Safety, tolerability, and feasibility of antifungal prophylaxis with micafungin at 2 mg/kg daily in pediatric patients undergoing allogeneic hematopoietic stem cell transplantation

Kentaro Yoshikawa[1], Yozo Nakazawa[1], Yoshihiko Katsuyama[2], Koichi Hirabayashi[1], Shoji Saito[1], Tomonari Shigemura[1], Miyuki Tanaka[1], Ryu Yanagisawa[1], Kazuo Sakashita[1], and Kenichi Koike[1]

[1]Department of Pediatrics, Shinshu University School of Medicine, and [2]Department of Pharmacy, Shinshu University Hospital, Matsumoto, Japan

Correspondence to: Yozo Nakazawa, M.D., Ph.D.

Department of Pediatrics, Shinshu University School of Medicine,

3-1-1, Asahi, Matsumoto 390-8621, Japan.

Phone: +81-263-37-2642 Fax: +81-263-37-3089

E-mail: yxnakaza@shinshu-u.ac.jp

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Abstract

Micafungin (MCFG) is used for prophylaxis of invasive fungal disease (IFD) after allogeneic hematopoietic stem cell transplantation (HSCT). However, the safety, efficacy, or optimal dosage/blood levels as prophylaxis is uncertain in pediatric HSCT-patients. In this study we prophylactically administered MCFG at 2 mg/kg once daily to 38 children and adolescents undergoing allogeneic HSCT. During MCFG prophylaxis, infusion reactions or adverse events (grades 2 to 5) related to MCFG use were not found in all the patients. Thus, MCFG prophylaxis was not discontinued and other antifungal agents were not added except 2 patients in whom probable or possible IFDs developed (completion rate, 94.7%). To elucidate the influence of HSCT-related complications/drugs on blood concentration of MCFG, we determined the plasma trough and peak levels in 13 and 10 among 38 patients, respectively. The mean trough and peak levels were 3.04 ± 1.21 μg/mL (569 samples) and 9.63 ± 3.62 μg/mL (44 samples), respectively. The peak levels were moderately correlated to the trough levels (R^2 = 0.466). In a patient the trough level of MCFG transiently increased up to 10.21 μg/mL during hepatic dysfunction due to acute graft-versus-host disease. The MCFG trough levels strongly correlated with T.Bil value (R^2 = 0.894). There was no relationship between the trough levels of MCFG and the circulating concentrations of tacrolimus (R^2 = 0.040). Additionally, MCFG levels were not influenced by treatment with cyclophosphamide or corticosteroids. These results indicate that prophylaxis with MCFG at 2 mg/kg once daily may be safe, tolerable, and feasible in pediatric HSCT-patients.

Introduction

Invasive fungal disease (IFD) mainly due to *Candida* and *Aspergillus* species causes significant morbidity and mortality in patients who receive allogeneic hematopoietic stem cell transplantation (HSCT), thus should be prevented by antifungal drugs [1]. However, appropriate antifungal prophylaxis is unclear in pediatric allogeneic HSCT-patients since there are few reports on the safety, efficacy, or pharmacokinetics [2].

Triazoles [fluconazole (FLCZ), itraconazole (ITCZ), voriconazole, and posaconazole] are antifungal agents for prophylaxis of IFD recommended in pediatric patients by the European Conference on Infections in Leukemia (ECIL) 4 (http://www.ebmt.org/Contents/Resources/Library/ECIL/Pages/ECIL.aspx). At present, FLCZ remains the first line therapy in pediatric HSCT, but it lacks activity against *Aspergillus* species [2]. In addition, the extensive use of FLCZ may lead to the development of azole resistance in *Candida* species [3]. ITCZ has broad-spectrum antifungal activity against both *Aspergillus* and *Candida* species, but the gastrointestinal absorption of the oral formulation is often poor in severely ill patients. Additionally, ITCZ has significant adverse interactions with calcineurin inhibitors (cyclosporine A and tacrolimus) [4]. Voriconazole, which has broad-spectrum antifungal activity, also alters the blood concentration of calcineurin inhibitors with a wide range of interindividual variability [5]. In addition, there is a report of occasional reversible visual disturbances associated with its use [6]. Posaconazole has not been approved in Japan.
Micafungin (MCFG) is a semisynthetic antifungal echinocandin-like lipopeptide that inhibits the synthesis of (1,3)-β-D-glucan, an essential polymeric polysaccharide in the cell wall of many pathogenic fungi [7]. A preclinical in vitro study on MCFG indicated that it has fungistatic activity against *Aspergillus* species and fungicidal activity against *Candida* species [8]. Administration of MCFG at a daily dosage of 50 mg (1 mg/kg for patients weighing ≤40kg) has been approved worldwide as the prophylaxis of *Candida* infection in children and adolescents undergoing allogeneic HSCT (<16 years of age) on the basis of van Burik et al.'s study [9] (http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/landing/epar_search.jsp&mid=WC0b01ac058001d125). However, the optimal dosage of prophylactic MCFG has not been fully investigated in pediatric HSCT-patients.

