the achievement of CR1 to relapse, death or last visit. Differences in OS and RFS between groups were compared by means of the log rank test. Cumulative incidences of relapse and non-relapse mortality were calculated with relapse considered as a competing risk for non-relapse mortality, and vice versa. Cox's regression model was used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs). All statistical analyses were performed with the SPSS software version 11.0.1 (SPSS, Chicago, IL, USA) and R software version 2.13.0 (The R Foundation for Statistical Computing).

Results and Discussion

The entire cohort consisted of 2,099 AML patients who had achieved CR with one or two courses of chemotherapy, among whom CBF AML, CN AML, MK$^-$ AML and MK$^+$ AML accounted for 21%, 49%, 27% and 4%, respectively. Table 1 shows the patients' characteristics according to these cytogenetic subgroups. Among the 73 patients with MK$^+$ AML, 68 (93%) had a cytogenetically unfavorable risk, while the remaining 5 had an unknown risk. In patients younger than 60 years, intensive therapy defined as "3+7" or its equivalent, was given to more than 95% in all of the cytogenetic subgroups. In patients aged 60 years or older, the proportion of those given intensive therapy seemed slightly lower in MK$^+$ AML but, nevertheless, 75% of them received intensive therapy.

Allogeneic HCT was performed in 32 patients with MK$^+$ AML, including 15 during CR1, 4 during second CR (CR2) and 13 during other disease phases. The details of patients who underwent allogeneic HCT in CR1 are summarized in the *Online Supplementary Table S1*. The median time from CR1 to transplantation was 93 days (range 14-540 days) for the 15 patients with MK$^+$ AML, which was significantly shorter than those in the other groups (P=0.011).

Figure 1A compares survival curves from the time of CR1 according to the cytogenetic subgroups. With a median follow up of 4.1 years for surviving patients, the 4-year probabilities of OS were 68% in CBF AML, 58% in CN AML, 46% in MK$^-$ AML and 14% in MK$^+$ AML, respectively (P<0.001). This significantly inferior OS in MK$^+$ AML patients can mainly be explained by a high risk of relapse, since the relapse rate was 86% at four years, which was significantly higher than those in the remaining groups (P<0.001). No patient with MK$^+$ AML survived four years without allogeneic HCT, and the difference in OS was more pronounced when patients undergoing allogeneic HCT were analyzed as censored cases (83%, 66%, 54% and 0% at four years in CBF AML, CN AML, MK$^-$ AML and MK$^+$ AML, respectively; P<0.001).

Next, we examined whether MK identified a very poor prognostic subset within 2 cytogenetically distinct subpopulations representing poor prognosis, i.e. unfavorable cyto-

Table 1. Patient's characteristics according to cytogenetic subgroup.

	CBF n=437	CN n=1,027	MK- n=562	MK+ n=73
Age, years				
Median	45	51	48	53
Range	16-70	16-70	16-70	20-70
Sex				
Male	279 (64%)	576 (56%)	311 (55%)	47 (64%)
Female	158 (36%)	451 (44%)	251 (45%)	26 (36%)
Cytogenetic risk by SWOG				
Favorable	411 (94%)	-	-	-
Intermediate	-	1,027 (100%)	64 (11%)	-
Unfavorable	26 (6%)	-	300 (53%)	68 (93%)
Unknown	-	-	198 (35%)	5 (7%)
WBC count, $\times 10^9$/L				
Median	11.2	13.0	8.5	4.4
Range	0.7-281.2	0.4-40.2	0.3-22.3	0.8-408.0
Dysplasia				
Yes	35 (8%)	220 (20%)	136 (24%)	33 (45%)
No	402 (92%)	807 (80%)	426 (76%)	40 (55%)
N. induction courses				
1 course	378 (86%)	825 (80%)	419 (75%)	56 (77%)
2 courses	59 (14%)	202 (20%)	143 (25%)	17 (23%)
Allogeneic HCT				
CR1	32 (7%)	256 (25%)	183 (33%)	15 (21%)
CR2	78 (18%)	106 (10%)	57 (10%)	4 (5%)
Other disease phase	66 (15%)	125 (12%)	87 (15%)	13 (18%)
Not performed	261 (60%)	540 (53%)	235 (42%)	41 (56%)

CBF: core binding factor AML; CN: cytogenetically normal AML; MK-: cytogenetically abnormal non-CBF AML without monosomal karyotype; MK+: cytogenetically abnormal non-CBF AML with monosomal karyotype; SWOG: Southwest Oncology Group; WBC: white blood cell count; HCT: hematopoietic cell transplantation; CR1: first complete remission; CR2: second complete remission.

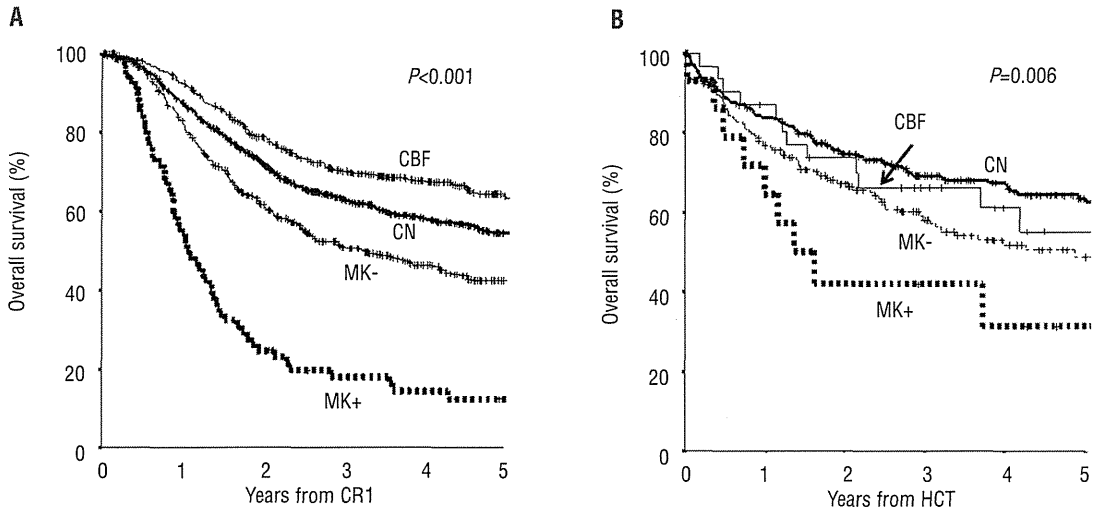

Figure 1. Kaplan-Meier curves for (A) OS after achieving CR1 for the entire cohort, and for (B) OS after allogeneic HCT for patients who underwent allogeneic HCT in CR1, according to the cytogenetic subgroups. CBF represents core binding factor AML; CN: cytogenetically normal AML; MK$^-$, cytogenetically abnormal non-CBF AML without monosomal karyotype; MK$^+$, cytogenetically abnormal non-CBF AML with monosomal karyotype. *P* values are presented for comparisons among the 4 groups.

genetics and CK. MK accounted for 17% of those with unfavorable cytogenetics (68 of 394), and 41% of those with CK (39 of 96). Among patients with unfavorable cytogenetics, there was a statistically significant difference in OS between those with and without MK (16% *vs.* 46% at four years, *P*<0.001; *Online Supplementary Figure S1A*). Similar findings were seen in patients with CK, with 4-year OS rates of 11% and 34% in those with and without MK (*P*<0.001; *Online Supplementary Figure S1B*).

Allogeneic HCT was performed during CR1 in 32 of 437 CBF AML patients (7%), 256 of 1,027 CN AML patients (25%), 183 of 562 MK$^-$ AML patients (33%), and 15 of 73 MK$^+$ AML patients (21%). Figure 1B shows Kaplan-Meier curves for OS after HCT in patients who were transplanted during CR1. These subgroups showed significantly different OS, with 4-year OS rates of 61%, 67%, 52% and 31% in CBF AML, CN AML, MK$^-$ AML, and MK$^+$ AML, respectively (*P*=0.006). A statistically significant difference was observed in terms of post-transplant relapse (*P*=0.025) (*Online Supplementary Table S2*). Non-relapse mortality in patients with MK$^+$ AML appeared to be higher than those in the other groups, but these differences were not statistically significant (*P*=0.595). Table 2 shows results of univariate and multivariate analyses on factors associated with post-transplant OS in patients undergoing allogeneic HCT in CR1. After adjusting for other covariates, MK remained significantly associated with inferior post-transplant OS (HR 3.12; 95% CI, 1.58-6.15; *P*=0.001, with reference to CN AML).

