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Ⅲ．　研究成果の刊行物（論文別刷）

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

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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.

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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

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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.

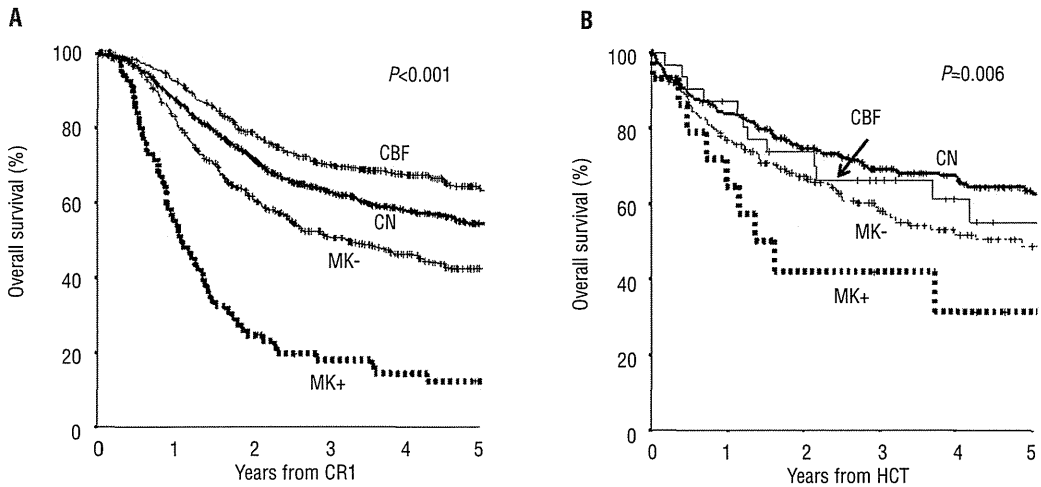

Figure 1. Kaplan-Meier curves for (A) OS after achieving CR1 for the entire cohort, and for (B) OS after allogeneic HCT for patients who underwent allogeneic HCT in CR1, according to the cytogenetic subgroups. CBF represents core binding factor AML; CN: cytogenetically normal AML; MK$^-$, cytogenetically 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.

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IMAGES IN HEMATOLOGY

Ocular palsy associated with aggressive NK-cell leukemia

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A 22-year-old woman was referred to our hospital with a 1-month history of recurrent fever, subacute onset of bilateral orbital pain, and diplopia. Physical examination revealed the limits of supraduction of her right eye and infraduction of her left eye. A bone marrow aspirate from the ilium showed scattered large immature-looking lymphocytes (15.8%) with pale cytoplasm, fine nuclear chromatin, and nucleoli (Fig. 1). These cells were stained positively with CD56, TIA-1, granzyme, and perforin, and were stained negatively with CD3. In situ hybridization for EBER demonstrated that the cells were EBV-positive. A diagnosis of aggressive NK-cell leukemia was made.

Magnetic resonance imaging of orbital cavity showed marked enlargement, and diffuse high intensity signals in the right superior rectus muscle and left inferior rectus muscle on fat-saturated T2-weighted image (Fig. 1). These lesions were diffusely contrast-enhanced on fat-saturated T1-weighted image (Fig. 2).

After two courses of dexamethasone, methotrexate, ifosfamide, L-asparaginase, and etoposide chemotherapy, she underwent cord blood transplantation with a preparative regimen comprised of etoposide, cyclophosphamide, and total body irradiation. She achieved complete remission, and her ocular manifestations completely resolved.

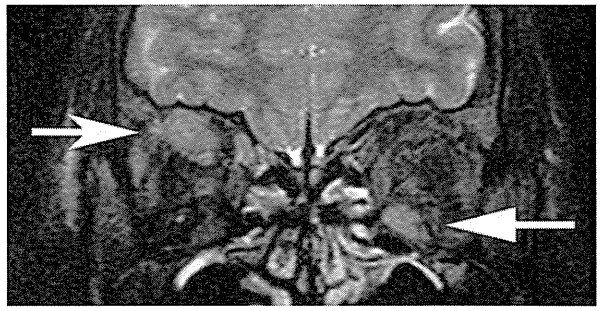

Fig. 1 MRI of orbital cavity showed the marked enlargement, and diffuse high intensity signal in the right superior rectus muscle and left inferior rectus muscle on fat-saturated T2-weighted image

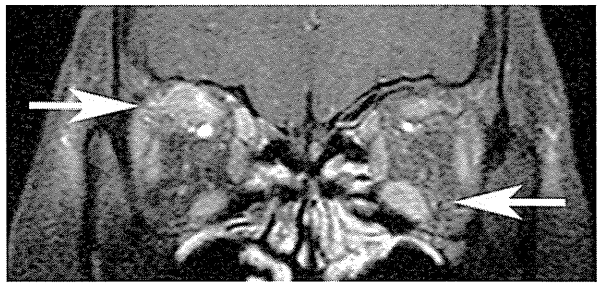

Fig. 2 Eye lesions were diffusely contrast-enhanced on fat-saturated T1-weighted image

We could not perform biopsy of the lesions due to the poor general condition of the patient, and the exact etiology

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of her ocular manifestation remains to be elucidated. While her ocular symptoms occurred in parallel to the progression of the leukemia, a number of possibilities for the cause of her ocular involvement can be raised, including tumor infiltration, infection, or paraneoplastic myositis. An accumulation of cases of ocular involvement associated with NK-cell leukemia is required.