In this study, we evaluated the safety of prophylactic administration of MCFG at a dose of 2 mg/kg once daily in children and adolescents undergoing allogeneic HSCT as the primary endpoint. We also investigated the prophylactic efficacy and the influence of HSCT-related complications/drugs on the plasma concentrations of MCFG.

Patients and methods

Study enrolment

In this study, we enrolled 38 patients who underwent allogeneic HSCT in the laminar air flow rooms with HEPA filters of Department of Pediatrics, Shinshu University Hospital, between June 2002 and May 2011, and who had no active IFD at the start of preparative conditionings regardless of a past history of IFD. The study protocol was approved by the institutional review board of the Shinshu University School of Medicine. Written informed consent was obtained from parents and patients aged >16 years.

Patients, donors, and HSCT procedures

Patient characteristics were summarized in Table 1. Of the 38 patients, 8, 14, and 7 patients with acute leukemia were in first complete remission, second complete remission, and non-remission, respectively. Seventeen patients were transplanted from related donors in whom 7 donors were 2- or 3-human antigen leukocyte (HLA)-mismatched with their recipients, while 21 patients were transplanted from unrelated donors. All the 38 patients received the preparative regimens including total body irradiation. Five patients were treated with reduced-intensity conditioning regimens.

For prophylaxis of acute graft-versus-host disease (GVHD), we used either of a combination of short-term methotrexate (sMTX) and cyclosporin A, or a combination of sMTX and tacrolimus with or without methylprednisolone (mPSL, 0.5–1 mg/kg) in bone marrow transplantation. We used tacrolimus and mPSL in cord blood transplantation. After engraftment, mPSL dosage was tapered up to day 30 and then discontinued in the absence of grades II-IV acute GVHD.

As supportive care, granulocyte colony-stimulating factor was daily administered intravenously to patients with acute lymphoblastic leukemia and non-malignant disorders from day 5, and its use was discontinued after the neutrophil count exceeded 1,500/μL. Oral antibiotics were administered to sterilize the bowel. Acyclovir was administered intravenously during the peritransplant period. Intravenous gamma-globulin was administered at 200 mg/kg every 2 weeks up to day 60 and thereafter monthly until 1 year. Trimethoprim/sulfamethoxazole was used for prophylaxis against *Pneumocystis jirovecii* infection after engraftment.

Myeloid recovery was defined as the first of 3 consecutive days with an absolute neutrophil count ≥500/μL. Engraftment syndrome (ES) was defined as described previously [10].

During continuous drip infusion of tacrolimus, blood levels were measured almost every day, and the dose was adjusted to maintain the target range (10−15 ng/mL). The concentration of tacrolimus in whole blood was measured with a microparticle-based enzyme immunoassay using the IMx analyzer (Abbott Japan, Tokyo, Japan) [10].

Screening for fungal infection

In all the 38 patients, β-D-glucan in serum was measured weekly up to 60 days and thereafter at least every 2 weeks (cutoff values, <2.84 μg/mL). In 17 patients who underwent HSCT from March 2008, galactomannan antigen in serum was also tested weekly up to 60 days and thereafter at least every 2 weeks (cutoff values, <0.5 μg/mL). If fever (≥38°C) developed during HSCT, blood culture was performed repeatedly, and if fever persisted beyound72 hours, chest CT scan was performed.

Prophylaxis with MCFG

MCFG was administered once daily at a dose of 2 mg/kg by one hour-infusion, started by 5 days before HSCT, and continued until at least the achievement of neutrophils engraftment. MCFG was discontinued if the patients fulfilled all of the following: 1) the patients have no or grade I acute GVHD, or recovered from grades II-IV acute GVHD, 2) the patients are treated without corticosteroids or with prednisolone under 1 mg/kg per day, 3) the patients are able to take ITCZ orally.

Toxicity grading

Systemic and regional toxicity were graded according to the Common Terminology Criteria for Adverse Events (CTCAE) ver. 4.0 (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm). The highest grade reached during infusion of MCFG was defined for each patient as the highest grade of organ toxicity. According to these

criteria, grades 1 to 5 indicate the severity of the adverse events.