MK is a recently proposed subgroup of cytogenetic abnormalities that confers a very unfavorable prognosis in AML.[8] Reported CR rates have been quite low, ranging between 18 and 48%,[8-10] and this represents a major cause of the poor prognosis. Since patients who fail to achieve CR generally have a very unfavorable prognosis regardless of the presence or absence of MK, we decided to restrict our analysis to patients who had achieved CR. In our patient population, MK was observed in 4%; this was lower than the values reported previously (6-13%).[7-9] The most probable explanation for this could be the fact that our cohort included only patients who had achieved CR, while the other studies included newly diagnosed patients.

Our data clearly demonstrated that MK confers a significantly worse prognosis in patients who have achieved CR. Notably, MK identified patients with a worse prognosis

Table 2. Factors associated with post-transplant OS in patients who underwent allogeneic HCT in CR1.

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Cytogenetic subgroup				
CBF	1.14 (0.62-2.09)	0.671	1.17 (0.63-2.15)	0.622
CN	1.00	-	1.00	-
MK$^-$	1.43 (1.05-1.96)	0.023	1.45 (1.06-1.98)	0.021
MK$^+$	2.74 (1.42-5.28)	0.003	3.12 (1.58-6.15)	0.001
Age				
As a numerical variable (1 year older)	1.01 (1.00-1.02)	0.294	1.01 (0.99-1.02)	0.377
Sex				
Male	1.00	-	1.00	-
Female	1.08 (0.81-1.45)	0.597	1.16 (0.86-1.57)	0.327
WBC count				
As a numerical variable (10×10^9/L lower)	1.02 (1.00-1.03)	0.037	1.02 (1.01-1.04)	0.007
Donor				
Related*	1.00	-	1.00	-
Other	1.39 (1.04-1.87)	0.026	1.47 (1.09-1.98)	0.011
Conditioning				
Myeloablative	1.00	-	1.00	-
Reduced-intensity	1.13 (0.81-1.58)	0.465	1.04 (0.70-1.56)	0.846

*HR: hazard ratio; CI: confidence interval; CBF: core binding factor AML; CN: cytogenetically normal AML; MK$^-$: cytogenetically abnormal non-CBF AML without monosomal karyotype; MK$^+$: cytogenetically abnormal non-CBF AML with monosomal karyotype; WBC: white blood cell count. *"Related" indicates a matched or 1 antigen-mismatched family donor.*

even among those with unfavorable cytogenetics or those with CK. The detrimental prognostic impact of MK was primarily due to high relapse rates and, importantly, similar results were seen in patients who received allogeneic HCT in CR1. Post-transplant relapse occurred more than 20% more frequently in MK+ AML patients than in those in each of the remaining cytogenetic subgroups. This finding is consistent with published studies.[11,14] Investigators at the University of Minnesota analyzed 134 AML patients, including 17 patients with MK who were allografted in CR1, and showed that the MK classification could significantly predict the risk of post-transplant relapse.[14] A report from the Fred Hutchinson Cancer Research Center described the outcome of 35 patients with MK and 193 patients without MK who underwent allogeneic HCT in CR1, in which the 4-year OS rates were 30 and 65% in those with and without MK.[11] Those results taken together with our present results suggest that allogeneic HCT may be able to improve but not completely override the poor prognosis with MK+ AML. It is widely recognized that allogeneic HCT in CR1 is the treatment of choice for patients with AML at cytogenetically unfavorable risk,[15-17] if they have a suitable donor and are fit enough to undergo the procedure. In this study, allogeneic HCT was given to only 21% of patients with MK+ AML during CR1. This low transplantation rate could partly be due to a short CR1 duration, which likely decreased the chance of receiving allogeneic HCT in CR1. A significantly shorter time to transplantation in our MK+ AML patients might reflect the short duration of their CR1 that precluded an implementation of allogeneic HCT after a relatively long interval after achieving CR. Despite a considerable risk of relapse even after transplantation, it is still conceivable that these cytogenetically very unfavorable patients would benefit from allogeneic HCT. We observed that no patient survived long-term without allogeneic HCT, which is in line with reports from the SWOG study.[9]

Our study has several limitations and the results must, therefore, be interpreted with caution. These limitations include the retrospective nature of the study, and the relatively small number of patients with MK+ AML, especially of those who underwent allogeneic HCT in CR1, leaving room for selection bias or chance effect. However, given that MK+ AML accounted for only 4% of our AML patients in CR, it would be quite impractical to conduct a prospective comparison to assess the role of allogeneic HCT in CR1. Under such conditions, the findings from a large-scale retrospective study could have important implications.

In summary, our data confirm that MK exerts a significantly adverse effect on post-remission outcome in AML patients treated with and without allogeneic HCT in CR1. Although our results suggest that allogeneic HCT is already an available treatment of choice, the development of alternative therapies is warranted for this patient population.

Authorship and Disclosures

The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at www.haematologica.org.

Financial and other disclosures provided by the authors using the ICMJE (www.icmje.org) Uniform Format for Disclosure of Competing Interests are also available at www.haematologica.org.

References

1. Estey E, Dohner H. Acute myeloid leukaemia. Lancet. 2006;368(9550):1894-907.
2. Dohner H, Estey EH, Amadori S, Appelbaum FR, Buchner T, Burnett AK, et al. Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. Blood. 2010;115(3):453-74.
3. Burnett A, Wetzler M, Lowenberg B. Therapeutic advances in acute myeloid leukemia. J Clin Oncol. 2011;29(5):487-94.
4. Grimwade D, Walker H, Oliver F, Wheatley K, Harrison C, Harrison G, et al. The importance of diagnostic cytogenetics on outcome in AML: analysis of 1,612 patients entered into the MRC AML 10 trial. The Medical Research Council Adult and Children's Leukaemia Working Parties. Blood. 1998; 92(7):2322-33.
5. Slovak ML, Kopecky KJ, Cassileth PA, Harrington DH, Theil KS, Mohamed A, et al. Karyotypic analysis predicts outcome of preremission and postremission therapy in adult acute myeloid leukemia: a Southwest Oncology Group/Eastern Cooperative Oncology Group Study. Blood. 2000;96(13): 4075-83.
6. Byrd JC, Mrozek K, Dodge RK, Carroll AJ, Edwards CG, Arthur DC, et al. Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: results from Cancer and Leukemia Group B (CALGB 8461). Blood. 2002;100(13):4325-36.
7. Grimwade D, Hills RK, Moorman AV, Walker H, Chatters S, Goldstone AH, et al. Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. Blood. 2010;116(3):354-65.
8. Breems DA, Van Putten WL, De Greef GE, Van Zelderen-Bhola SL, Gerssen-Schoorl KB, Mellink CH, et al. Monosomal karyotype in acute myeloid leukemia: a better indicator of poor prognosis than a complex karyotype. J Clin Oncol. 2008;26(29):4791-7.
9. Medeiros BC, Othus M, Fang M, Roulston D, Appelbaum FR. Prognostic impact of monosomal karyotype in young adult and elderly acute myeloid leukemia: the Southwest Oncology Group (SWOG) experience. Blood. 2010;116(13):2224-8.
10. Perrot A, Luquet I, Pigneux A, Mugneret F, Delaunay J, Harousseau JL, et al. Dismal prognostic value of monosomal karyotype in elderly patients with acute myeloid leukemia: a GOELAMS study of 186 patients with unfavorable cytogenetic abnormalities. Blood. 2011;118(3):679-85.
11. Fang M, Storer B, Estey E, Othus M, Zhang L, Sandmaier BM, et al. Outcome of patients with acute myeloid leukemia with monosomal karyotype who undergo hematopoietic cell transplantation. Blood. 2011;118(6): 1490-4.
12. Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood. 2009;114(5):937-51.
13. International Standing Committee on Human Cytogenetic Nomenclature. Shaffer LG, Slovak ML, Campbell LJ. ISCN 2009: an international system for human cytogenetic nomenclature (2009). Basel; Unionville, CT: Karger; 2009.
14. Oran B, Dolan M, Cao Q, Brunstein C, Warlick E, Weisdorf D. Monosomal karyotype provides better prognostic prediction after allogeneic stem cell transplantation in patients with acute myelogenous leukemia. Biol Blood Marrow Transplant. 2011;17(3): 356-64.
15. Yanada M, Matsuo K, Emi N, Naoe T. Efficacy of allogeneic hematopoietic stem cell transplantation depends on cytogenetic risk for acute myeloid leukemia in first disease remission: a metaanalysis. Cancer. 2005;103(8):1652-8.
16. Cornelissen JJ, van Putten WL, Verdonck LF, Theobald M, Jacky E, Daenen SM, et al. Results of a HOVON/SAKK donor versus no-donor analysis of myeloablative HLA-identical sibling stem cell transplantation in first remission acute myeloid leukemia in young and middle-aged adults: benefits for whom? Blood. 2007;109(9):3658-66.
17. Koreth J, Schlenk R, Kopecky KJ, Honda S, Sierra J, Djulbegovic BJ, et al. Allogeneic stem cell transplantation for acute myeloid leukemia in first complete remission: systematic review and meta-analysis of prospective clinical trials. JAMA. 2009;301 (22):2349-61.