Conflict of interest None declared.

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ORIGINAL ARTICLE

A randomized controlled trial of plasma real-time PCR and antigenemia assay for monitoring CMV infection after unrelated BMT

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Preemptive therapy is the standard strategy for preventing CMV disease after allogeneic hematopoietic SCT. In this study, unrelated BMT recipients were randomly assigned to a plasma real-time PCR group or an antigenemia group to compare the value of these monitoring tools for CMV reactivation. Ganciclovir (GCV) was started at 5 mg/kg/day when PCR reached 300 copies per ml or when antigenemia reached three positive cells per two slides. A total of 88 patients were randomized into the antigenemia group ($n=45$) or the PCR group ($n=43$). A significantly higher number of patients reached the threshold in the antigenemia group than in the PCR group (73.3 vs 44.2%, $P=0.0089$). However, only three patients (one in the antigenemia group and two in the PCR group) developed early CMV disease. These patients exclusively had colitis and were successfully treated with GCV or foscarnet. The median number of antigenemia-positive cells at the start of GCV was 47 in the PCR group. These findings suggest that antigenemia assay with the current cutoff was too sensitive and led to unnecessary use of GCV. However, the appropriateness of the threshold may be different by the methodology used, and therefore, it is difficult to generalize.

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Keywords: CMV; antigenemia; real-time PCR; preemptive therapy

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Introduction

Cytomegalovirus infection is a frequent complication after allogeneic hematopoietic SCT. Universal prophylaxis with ganciclovir (GCV) did not improve the transplantation outcome because of neutropenia caused by GCV.[1,2] Therefore, the initiation of GCV triggered by the detection of CMV reactivation is currently the standard strategy for preventing CMV disease.[3–5] A CMV antigenemia assay has been widely used to monitor CMV reactivation. However, the details of preemptive therapy still need to be clarified, including the threshold number of antigenemia-positive cells for deciding when to start GCV, the dose and duration of GCV and so on. We previously showed that a risk-adapted preemptive therapy, in which the cutoff number of antigenemia-positive cells for deciding when to start GCV was changed according to the risk for CMV disease, was appropriate in allogeneic SCT recipients, but the incidence of neutropenia was still high.[6] Therefore, in the next study, we evaluated the feasibility of preemptive therapy with low-dose GCV, and the findings showed that the initial dose of GCV could be safely decreased to 5 mg/kg.[7]

The PCR used to detect CMV DNA has also been investigated for its ability to monitor CMV reactivation.[8] PCR using whole blood samples might be too sensitive as a trigger for deciding when to start preemptive therapy compared with an antigenemia assay or PCR using plasma samples.[9,10] However, the recent development of real-time PCR has enabled the quantification of CMV DNA. Several studies have shown the feasibility of preemptive therapy guided by real-time PCR monitoring using either whole blood or plasma samples.[11–14] As for whole blood real-time PCR, Gerna *et al.* performed two randomized controlled trials of PCR and antigenemia, one in young patients (0–25 years old) and the other in older patients (20–67 years old).[12,13] They showed that a threshold value of 10 000

copies per ml for determining when to start GCV by whole blood PCR significantly reduced the use of GCV compared with a threshold in which GCV is started at any level of positive antigenemia. However, the study included heterogeneous patients in terms of donor type, stem cell source and GVHD prophylaxis. In particular, antithymocyte globulin was used in approximately half of the patients, and this may have strongly affected the incidence of CMV reactivation and disease.[15,16] In addition, preemptive therapy guided by antigenemia assay could be more appropriately performed by using a cutoff based on the number of positive cells.

Therefore, we performed a randomized controlled trial of plasma real-time PCR with a cutoff of 300 copies per ml and an antigenemia assay with a cutoff of three positive cells per two slides in a homogenous population of unrelated BMT recipients who received GVHD prophylaxis with a calcineurin inhibitor and MTX.