Definition of IFD and the prophylactic success

IFD was classified into proven, probable, or possible IFD according to the Invasive Fungal Infections Cooperative Group of the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) [11]. Prophylactic success of IFD was defined as the absence of proven, probable, or possible IFD through the end of MCFG administration and as the absence of proven or probable IFD through the end of the 4-week period after prophylactic treatment according to the criteria reported by van Burik et al [9].

Determination of plasma concentrations of MCFG

In 13 of the 38 enrolled patients, we measured the plasma concentration of MCFG from 3 days after the start of MCFG prophylaxis. Blood samples for determining trough levels were collected within 2 hours before the daily infusion of MCFG at least 3 times a week between days -8 and 96 after HSCT prophylaxis in accordance with blood chemistry tests and measurements of tacrolimus. In 10 of the 13 patients the above, blood samples for determining peak levels were also collected 1 hour after the infusion of MCFG 4 or 5 times between days –8 and 31 after HSCT. The plasma samples were separated from the collected blood, and were kept at −80°C until analysis.

MCFG solution (100 μg/mL) and FR195743 solution (internal standard; 100 μg/ml) were donated by Astellas Pharma Inc. (Tokyo, Japan). Plasma concentrations of MCFG were determined by high performance liquid chromatography according to the method reported by Yamato et al. [12] with some modifications. The minimal concentration of MCFG detectable by this method was 0.05 μg/mL.

Statistical analysis

To determine the significance of differences in continuous variables between 2 independent groups, the unpaired t-test (for normally distributed data) or the Mann-Whitney U-test (when the data were not normally distributed). The correlation between 2 parameters was analyzed using regression analysis. Analysis was performed using PASW Statistics, Version 18.0. (SPSS, Inc., Chicago, IL), and statistical significance was defined as a p-value <0.05.

Results

Antifungal prophylaxis with MCFG in patients who received allogeneic HSCT

Thirty-eight patients aged 0.4 to 18.7 (median, 7.3) years received MCFG prophylaxis (2mg/kg once daily). All patients achieved sustained neutrophil engraftment on day 20.8 ± 8.1. Twenty-five patients suffered from grades II–IV acute GVHD, and most of the patients were treated with of mPSL (1–2 mg/kg/day) and/or mycophenolate mofetil (MMF, 30–90 mg/kg/day).

Three patients died of leukemic relapse during MCFG prophylaxis on days 34, 56, and 185, respectively, but no IFD was found at the time of death.

As shown in Table 2, three patients had suffered IFD before HSCT, but none developed post-transplant IFD. Breakthrough IFD occurred in 2 patients during MCFG prophylaxis. On day 59 day after HSCT, one patient without

neutropenia was diagnosed with probable IFD on the basis of chest CT findings scan and the galactomannan antigen test (1.2; normal controls, <0.5). The patient was treated with intravenous infusion of a combination of liposomal amphotericin B (L-AMB) and ITCZ. Three months later, his pulmonary lesions were improved. The other patient had been administered with immunosuppressants including MMF and infliximab for grade IV GVHD since day 24. On day 120, his absolute neutrophil count showed 18/μL, and he was diagnosed with developed possible IFD on the basis of positive findings of CT scan and negative results of mycological tests. The patient was treated with intravenous infusion of L-AMB, and his pulmonary lesions improved after 2.5 months.

In the remaining 33 patients without leukemic relapse or breakthrough IFD, intravenous administration of MCFG was replaced with oral administration of ITCZ between days 25 and 115 after HSCT.

During MCFG prophylaxis, persistence of fever beyond 72 hours was observed in 12 of 38 patients, and new onset fever developed in 3 of 38 patients while receiving broad-spectrum antibiotic treatment. However, MCFG was not discontinued and other antifungal agents were not added in the patients except those presenting probable or possible IFD (completion rate, 94.7%). In 38 patients, median duration of MCFG prophylaxis was 68 days (range, 34–196 days).

Adverse effects of MCFG

Infusion-related reactions were not observed in all patients. As shown in Table 3, signs of hepatic stress were observed during MCFG prophylaxis: AST, ALT, and total bilirubin (T-Bil) were elevated at grade 2 or higher based on CTCAE in 11 (28.9%), 22 (57.9%), and 6 (15.8%) of 38 cases, respectively. Three patients developed renal abnormalities of grade 2 or 3. However, these grades 2 to 4 adverse events developed within a week after the occurrence of acute GVHD, viral infection, or thrombotic, microangiopathy, but not within 3 weeks after the start of MCFG prophylaxis. As shown in Figure 1, T.Bil values were slightly increased on day 1 after HSCT (between 6 and 20 days after the start of MCFG prophylaxis), and recovered 7 days after the end of the prophylaxis to the baseline before HSCT. We did not observe any increase of AST, ALT, or Cr values on day 1 after HSCT. No patients discontinued MCFG prophylaxis because of adverse effects or abnormal laboratory findings.