Rapid T-cell chimerism switch and memory T-cell expansion are associated with pre-engraftment immune reaction early after cord blood transplantation

Cord blood (CB) contains immature immune cells and is thought to be less active in inducing allogeneic immune reaction than other sources of stem cells. However, a high incidence of immune-mediated complications has been reported, such as pre-engraftment immune reaction (PIR) and haemophagocytic syndrome (HPS) early after cord blood transplantation (CBT) (Kishi *et al*, 2005; Narimatsu *et al*, 2007; Frangoul *et al*, 2009; Takagi *et al*, 2009; Patel *et al*, 2010). In addition, we reported that human leucocyte antigen (HLA) disparity in the graft-*versus*-host (GVH) direction adversely affected engraftment kinetics when single calcineurin inhibitors were used for GVH disease (GVHD) prophylaxis (Matsuno *et al*, 2009). These observations suggested that the GVH reaction plays a critical role in engraftment. Here, we report the engraftment kinetics of donor-derived T cells using a multicolour flow cytometry-based method (HLA-Flow method) (Watanabe *et al*, 2008) and also describe the results of naïve/memory T-cell phenotype analyses early after CBT.

Between November 2009 and September 2010, 73 adult patients underwent single-unit CBT at Toranomon hospital. This study reports 41 patients who were eligible for chimerism analysis using the HLA-Flow method and survived more than 14 d after CBT. Characteristics of the patients and CB are summarized in Table SI. All patients provided written informed consent, and the study was conducted in accordance with institutional review board requirements. Peripheral blood was collected at 1, 2, 3, 4, and 8 weeks after CBT. Anti-HLA monoclonal antibodies in combination with lineage-specific antibodies were used to analyse the lineage-specific chimerism as previously reported (Watanabe *et al*, 2008). Anti-HLA antibodies specific for donor and recipient HLA in all patients are summarized in Table SII. At 2, 4, and 8 weeks after CBT, T-cell subsets were analysed using the following monoclonal antibodies: peridinin-chlorophyll-protein – cyanin 5·5 (PerCP-Cy5·5)-CD8, phycoerythrin – cyanin 7 (PE-Cy7)-CCR7, allophycocyanin (APC)-CD4, APC-Cy7-CD3 (BD Pharmingen, San Jose, CA, USA), and Pacific Blue-CD45RA (CALTAG, Carlsbad, CA, USA). Absolute numbers of $CD4^+$ T cells ($CD3^+CD4^+$), $CD8^+$ T cells ($CD3^+CD8^+$), and naïve ($CD45RA^+CCR7^+$) and memory ($CD45RA^-CCR7^{+/-}$) T cells were calculated by multiplying the peripheral lymphocyte counts by the percentage of positive cells. PIR was characterized by non-infectious high-grade fever (>38·5°C) coexisting with skin eruption, diarrhoea, jaundice and/or body weight gain greater than 5% of baseline, developing 6 or more days before engraftment (Kishi *et al*, 2005; Uchida *et al*, 2011). Cumulative incidence of neutrophil engraftment, PIR, and GVHD were calculated using Gray's method. Intergroup comparisons were performed using the Mann–Whitney *U*-test.

We analysed lineage-specific chimerism for 32, 40, 40, 34, and 34 patients at a median of 8 (range, 7–11; week 1), 15 (14–20; week 2), 22 (21–25; week 3), 29 (28–36; week 4), and 57 (56–62; week 8) days post-transplant, respectively. Fig 1A shows representative results for $CD4^+$ T-cell chimerism. $CD4^+$ and $CD8^+$ T-cell chimerism results in all patients are shown in Fig 1B. Of 41 enrolled patients, 37 achieved neutrophil engraftment at a median of 19 d (range, 13–38 d). Thirty-nine patients achieved donor-dominant T-cell chimerism (>90%) by 3 weeks after CBT, whereas the remaining two patients, with recipient-dominant T-cell chimerism (>90%) at every point tested, developed graft failure because of early relapse (day 14 post-transplant) and rejection, respectively. Among the 39 patients who achieved donor-dominant T-cell chimerism, two died before engraftment due to non-relapse causes on day 28 (infection) and day 25 (diffuse alveolar haemorrhage), respectively. Among those with donor-dominant chimerism, 24 (63%) of 38 evaluable patients developed PIR at a median of 8 (6–11) days after CBT. Patients who achieved donor-dominant T-cell chimerism (>90%) at 1 week had a higher incidence of PIR compared to those who did not ($P = 0{\cdot}017$, Fig 1C). In a representative patient at 2 weeks after CBT, rapid conversion from naïve to memory phenotype was observed in both $CD4^+$ and $CD8^+$ T cells (Fig 2A). Fig 2B shows the relative proportion of naïve $CD4^+$ and $CD8^+$ T cells at 2, 4, and 8 weeks after CBT in 37 evaluable patients who achieved donor-dominant T-cell chimerism. Patients who developed PIR had significantly more lymphocytes, $CD4^+$ T cells, $CD8^+$ T cells, $CD4^+$ memory T cells, and $CD8^+$ memory T cells at 2 weeks after CBT compared with those without PIR (Fig 2C and data not shown).

Our data confirmed that a majority of patients achieved donor-dominant T-cell chimerism around 2 weeks after CBT. We also found that early recipient-type T-cell chimerism was closely associated with graft rejection. A remarkable finding was that a rapid recipient-to donor-dominant switch

© 2012 Blackwell Publishing Ltd, *British Journal of Haematology*
doi:10.1111/bjh.12097

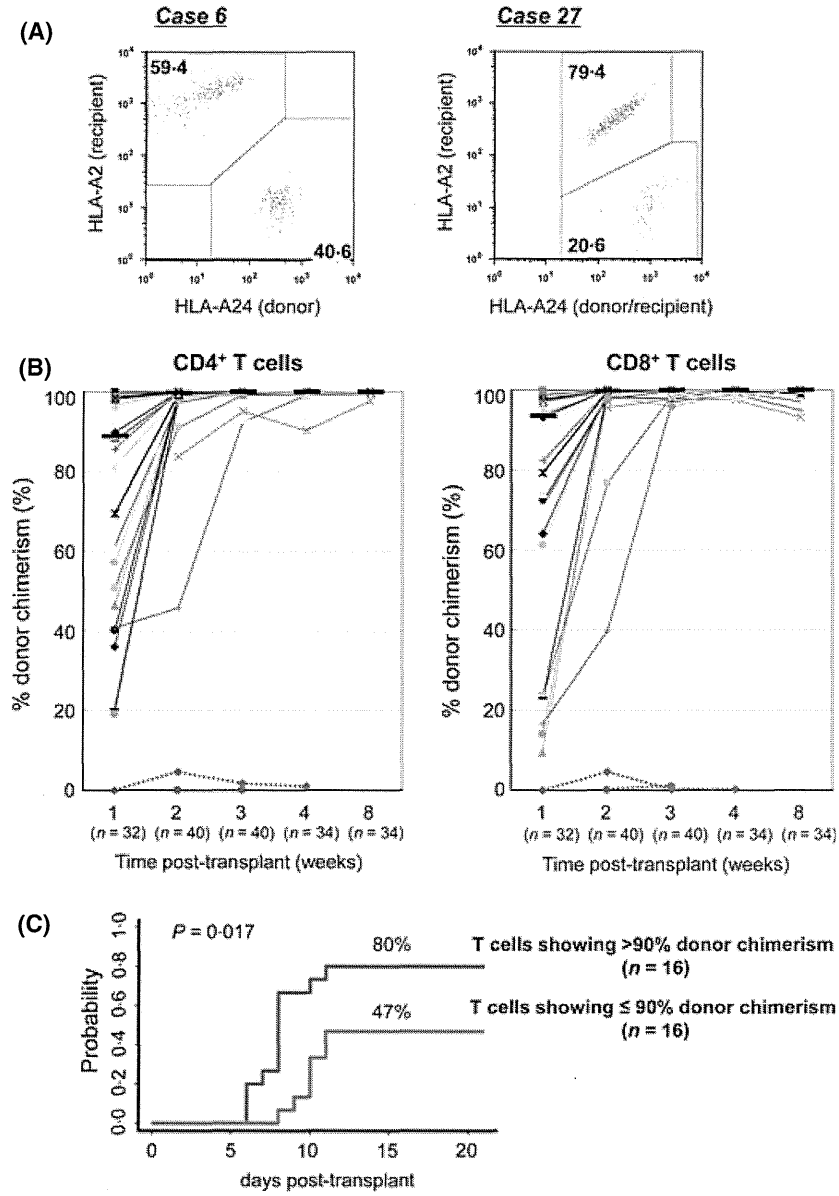

Fig 1. T-cell chimerism analysed by HLA-Flow method. (A) Chimerism analysis by the HLA-Flow method separated donor- vs. recipient-derived cells among $CD4^+$ T cells at 1 week after cord blood transplant (CBT). In Case 6, human leucocyte antigen (HLA)-A2 was recipient-specific and HLA-A24 was donor-specific. In Case 27, HLA-A2 was recipient-specific, whereas HLA-A24 was shared by both donor and recipient, indicating that HLA-A2-negative and HLA-A24-positive cells were donor-derived. (B) The median percentages of donor-derived $CD4^+$ T cells and $CD8^+$ T cells at 1 week after CBT were 88·9%, and 93·5%, respectively. Red dotted lines indicate recipient-dominant chimerism in two patients who developed graft failure. (C) Cumulative incidence of pre-engraftment immune reaction (PIR) according to chimerism status of T cells at 1 week after CBT

of T-cell chimerism at 1 week post-transplant was associated with a higher incidence of PIR, supporting a hypothesis that PIR could be an early variant form of GVH reaction caused by donor-derived T cells. CB T cells are naïve and do not include pathogen-specific effector T cells. Grindebacke *et al* (2009) demonstrated that about 80% of $CD4^+$ T cells kept the naïve phenotype during the first 18 months after birth. In contrast, we found a rapid conversion from naïve to memory phenotype at 2 weeks after CBT. In addition, PIR could be associated with peripheral expansion of donor-derived memory T cells. Recently, Gutman *et al* (2010) reported that $CD8^+$ T cells predominately expressed effector memory or effector phenotype early after double-unit CBT, reflecting an immune response of the dominant unit against the non-engrafting unit. These findings suggest that donor-derived naïve T cells will be activated by alloantigens and differentiate into mature cells early after CBT. Most of the present patients with PIR responded promptly after a

© 2012 Blackwell Publishing Ltd,

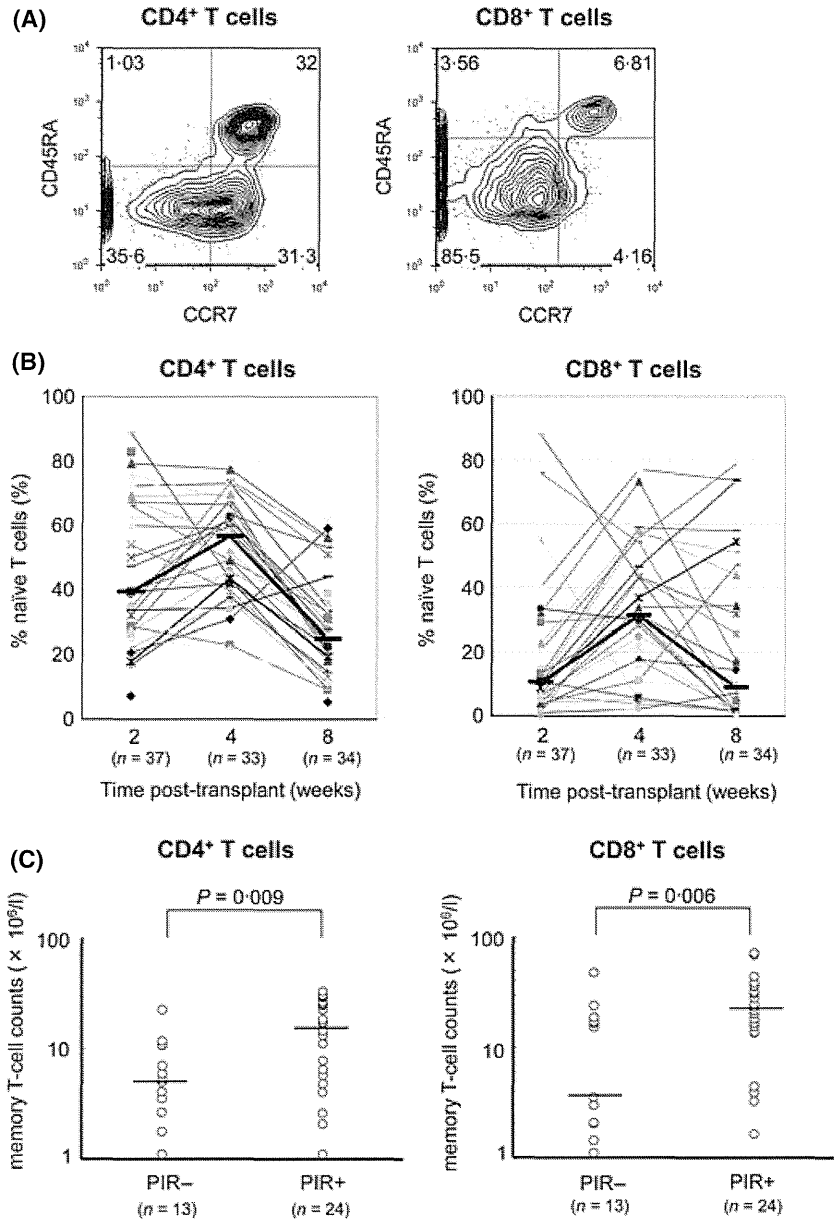

Fig 2. Conversion from naïve to memory T-cell phenotype. (A) A rapid conversion from naïve phenotype ($CD45RA^{+}CCR7^{+}$) to memory phenotype ($CD45RA^{-}CCR7^{+/-}$) in a representative sample at 2 weeks after cord blood transplant (CBT) (Case 5). (B) Relative proportion of naïve $CD4^{+}$ and $CD8^{+}$ T cells at 2, 4, and 8 weeks after CBT. Bold horizontal lines denote median values. (C) Memory T-cell counts at 2 weeks after CBT in patients with or without pre-engraftment immune reaction (PIR).

short course of steroid treatment, and none experienced graft failure due to HPS. This observation could be attributed to more intensive immunosuppression from adding mycophenolate mofetil to tacrolimus in the majority of patients (Uchida *et al*, 2011). Although neither the T-cell chimerism nor the memory T-cell counts affected the incidence of acute GVHD, steroid treatment for PIR could suppress the onset of acute GVHD. In conclusion, rapid T-cell chimerism switch and donor-derived memory T-cell expansion were associated with PIR, supporting a significant role of donor-derived T cells in the pathogenesis of the early immune reaction after CBT.