Blood concentrations of MCFG

We measured the plasma concentration of MCFG in 13 children aged 1.3 to 13.5 (median, 7.3) years. As shown in Figure 2 and 3A, the mean trough level of 569 samples from 13 patients and the peak level of 44 samples from 10 patients were 3.04 ± 1.21 μg/mL and 9.63 ± 3.62 μg/mL, respectively. The peak levels were moderately correlated to the trough levels (coefficient of determination, $R^2 = 0.466$) (Figure 3A).

Influence of HSCT-related complications on trough levels of MCFG

Patient no. 8 developed ES and acute GVHD graded as 3 (skin, stage 1; liver, stage 2; gut, stage 3). As presented in Figure 4A, the mean trough level of MCFG was 2.59 ± 0.53 μg/mL between days -15 and 25, markedly increased to 8.69 ± 1.26 μg/mL between days 30 and 45 (maximum, 10.21 μg/mL on day 35), then decreased to 1.4 μg/mL on day 52. The duration of increased MCFG levels coincided with that of hepatic dysfunction due to acute GVHD, but not with that of ES. The mean peak level of MCFG also increased from a mean of 7.72 ± 0.34 μg/mL on days 8–22 to 13.85 μg/mL on day 30. The patient did not complain of headache, arthralgia, angialgia, insomnia, or rash between days 30 and 45. The maximal

levels of T-Bil, direct (D)-Bil, indirect (ID)-Bil, ALT, and γ-GTP were 5.5 mg/dl, 3.9 mg/dL, 1.6 mg/dL, 773 IU/L, and 886 IU/L, respectively. Regression analysis revealed strong positive correlations of T-Bil, D-Bil, and ID-Bil values with MCFG trough levels (R^2 = 0.894, R^2 = 0.890, and R^2 = 0.871, respectively). The ALT and γ-GTP values correlated with MCFG trough levels to a lesser extent (R^2 = 0.624 and R^2 = 0.358, respectively). Serum creatinine levels were within the normal range between days −15 and 59.

Patient no. 11 also developed acute GVHD graded as 3 (skin, stage 1; liver, stage 1; gut, stage 3). As presented in Figure 4B, the mean trough level of MCFG was 2.16 ± 0.29 μg/mL between days 0 and 14 of HSCT, which increased slightly to 4.75 μg/mL on days 23 and 24. The maximum levels of T-Bil and ALT were 2.01 mg/dL and 1,651 IU/L, respectively. The fluctuation in MCFG levels showed only a moderate relationship to T-Bil and ALT (R^2 = 0.266 and R^2 = 0.292, respectively) and no correlation to D-Bil and ID-Bil (R^2 = 0.046 and R^2 = 0.009, respectively).

Of the remaining 11 patients in whom the trough levels of MCFG were examined, ES developed in 5, and grades II to III acute GVHD with no liver involvement in 6. The mean trough levels of MCFG during the period of ES or acute GVHD were 1.49–3.18 μg/mL in these patients.

Effects of other drugs on trough levels of MCFG

Because MCFG is used together with calcineurin inhibitors, which are substrates of cytochrome CYP3A4, cytotoxic agents, or immunosuppressants in the allogeneic HSCT setting, we assessed for possible drug-drug interactions on MCFG. In 12 patients (including case no. 8), we compared MCFG levels before and after initiation of tacrolimus administration. There were no differences in the trough levels of MCFG before or 1 day after administration of tacrolimus (2.85 ± 0.54 μg/mL vs. 2.75 ± 0.45 μg/mL, p = 0.284). We then analyzed the correlation of tacrolimus levels with MCFG levels using 548 samples from 13 patients. As presented in Figure 3B, the trough levels of MCFG did not correlate with the blood concentration of tacrolimus (R^2 = 0.040).

The trough levels of MCFG were not influenced by treatment with CY as a preparative conditioning (CY infusion period vs. 1 day after HSCT, n = 5), by treatment with mPSL or prednisolone (PSL) for ES (4 days before vs. 1 day after treatment with mPSL or PSL, n = 6), or by treatment with mPSL or PSL for acute GVHD (4 days before vs. 5 days, 10 days, and 15 days after treatment for acute GVHD, n = 8).