Acknowledgements

The authors thank Madoka Narita for data collection and her skilful secretarial assistance; Eri Watanabe, Mari Muto, and Stephanie Napier for their technical expertise. We also thank all physicians (Shigeyoshi Makino and Hideki Araoka), nurses, pharmacists (Yumiko Uchida and Tadaaki Ito), data managers (Naomi Yamada, Kaori Kobayashi and Rumiko Tsuchihashi), and support personnel for their care of patients involved in this study. This work was supported in part by a Grant-in-Aid from the Japanese Ministry of Health, Labour, and Welfare (H21-Clinical Research-Ippan-020).

Author contributions

NM, HY, NW, HN, and ST designed the study; NM, HY, and NW performed the research; NM and HY analysed data; HY, NU, HO, AN, TI, K Ishiwata, NN, MT, Y A-M, K Izutsu, KM, AW, and ST performed transplantation; AY reviewed histopathological findings; and NM, HY, NW, NU, HN, and ST contributed to writing the paper.

Competing interests

The authors have no competing interests.

Naofumi Matsuno[1,2]*
Hisashi Yamamoto[2]*
Nobukazu Watanabe[1]
Naoyuki Uchida[2]
Hikari Ota[2]
Aya Nishida[2]
Taichi Ikebe[2]
Kazuya Ishiwata[2]
Nobuaki Nakano[2]
Masanori Tsuji[2]
Yuki Asano-Mori[2]
Koji Izutsu[2]
Kazuhiro Masuoka[2]
Atsushi Wake[2]
Akiko Yoneyama[3]
Hiromitsu Nakauchi[4]
Shuichi Taniguchi[2,5]

[1]*Laboratory of Diagnostic Medicine, Division of Stem Cell Therapy, Centre for Stem Cell Biology and Regenerative Medicine, the Institute of Medical Science, the University of Tokyo, Tokyo, Japan,* [2]*Department of Haematology, Toranomon Hospital, Tokyo, Japan,* [3]*Department of Infectious Diseases, Toranomon Hospital, Tokyo, Japan,* [4]*Division of Stem Cell Therapy, Centre for Stem Cell Biology and Regenerative Medicine, the Institute of Medical Science, the University of Tokyo, Tokyo, Japan and* [5]*Okinaka Memorial Institute for Medical Research, Tokyo, Japan*
E-mail: nwatanab@ims.u-tokyo.ac.jp

*The first two authors contributed equally to this work.

Keywords: cord blood transplantation, chimerism, human leucocyte antigen-Flow method, naïve and memory T-cell, pre-engraftment immune reaction.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table SI. Patient and cord blood characteristics.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

References

Frangoul, H., Wang, L., Harrell, F.E., Jr, Ho, R. & Domm, J. (2009) Preengraftment syndrome after unrelated cord blood transplant is a strong predictor of acute and chronic graft-*versus*-host disease. *Biology of Blood and Marrow Transplantation*, **15**, 1485–1488.

Grindebacke, H., Stenstad, H., Quiding-Jarbrink, M., Waldenstrom, J., Adlerberth, I., Wold, A.E. & Rudin, A. (2009) Dynamic development of homing receptor expression and memory cell differentiation of infant CD4+CD25high regulatory T cells. *Journal of Immunology*, **183**, 4360–4370.

Gutman, J.A., Turtle, C.J., Manley, T.J., Heimfeld, S., Bernstein, I.D., Riddell, S.R. & Delaney, C. (2010) Single-unit dominance after double-unit umbilical cord blood transplantation coincides with a specific CD8+ T-cell response against the nonengrafted unit. *Blood*, **115**, 757–765.

Kishi, Y., Kami, M., Miyakoshi, S., Kanda, Y., Murashige, N., Teshima, T., Kusumi, E., Hara, S., Matsumura, T., Yuji, K., Masuoka, K., Wake, A., Morinaga, S., Kanemaru, M., Hayashi, T., Tanaka, Y. & Taniguchi, S. (2005) Early immune reaction after reduced-intensity cord-blood transplantation for adult patients. *Transplantation*, **80**, 34–40.

Matsuno, N., Wake, A., Uchida, N., Ishiwata, K., Araoka, H., Takagi, S., Tsuji, M., Yamamoto, H., Kato, D., Matsuhashi, Y., Seo, S., Masuoka, K., Miyakoshi, S., Makino, S., Yoneyama, A., Kanda, Y. & Taniguchi, S. (2009) Impact of HLA disparity in the graft-*versus*-host direction on engraftment in adult patients receiving reduced-intensity cord blood transplantation. *Blood*, **114**, 1689–1695.

Narimatsu, H., Terakura, S., Matsuo, K., Oba, T., Uchida, T., Iida, H., Hamaguchi, M., Watanabe, M., Kohno, A., Murata, M., Sawa, M., Miyamura, K. & Morishita, Y. (2007) Short-term methotrexate could reduce early immune reactions and improve outcomes in umbilical cord blood transplantation for adults. *Bone Marrow Transplantation*, **39**, 31–39.

Patel, K.J., Rice, R.D., Hawke, R., Abboud, M., Heller, G., Scaradavou, A., Young, J.W. & Barker, J.N. (2010) Pre-engraftment syndrome after double-unit cord blood transplantation: a distinct syndrome not associated with acute graft-*versus*-host disease. *Biology of Blood and Marrow Transplantation*, **16**, 435–440.

Takagi, S., Masuoka, K., Uchida, N., Ishiwata, K., Araoka, H., Tsuji, M., Yamamoto, H., Kato, D., Matsuhashi, Y., Kusumi, E., Ota, Y., Seo, S., Matsumura, T., Matsuno, N., Wake, A., Miyakoshi, S., Makino, S., Ohashi, K., Yoneyama, A. & Taniguchi, S. (2009) High incidence of haemophagocytic syndrome following umbilical cord blood transplantation for adults. *British Journal of Haematology*, **147**, 543–553.

Uchida, N., Wake, A., Nakano, N., Ishiwata, K., Takagi, S., Tsuji, M., Yamamoto, H., Kato, D., Matsuno, N., Masuoka, K., Araoka, H., Asano-Mori, Y., Izutsu, K., Makino, S., Yoneyama, A. & Taniguchi, S. (2011) Mycophenolate and tacrolimus for graft-*versus*-host disease prophylaxis for elderly after cord blood transplantation: a matched pair comparison with tacrolimus alone. *Transplantation*, **92**, 366–371.

Watanabe, N., Takahashi, S., Ishige, M., Ishii, Y., Ooi, J., Tomonari, A., Tsukada, N., Konuma, T., Kato, S., Sato, A., Tojo, A. & Nakauchi, H. (2008) Recipient-derived cells after cord blood transplantation: dynamics elucidated by multicolour FACS, reflecting graft failure and relapse. *Biology of Blood and Marrow Transplantation*, **14**, 693–701.

© 2012 Blackwell Publishing Ltd,

Int J Hematol (2012) 96:10–25
DOI 10.1007/s12185-012-1086-x

REVIEW ARTICLE

Mycophenolate mofetil: fully utilizing its benefits for GvHD prophylaxis

Kentaro Minagawa · Motohiro Yamamori · Yoshio Katayama · Toshimitsu Matsui

Received: 1 March 2012 / Revised: 18 April 2012 / Accepted: 19 April 2012 / Published online: 17 May 2012
© The Japanese Society of Hematology 2012

Abstract Mycophenolate mofetil (MMF) has been widely used for the prophylaxis of graft-versus-host disease (GvHD) in hematopoietic stem cell transplantation (HSCT), based on clinical evidence established in organ transplantations. MMF is not a cytotoxic, but rather a cytostatic agent, and there have been several reports of significant advantages in engraftment as well as greatly reduced stomatitis compared to methotrexate (MTX). MMF has been preferred for MTX-free immunosuppression, especially in reduced intensity conditioning, but it is suitable for GvHD prophylaxis for any type of HSCT. Some clinicians doubt its effectiveness, due to the lack of advantage over MTX in acute GvHD prophylaxis, especially in myeloablative conditioning. Pharmacokinetics studies of mycophenolic acid (MPA), the active form of MMF, show large inter- and intra-patient variation, which make interpretations of its clinical usefulness difficult. Nevertheless, several studies, including ours, have demonstrated that relatively higher area under the curve (AUC) of the MPA group leads to significant suppression of acute GvHD in prophylactic use. We propose a model algorithm for optimal dose finding using therapeutic drug monitoring (TDM) for MPA. Preemptive strategies depending on plasma MPA levels could yield more effective approaches to GvHD prophylaxis, alternative to MTX.

Keywords Mycophenolate mofetil (MMF) · Mycophenolate acid (MPA) · Graft-versus-host disease (GvHD) · Therapeutic drug monitoring (TDM)

K. Minagawa (✉) · Y. Katayama
Division of Hematology, Department of Medicine,
Kobe University Graduate School of Medicine,
7-5-1 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan
e-mail: kminagaw@med.kobe-u.ac.jp

M. Yamamori
Department of Clinical Pharmacy, School of Pharmacy and Pharmaceutical Sciences,
Mukogawa Women's University, Nishinomiya, Japan

T. Matsui
Department of Hematology, Ono Municipal Hospital,
Ono, Hyogo 675-1332, Japan

Introduction

The prophylaxis for graft-versus-host disease (GvHD) after hematopoietic stem cell transplantation (HSCT) has been developed in the last two decades. Although a combination of calcineurin inhibitor (CI), such as cyclosporin (CsA) or tacrolimus (FK506), plus short-term methotrexate (MTX) has been widely used in clinical practice [1–6], other immunosuppressive drugs, such as steroids, anti-thymocyte globulin (ATG), and campath-1H, are also used as alternative or additional immunosuppressants. Mycophenolate mofetil (MMF) is a type II inosine monophosphate dehydrogenase inhibitor that exerts its immunosuppressive effect by blocking the production of guanosine nucleotide synthesis through the de novo pathway [7, 8]. MMF is widely used for prevention of rejection in organ transplantations. MMF is highly selective, specifically in the suppression of lymphocytes but not myeloid cells; hence, it enables faster engraftment and causes less cytotoxicity, especially stomatitis, compared to MTX. Many clinicians who conduct HSCT favor this drug for GvHD prophylaxis. In addition, MMF has also been utilized as first-line or salvage treatment of acute GvHD, as well as chronic GvHD [9–20]. Despite the increasing infectious complications associated with combined MMF and prednisone regimen, current data have suggested that MMF is an active agent in

the treatment of acute and chronic GvHD. MMF dosage for GvHD prophylaxis ranges from 1 to 3 g/day according to institutions, because the optimal dose is not provided by therapeutic drug monitoring (TDM). Several studies fail to establish the range of MMF dosage because the concentration of mycophenolic acid (MPA), which is the active and hydrolyzed form of MMF, differs in each individual. Moreover, plasma MPA concentrations after HSCT are apparently lower than after organ transplantation. In this review, the efficacy/limitations in the field of HSCT and analysis of MMF usage based on conditioning, timing, and stem cell source are discussed.

MMF pharmacokinetics and pharmacodynamics in HSCT

Figure 1 shows the pharmacodynamics of MMF. MMF is a morpholinoethyl ester formulation, which enhances the bioavailability of MPA. MPA was first isolated from a *Penicillium* culture, but its efficacy as an antibiotic was limited [21]. MPA was later shown to be a potent inhibitor of nucleic acid synthesis, largely by its ability to inhibit the enzyme IMP dehydrogenase (IMPDH) selectively, reversibly, and noncompetitively. IMPDH is the rate-limiting enzyme in the de novo synthesis of guanosine monophosphate (GMP) from IMP. Then it was focused for anti-tumor activity [22–26].

The GMP synthesis in lymphocytes is highly dependent on the de novo pathway, while other cells utilize the salvage pathway. Therefore, the blockade of GMP synthesis leads to prevention of T-cell activation, as well as B-cell activation. Thereafter, MMF has been widely used as an immunosuppressive agent.

The bioavailability of MMF after oral administration in healthy individuals was reported to be approximately 94 % [27]. Once orally or intravenously administered, MMF rapidly undergoes de-esterification to form its active compound, MPA [7]. Maximum peak concentrations (C_{max}) of MPA generally occur within 1 or 2 h after MMF administration. MPA is primarily metabolized in the liver by uridine diphosphate glucuronosyl transferases (UGTs) to form the metabolites phenolic MPA-glucuronide (MPAG) and, to a lesser extent, acyl-MPAG (AcMPAG). The latter is pharmacologically active and has been linked to the occurrence of MMF-related adverse effects. The excretion of MPAG is primarily renal. Over 90 % of the administered dose is eventually excreted in the urine,

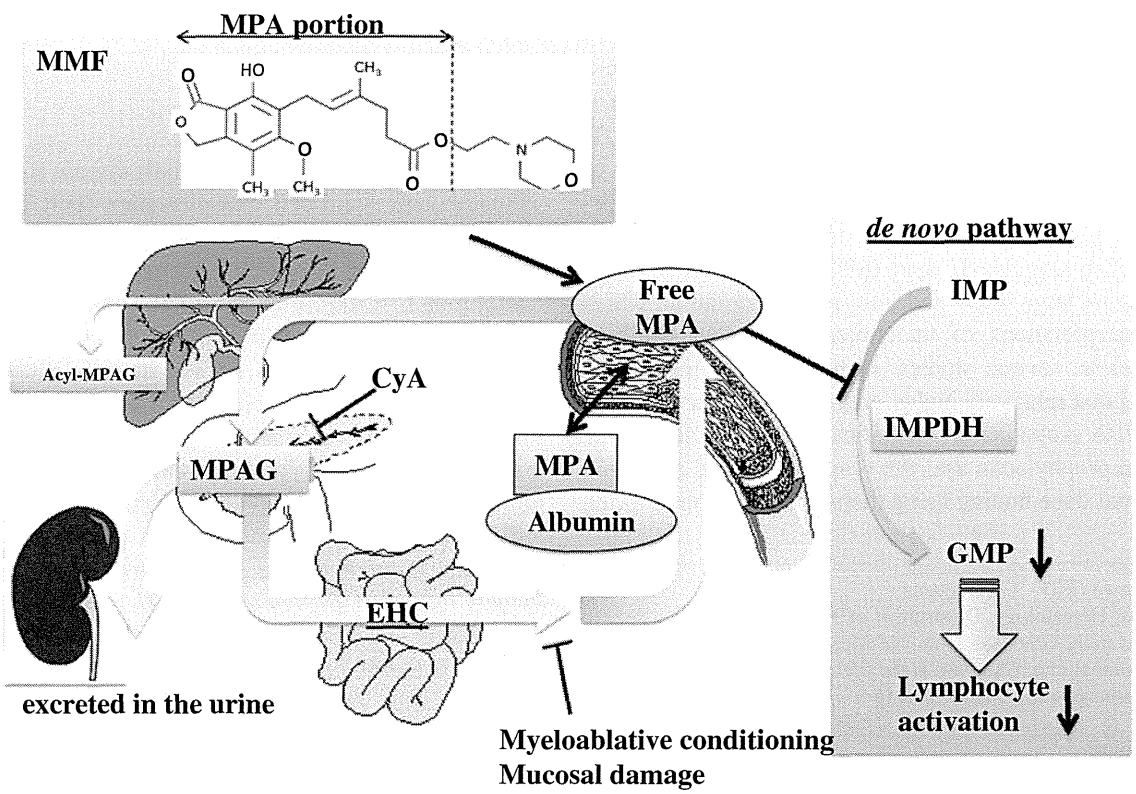

Fig. 1 Summary of pharmacology and pharmacodynamics of MMF. After MMF is administered, MMF is hydrolyzed to MPA, which is the active form, and causes reversible inhibition of IMPDH. MPA is reabsorbed via the enterohepatic circulation. *MMF* mycophenolate mofetil, *MPA* mycophenolic acid, *IMPDH* inosine monophosphate dehydrogenase, *IMP* inosine monophosphate, *GMP* guanosine monophosphate, *MPAG* MPA glucuronide, *UGTs* uridine diphosphate glucuronosyl transferases, *EHC* enterohepatic circulation, *CsA* cyclosporine