Discussion

In the present study, we prophylactically administered a daily dose of 2 mg/kg of MCFG for 34–196 days (median, 68 days) in 38 pediatric patients undergoing allogeneic HSCT, and evaluated the safety. We found no infusion-related reactions or moderate to severe adverse events related to MCFG use during the MCFG prophylaxis, although we observed a slight, but significant increase of T.Bil values (Figure 1). Therefore, we did not discontinue the administration of MCFG in all the patients except those in whom probable or possible IFD developed. Prophylactic administration of MCFG at a dose of 1 mg/kg once daily (maximum, 50 mg) has been approved and used worldwide in children and adolescents undergoing allogeneic HSCT: however, our results indicated that MCFG administration at a dose of 2 mg/kg once daily would be also safe and tolerable in pediatric HSCT-patients.

In our study, probable or possible IFD developed during MCFG prophylaxis in 2 patients (Table 2). Both improved after the treatment with L-AMB with or without ITCZ. Accordingly, the failure rate for prophylaxis with MCFG was 5.3% (including one possible case), similar to or superior to the data reported by other investigators in pediatric HSCT (4.3% to 24%) [13−18]. Before this study, we had used oral /intravenous FLCZ or oral ITCZ for IFD prophylaxis in 28 patients. There was no significant difference in the prophylaxis failure rate between past and present regimens (8.3% vs. 5.3%, p=0.714); however, 2 probable/possible IFD-patients in the present study improved without sequelae, while 2 proven/probable IFD patients receiving the past regimen died of systemic aspergillosis, or has still suffered from a post-operative complication of severe arthritis probably due to yeast species. Our data from a small number of patients suggested that MCFG at a dose of 2 mg/kg once daily appears to be effective as prophylaxis in pediatric patients undergoing allogeneic HSCT.

Shimoeda et al. [19] proposed a blood trough concentration of ≥5 μg/mL MCFG for the treatment of aspergillosis in adult patients with hematologic diseases. In addition, they reported a mean blood trough level of 3.45 ± 1.63 μg/mL MCFG in 20 patients who did not contract a fungal infection (mean daily dosage, 2.0 ± 0.7 mg/kg). In our study, a mean trough level of 3.04 ± 1.21 μg/mL MCFG was obtained for the prophylaxis of IFD in 13 pediatric HSCT-patients who were administration of MCFG at a dose of 2 mg/kg once daily. In only one patient (case no. 8), who had acute GVHD-mediated liver dysfunction (stage 2), the trough level of MCFG increased to 8.69 ± 1.26 μg/mL between days 30 and 45 (with a maximum of 10.21 μg/mL on day 35). Of note, the MCFG trough levels strongly correlated with each of T-Bil, D-Bil, and I-Bil values. In a patient with acute GVHD-mediated liver involvement (stage 1), the fluctuation in MCFG levels showed only a moderate relationship to T-Bil, but no correlation to D-Bil or ID-Bil. There have been controversial findings regarding dose adjustment for patients with liver dysfunction. Hebert et al. [20] found no differences in body weight-adjusted clearance, volume of distribution, or half-life of MCFG between patients with moderate hepatic dysfunction and healthy control subjects. Therefore, they concluded that MCFG dose should not be reduced for patients with moderate hepatic dysfunction. Nakagawa et al. [21] provided a similar recommendation. However, in both studies, increases in serum T-Bil levels in the subjects were relatively mild (1.6 ± 1.6 mg/dL and 0.8 ± 0.4 mg/dL, respectively). On the other hand, Shimoeda et al. [19] and Muraki et al. [22] mentioned the necessity of MCFG dose reduction in patients with significant elevations of T-Bil caused by biliary stasis. Excessive conjugated bilirubin may competitively inhibit the multidrug resistance-associated protein 2-mediated transport of MCFG into bile duct [22]; the same mechanism may have caused the increases in serum T-Bil values and MCFG trough levels in this patient. Nevertheless, they did not report occurrence of adverse effects at the time of higher circulating MCFG concentrations. In the present study, the patient whose trough levels of MCFG increased to ≥8 μg/mL did not report the most commonly recognized side effects of MCFG toxicity (headache, arthralgia, angialgia, insomnia, and rash) [23]. Our data indicated that MCFG at a dose of 2 mg/kg once daily would be tolerable in pediatric HSCT-patients even if hyperbilirubinemia developed because of acute GVHD.

We recently reported a higher tacrolimus clearance rate in pediatric patients with ES (but not acute GVHD) and younger age at allogeneic HSCT [10]. In the present study, there was no correlation between the trough levels of MCFG and the circulating concentrations of tacrolimus, consistent with the findings of Fukuoka et al [24]. In addition, the trough levels

of MCFG at the time of treatment with CY as a preparative conditioning, at the time of treatment with mPSL for ES, and at the time of treatment with mPSL for acute GVHD, were not different from the control values.

Kusuki et al. [25] reported that 13 of 14 children who received daily 3 mg/kg of MCFG achieved succeeded prophylaxis for post-HSCT IFD, but did not show enough patient safety data. In this study, we showed that prophylactic treatment of MCFG at a dose of 2mg/kg once daily would be safe, tolerable, and feasible in pediatric patients undergoing HSCT. We will further study the efficacy of this prophylaxis in a larger cohort.

Conflict of interest

The authors declare no conflicts of interest.

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Table 1 Patient characteristics

Age in years, median (range)	7.3 (0.4 - 18.7)
Sex, M/F	20/18
Weight (kg), mean	21.0 ± 11.9
Diseases	
ALL	15
AML	14
MLL	1
BPDCN	1
Non-Hodgkin lymphoma	1
MDS	2
AA	1
HLH	1
Severe congenital neutropenia	1
Congenital metabolic disorder	1
Stem cell source	
Related BM	
0-1 mismatched allele	10
2-3 mismatched alleles	7
Unrelated BM	9
Unrelated CB	12
Conditioning	
Fludarabine + CY + TBI (≤6Gy)	5
Fludarabine + CY + TBI (8-12Gy)	32
Etoposide + CY + TBI (12 Gy)	1
GVHD prophylaxis	
Cyclosporin A + sMTX	4
Tacrolimus + mPSL	12
Tacrolimus + mPSL + sMTX	17
Tacrolimus + sMTX	5

Abbreviations: AA, aplastic anemia; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; BM, bone marrow; BPDCN, blastic plasmacytoid dendritic cell neoplasm; CB, cord blood; CY, cyclophosphamide; GVHD, graft-versus-host disease; HLH, hemophagocytic lymphohistiocytosis; MDS, myelodysplastic syndrome; MLL, mixed lineage leukemia; mPSL, methylprednisolone; sMTX, short-term methotrexate; TBI, total body irradiation

Table 2 Incidence of invasive fungal disease (IFD) before and after micafungin (MCFG) prophylaxis

	MCFG prophylaxis		
	Before	During	4 weeks after
IFI			
Proven	1	0	0
Probable	1	1	0
Possible	1	1	0
Absent	35	36	33
Unevaluable	0	0	5*

* Three patients died of leukemic relapse, and two had IFD during MCFG prophylaxis.

Table 3 Adverse events according to the Common Terminology Criteria for Adverse Events (CTCAE) v4.0

Organ toxicity	0	1	2	3	4	5
AST increased	5	22	3	8	0	-
ALT increased	3	13	9	9	4	-
Blood bilirubin increased	22	10	4	2	0	-
Acute kidney injury	30	5	1	2	0	0

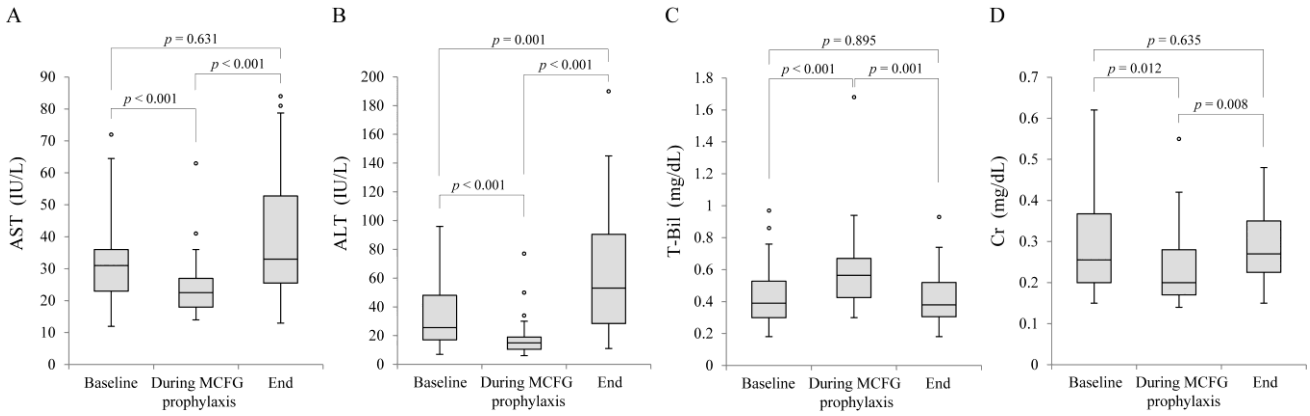


Figure 1 Kinetics of hepatic and renal function parameters at baseline, during MCFG prophylaxis, and at end of the prophylaxis