mostly as MPAG. The pharmacokinetics of MMF is complicated by the enterohepatic circulation (EHC) of MPAG, which is excreted into the bile and subsequently hydrolyzed in the intestine and reabsorbed as MPA giving rise to a secondary peak of MPA 6–12 h after MMF administration. However, most studies reported that the second peak of MPA was not found in HSCT, because mucosal damage partly due to conditioning regimen interfered with EHC [28, 29]. Even though EHC is active, detection of the second peak is difficult because of the low plasma MPA levels of HSCT patients 6 h after MMF administration. Furthermore, MPA binds extensively to plasma albumin, and the free MPA fraction is less than 3 %. The free MPA fraction is thought to be responsible for the immunosuppressive effect of MPA. Systemic exposure to MPA when MMF is given in combination with CsA is approximately 30–40 % lower than when given alone or with FK506 or sirolimus. It is because CsA inhibits multidrug resistance associated protein 2 (MRP-2), which has been reported to be responsible for the biliary excretion of MPAG and subsequent MPA EHC [30, 31]. The interference associated with the concomitant use of CsA would be one reason for the inter-patient, as well as intra-patient variations in the plasma MPA levels. While the recommended target range for the MPA area under the curve ($AUC_{0-12\ h}$) in renal transplant recipients is 30–60 mg h/L [32], no standard target range in HSCT has been proposed.

Generally, the plasma MPA levels in HSCT are lower than those in organ transplantation, such as renal transplantation. Considerations on MMF pharmacokinetics for HSCT are listed in Table 1. The major factors are bioavailability and MPA clearance. Indeed, the intestinal mucosal damage due to the myeloablative regimen, including total body irradiation (TBI) and high-dose chemotherapy, and/or the use of broad-spectrum antibiotics deteriorate the bioavailability after oral administration of MMF. However, because the intravenous formulation of MMF still achieved ten times lower trough blood level of MPA in myeloablative HSCT compared with healthy volunteers [33], the bioavailability of MMF might not be mainly the reason for the lower MPA levels. For MPA clearance, a higher clearance has been reported to be associated with decreased EHC, lower serum albumin levels [34, 35], and in combination with CsA [30, 31]. Pediatric HSCT study using intravenous and oral MMF in combination with FK506 showed that there was a trend in increased MPA clearance following myeloablative conditioning, which caused a more severe mucosal damage and decreased EHC compared to reduced intensity conditioning (RIC) [36]. However, there was no difference in the plasma MPA concentrations in both oral and intravenous regimens. Recently, a pharmacokinetics study has revealed that MPA clearance was increased in HSCT patients compared to renal transplant recipients [37]. Interestingly, the correlation between MPA clearance and CsA trough levels and plasma albumin was significant in a multivariate model. One explanation for the lower MPA levels in HSCT patients compared to those who received organ transplant could be the high MPA clearance as a result of combination with CsA and the high trough and low plasma albumin levels. However, another report on patients treated with RIC did not find a positive correlation between total MPA AUC and serum albumin levels [38]. Therefore, there is still no clear explanation for the lower MPA concentration in HSCT patients.

Beginning of utilizing MMF for HSCT

The usefulness of MMF in the field of organ transplantation, especially renal transplantation, by randomized

Table 1 Issues to consider in the use of MMF for acute GvHD prophylaxis and pharmacokinetics of MPA in HSCT

Issues	Comments
Patient/disease-oriented issues	
Conditioning	RIC is more favorable than myeloablative conditioning
Stem cell source	Any type of sources is feasible
Stomatitis	Oral mucosal damage is milder than MTX
Engraftment	Usually faster than MTX
Infectious status	Infections might be increased
Factors affecting pharmacokinetics	
MPA clearance	Might be higher when myeloablative conditioning rather than RIC is used
Serum albumin level	MPA clearance is increased when serum albumin level is decreased
Bioavailability (if oral)	Intravenous formulation is available
Enterohepatic circulation (EHC)	Mucosal damage and antibiotics use reduce EHC
Combination drug for GvHD prophylaxis	CsA interferes with secretion of MPA in bile

MMF mycophenolate mofetil, *RIC* reduced intensity conditioning, *MTX* methotrexate, *MPA* mycophenolic acid, *CsA* cyclosporine, *GvHD* graft-versus-host disease

controlled studies had been reported since the mid-1990s [39–41]. In the late 1990s, Storb et al. conducted experimental studies to show the effectiveness of CsA plus MMF in canine HSCT after non-myeloablative conditioning [42, 43]. Subsequently, MMF was introduced for GvHD prophylaxis in human HSCT. The Seattle group showed that the combination of CsA plus MMF was comparable to CsA plus MTX. Thereafter, MMF has been practically employed in the HSCT field, especially for RIC [44, 45], with less clinical trials compared to the application in renal transplantation.

Conventional usage of MMF for acute GvHD prophylaxis

Generally, MMF is initiated at 15–45 mg/kg orally or intravenously twice or thrice daily (the dose is rounded off to the nearest multiple of 250 mg for internal use) from day 0 for 27–40 days, and then it is either stopped or tapered through day 96–180 [29, 38, 46–48]. A report from Fred Hutchinson Cancer Research Center demonstrated that MMF given thrice daily allowed durable engraftment in unrelated HSCT after RIC (fludarabine 90 mg/m^2 plus TBI 2 Gy) [49]. The infection rate with thrice daily administration was slightly higher than that with twice daily, but treatment-related mortality (TRM) was not increased in thrice daily administration. The probability of acute GvHD was similar in both groups. Another report from the same institute also documented that post-grafting immunosuppression with extended (up to day 180) MMF and shortened (up to day 80) CsA increased the incidence of acute GvHD in unrelated HSCT given RIC [50]. Our small cohort showed that extended MMF administration beyond day 30 is recommended depending on individual risk factors for GvHD, namely (1) HSCT from a mismatched donor, (2) concurrent acute GvHD, (3) eosinophilia (>0.5 $\times$ 10^9/L), or (4) fever without infection. The median extended dosing period of MMF was 64.5 days (50–94). The cumulative incidence of grade II–IV acute GvHD was significantly lower (12.5 %) compared to the cessation of MMF administration at day 30 (42.3 %)[51].

The optimal MMF dose is not elucidated so far, but 2–3 g daily of MMF in combination with CI has been mostly used in Western countries, and it might be acceptable to taper according to the patients' status beyond day 30 before CI withdrawal.

Engraftment issue

Primary as well as secondary engraftment failure is one of the most important concerns, especially in HSCT given RIC or cord blood transplantation (CBT). Previous reports demonstrated that the intensification of the immunosuppressive regimens was the key factor for promotion of engraftment. Conditioning with the use of immunosuppressive agents, such as ATG, campath-1H, fludarabine, cyclophosphamide, and TBI, is an effective way of engraftment [52–56]. As a multicenter experience, fludarabine plus low dose TBI in 38 cases of salvage HSCT for allograft rejection was reported to be well tolerated and resulted in an engraftment rate of 87 % [56]. In this study, MMF was employed for GvHD prophylaxis in combination with CI. Our preliminary experiences also found that the intensification of immunosuppression with MMF in combination with CI enabled the achievement of a successful engraftment as a salvage CBT [57]. Although the mechanism has not been elucidated, several reports have suggested that GvHD prophylaxis regimen containing MMF is associated with faster engraftment [58–61]. One possible explanation is that short-term MTX, but not MMF, often interferes with emerging neutrophil engraftment due to its cytotoxic effect.