Values of AST, ALT, T-Bil, and Cr in 38 patients were shown as box plots in A, B, C, and D, respectively. The parameters (n = 38) the day before the start of MCFG were indicated in Baseline. The parameters (n = 38) 6-20 days after the start of MCFG (on day 1 after HSCT) were indicated in During MCFG prophylaxis. The parameters (n = 33) 7 days after the end of MCFG were indicated in End. The statistical differences in each two groups among Baseline, During MCFG prophylaxis, and End were analyzed by Mann-Whitney test.

Figure 2 Trough plasma concentrations of MCFG in 13 patients before and after allogeneic HSCT

The trough levels of MCFG (given in μg/mL plasma) of 13 individual patients were measured, starting at −8 days before HSCT. Data on patient no. 8 are shown as black circles.

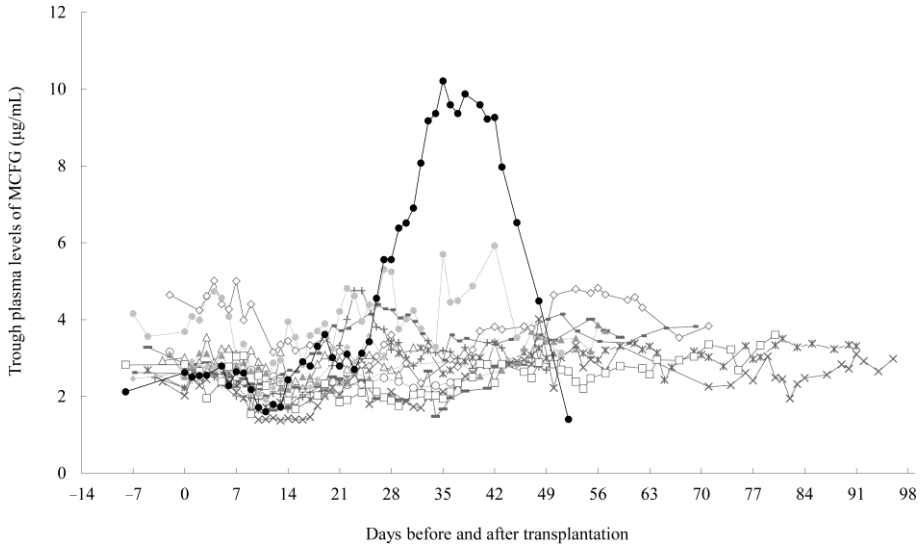
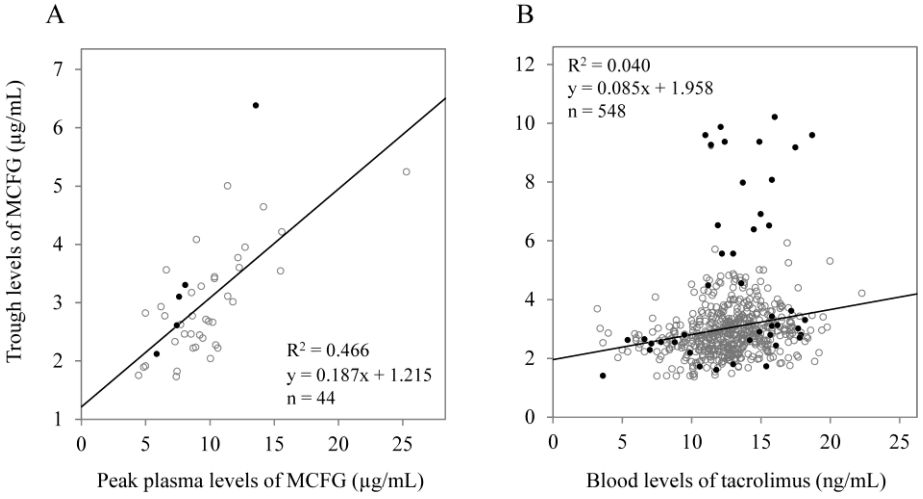


Figure 3 Influence of the peak levels of MCFG or blood concentrations of tacrolimus to the trough levels of MCFG

A. The peak levels of MCFG were moderately correlated to the trough levels of MCFG. B. blood concentrations of tacrolimus did not correlate with the trough levels of MCFG. Data on patient no. 8 are shown as black circles.