Another possibility is that MMF itself might enhance engraftment because patients with mean MPA concentration steady state (C_{ss}) less than 2.5 μg/mL were reported to come across graft rejection [38]. It is well known that treatment with thrice daily MMF significantly increased MPA C_{ss} in plasma [38, 49, 62], despite a negative report [63]. Because free MPA binds reversibly to IMPDH and interrupts de novo purine pathway transiently, high fractionated administration is reasonable to enhance its activity [45]. It is of interest whether the pharmacological increase in C_{ss} by *tid* could contribute to the clinical efficacies to prevent GVHD as well as graft failure.

Trends toward MTX-free immunosuppression

Stomatitis is a frequent complication of the conditioning regimen. Severe oral stomatitis occurs in up to 75 % of cases with myeloablative conditioning [64]. Severe stomatitis often causes problems and increases early mortality rate after HSCT [65]. MTX for GvHD prophylaxis impairs mucosal regeneration after conditioning-related mucosal damage, as well as faster engraftment. In RIC of patients over 55 years old, which is the upper limit for myeloablative conditioning in most facilities, or younger patients with concomitant complications, such as cardiac or infectious diseases, the lesser occurrence of stomatitis and early mortality and faster engraftment by utilizing an alternative immunosuppressant to MTX would be attractive for a safe and secure HSCT.

Thus, the concept of "MTX-free immunosuppression" has emerged. Sirolimus is the first available inhibitor of the

mammalian target of rapamycin (mTOR) used in HSCT. Since sirolimus poses less nephrotoxicity and neurotoxicity, combination therapy with CI is appealing. There have been many clinical trials of sirolimus and FK506 without MTX for GvHD prophylaxis mainly at Dana-Faber Cancer Institute so far [66–70]. These results indicate that sirolimus may reduce the incidence of acute GvHD, while the severity and incidence of stomatitis and cytomegalovirus (CMV) reactivation are decreased as compared to the MTX-containing regimen [68, 69, 71]. Unfortunately, these results have been discouraged by the increased risk of sinusoidal obstruction syndrome (SOS), especially with busulfan-based conditioning [72]. Moreover, thrombotic microangiopathy (TMA) and renal failure are also documented in GvHD prophylaxis and treatment trials using the sirolimus and CI combination [73–75]. Because MMF has not been shown to increase the risk of SOS or TMA, MTX-free immunosuppression, such as CI plus MMF, might be suitable for GvHD prophylaxis, especially in busulfan-containing regimen. Indeed, MMF has been used as part of the front-line regimen for the prevention of GvHD, especially in the setting of RIC regimen. Similarly to sirolimus, MMF reduces stomatitis [58, 76] and facilitates engraftment [58–61, 76–78].

A recent retrospective study in the comparison of CsA plus MMF and CsA plus MTX after RIC from human leukocyte antigen (HLA)-identical siblings showed a lower tendency of non-relapse mortality (NRM) at day 100 (6 vs. 18 %, $P = 0.04$) [79]. Comparisons of MTX, sirolimus, and MMF for GvHD prophylaxis are summarized in Table 2. Representative reports of comparison for prophylaxis on the use of CI plus MMF versus CI plus MTX are also shown in Table 3.

Why do some clinicians stay away from MMF as conventional prophylaxis?

MMF usage in myeloablative conditioning is controversial. A prospective randomized trial that compared CsA plus MTX and CsA plus MMF for GvHD prophylaxis in myeloablative HSCT showed significantly less severe stomatitis and more rapid neutrophil engraftment in the MMF arm. The incidence of grade II–IV acute GvHD was similar in the two arms (48 % in the MMF arm and 37 % in the MTX arm) [58] (Table 3). On the other hand, phase I/II study, in which 45 mg/kg/day MMF was given thrice daily for 27 days in combination with CsA, suggested the lack of a significant improvement in the prevention of GvHD compared with historical data for CsA and MTX after myeloablative HSCT from HLA-matched sibling donors [29]. In this study, the incidence of acute GvHD was 62 %. For some physicians, this is the rationale why the combination of CsA plus MMF is considered only when MTX is contraindicated. However, there are not significant differences on the pharmacokinetics of MMF in myeloablative conditioning versus RIC in adult HSCT to date. There is no clear explanation why the benefit of MMF is superior in RIC regimen compared to myeloablative conditioning.

Prophylactic use for related donors (especially from HLA-mismatched donors)

Some studies showed that GvHD prophylaxis by MMF plus CI for either myeloablative or RIC achieved successful engraftment and prevented GvHD similar to MTX plus CI [29, 80, 81] (Table 4). Of note, MMF is also used for HSCT from HLA-mismatched related donors, as well as HLA-matched ones. In Asia, Japanese, and Chinese recipients with no HLA-matched sibling donors received HSCT from two or three loci-mismatched related donor. The usual conditioning regimens, including ATG, and multiple immunosuppressants, such as MMF, were used for GvHD prophylaxis [82, 83]. Moreover, clinical trials from Johns Hopkins University recently evaluated the efficacy of high-dose, post-transplantation cyclophosphamide in addition to FK506 and MMF to prevent GvHD after non-T cell-depleted transplantation from partially HLA-mismatched related donors [84, 85].

Table 2 Comparison of MTX, sirolimus, and MMF

	Stomatitis	Engraftment	SOS/TMA	aGvHD prophylaxis	Infectious complications	Drug interaction
MTX	+++	+	+	+	+	+
Sirolimus	±	++	+++[a]	++	±	++
MMF	±	++	+	+	++	+

MTX methotrexate, *MMF* mycophenolate mofetil, *SOS* sinusoidal occlusive syndrome, *TMA* thrombotic microangiopathy, *aGvHD* acute graft-versus-host disease

[a] Especially if myeloablative regimens of busulfan and cyclophosphamide were used

Table 3 Representative reports of comparison on calcineurin inhibitor (CI) + MMF versus CI + MTX

References		*N*	Age (range)	Regimen	Donor	Additional prophylaxis	Neutrophil engraftment (range)	Grade II–IV acute GvHD (95 % CI)	Extensive chronic GvHD (95 % CI)	NRM (95 % CI)
Bornhauser et al. [59]	MMF arm	14	38 (21–63)	Myeloablative	Related	CsA	11 days (9–20)	46.5 %	N.A.	N.A.
	MTX arm	15	38 (20–58)				17 days (10–25)	60 %		
							$P = 0.023$			
Bolwell et. al. [58]	MMF arm	21	49 (19–60)	Myeloablative	Related	CsA	11 days (8–24)	48 %	63 %	N.A.
	MTX arm	19	46 (16–62)				18 days (11–28)	37 %	64 %	
							$P < 0.001$			
Neumann et. al. [61]	MMF arm	26	39 (22–57)	Myeloablative	Related	CsA	12 days	38 %	50 %	17 %
	MTX arm	67	32 (17–51)				18 days $P < 0.0001$	61 %	45 %	27 %
Pinana et. al. [79]	MMF arm	52	57 (18–71)	RIC	Related	CsA	15 days (11–27)	38 % (27–54)	39 % (27–58)	6 % (2–17)
	MTX arm	93	54 (23–70)				15 days (10–29)	33 % (25–45)	38 % (28–52)	19 % (12–29)
										$P = 0.04$
										(NRM at day 100)
Perkins et. al. [76]	MMF arm	42	49.9 (23–66.2)	Varies (mostly fludarabine/ busulfan)	Related/unrelated	FK506	15 days	78 %	38 %	Similar between the two arms
	MTX arm	47	54 (24.9–69.6)				16 days	79 %	45 %	
							Platelate recovery			
							15 days versus 17 days $P < 0.01$			

N.A. not applicable, *RIC* reduced intensity conditioning, *CsA* cyclosporine, *FK506* tacrolimus, *MTX* methotrexate, *MMF* mycophenolate mofetil, *CI* confidence interval, *NRM* non-relapse mortality, *GvHD* graft-versus-host disease