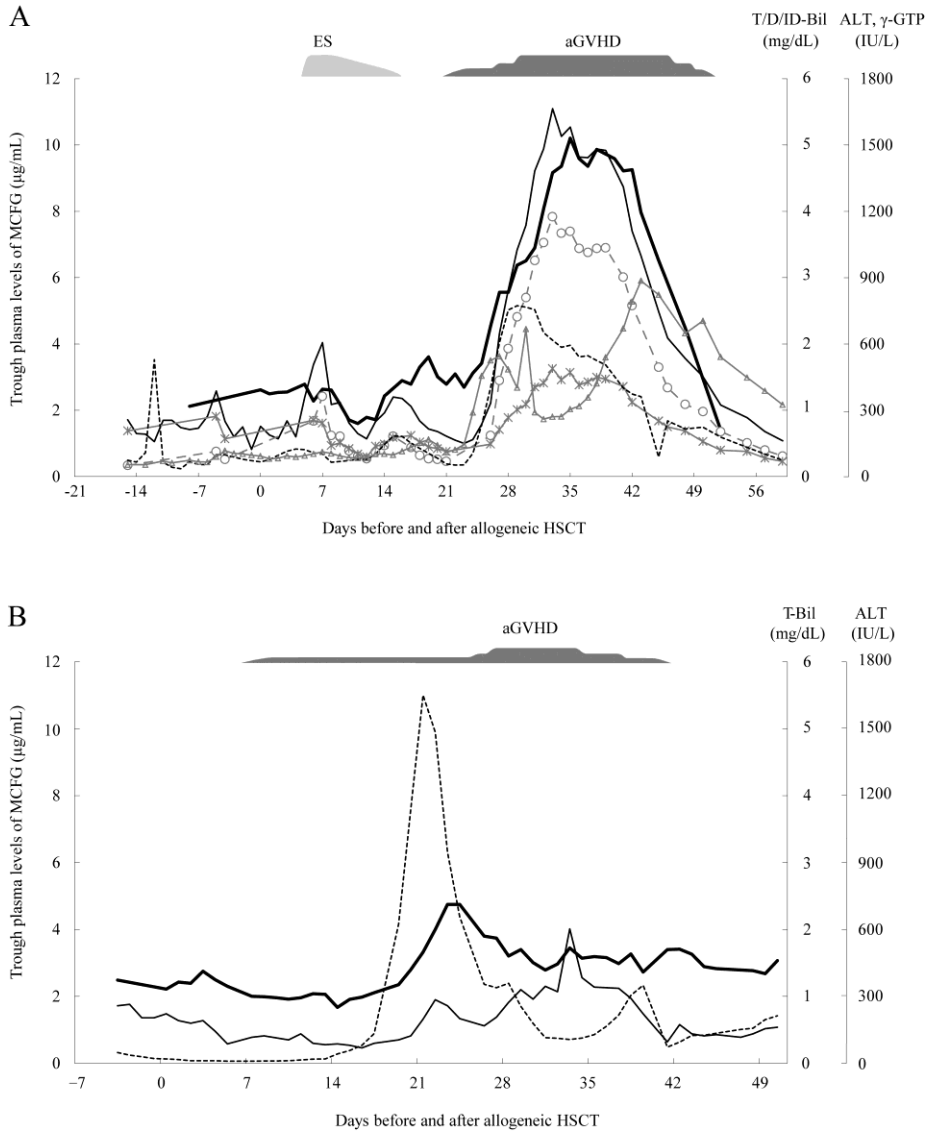


Figure 4 Influence of ES or acute GVHD on trough levels of MCFG

A. Influence of ES and acute GVHD on trough levels of MCFG in patient no. 8. Levels of T-Bil, D-Bil, and ID-Bil strongly correlated with the MCFG trough levels.

Abbreviations: aGVHD, acute graft-versus-host disease; ALT, alanine aminotransferase; D-Bil, direct bilirubin; ES, engraftment syndrome; tacrolimus, γ-GTP, γ-glutamyl transpeptidase; HSCT, hematopoietic stem cell transplantation; ID-Bil, indirect bilirubin; T-Bil, total bilirubin.

Trough plasma levels of MCFG; T-Bil;
D-Bil; ID-Bil; ALT; γ-GTP;

B. Influence of acute GVHD with liver involvement (graded 1) on trough levels of MCFG in patient no. 11. The fluctuation of MCFG levels showed weak relationship to T-Bil, but no correlation to D-Bil or ID-Bil. Abbreviations are the same as in Figure 3.

Trough plasma levels of MCFG; T-Bil; ALT;