Patients and methods

Patients

Patients were eligible for the study if they were between 20 and 55 years old, would undergo BMT without *in vivo* or *ex vivo* T-cell depletion from an HLA-matched unrelated donor using a myeloablative conditioning regimen and had a good performance status without significant organ dysfunction, as defined in the protocol. Either the donor, the recipient or both must have been seropositive for CMV. Prophylaxis against GVHD was limited to a combination of CYA and MTX, but a combination of tacrolimus and MTX was allowed after June 2002. Patients were enrolled before starting a conditioning regimen, but randomization was performed between day 10 and day 12 after transplantation to exclude patients who developed significant organ dysfunction early after transplantation. This study was approved by the institutional review board of each participating center and a written informed consent was obtained from each patient (UMIN-CTR C000000347).

CMV monitoring methods

Cytomegalovirus antigenemia assay was performed as described previously.[17] In brief, 1.5×10^5 peripheral blood leukocytes were attached to a slide using a cytocentrifuge and fixed with formaldehyde. The cells were sequentially immunostained with MoAb C10/11 (Clonab CMV; Biotest, Dreieich, Germany) and reacted with goat alkaline phosphatase-labeled anti-mouse Ig (Mitsubishi Kagaku Iatron Inc, Tokyo, Japan). Under a light microscopy, CMV-positive cells were counted and the results are presented as the sum of the number of positive cells per two slides.

Real-time PCR was performed using primers and a TaqMan probe for immediate early genes using serum samples.[18] Briefly, DNA extracted from 100 μl of plasma was subjected to PCR using TaqMan Universal PCR Master Mix (PE Biosystems, Foster City, CA, USA) and the PCR product was detected as an increase in the fluorescent intensity using ABI Prism 7700 (PE Biosystems). Real-time fluorescent measurements were taken and a threshold cycle (CT) value for each sample was calculated by determining the point at which the fluorescence exceeded 10 times the baseline fluorescence. A standard curve was constructed using the CT values obtained from serially diluted DNA extracted from a plasmid that contains the respective region of CMV. The CT values from the clinical samples were plotted on the standard curve and the copy number was calculated automatically using Sequence Detection System version 1.6 (PE Biosystems).

Preemptive therapy against CMV disease

Patients were randomly assigned to the antigenemia group or the PCR group using a random block design. Assignment was stratified by the institute, age and the presence or absence of GVHD at the time of randomization. CMV reactivation was monitored weekly by both the antigenemia assay and PCR in all patients, but only the results of the assigned monitoring method were returned to the physicians. Preemptive therapy with GCV was started at an induction dose of 5 mg/kg/day when three or more CMV-positive cells per two slides were detected in the antigenemia group and 300 or more CMV DNA copies per ml were detected in the PCR group. The dose of GCV was increased to 10 mg/kg/day when a rising CMV load was observed. The dose of GCV was decreased to 5 mg/kg/day when a declining CMV load was observed in patients who were receiving GCV at 10 mg/kg/day. A rising and declining CMV load was defined as an increase and decrease in the CMV load by 50% or more of the previous value, respectively. However, changes in antigenemia-positive cells by less than five cells per two slides and changes in the DNA copy number by less than 500 copies per ml were regarded as a stable CMV load. When the CMV load fell below the threshold to start GCV, the dose of GCV was decreased to 5 mg/kg/day, if the patient was receiving GCV at 10 mg/kg/day, and GCV was discontinued if the patient was receiving GCV at 5 mg/kg/day. The dose of GCV was adjusted according to the renal function.[19] CMV monitoring was continued until all of the following three requirements were fulfilled: (i) More than 100 days had passed after transplantation; (ii) More than 2 weeks had passed after the last administration of GCV; and (iii) Absence of the use of (methyl-)prednisolone at 0.5 mg/kg/day or more.[20]

Definition of CMV disease

All patients with symptoms compatible with CMV disease such as interstitial pneumonia, colitis and gastritis underwent extensive pathological and microbiological examination of biopsy specimens. The diagnosis of CMV disease was made by histopathological examination and immunochemical staining of biopsy specimens. However, CMV retinitis was diagnosed when CMV DNA was detected by PCR using aqueous humor samples associated with characteristic retinal changes by ophthalmoscopy. Early and late CMV diseases were defined as those occurring before and after day 100, respectively.

Statistical considerations

The primary end point of the study was the incidence of early CMV disease. We defined success as the absence of CMV disease before day 100. Noninferiority was predefined as a difference in the success rates between the antigenemia group and the PCR group of no more than 10 percentage points. On the basis of the assumption of a success rate of 95% in the PCR group and 90% in the antigenemia group, 39 patients in each treatment group were required to show noninferiority with an alpha error of 5% and a power of 80%, which permitted a 10% difference in the success rate. On the basis of the assumption of a 20% loss of patients between the enrollment and randomization, a total of 96 patients needed to be enrolled in this study. Comparisons for dichotomous and continuous variables between groups were performed with Fisher's exact test and *t*-test, respectively. Pearson's correlation coefficient was calculated to compare the results of the two monitoring methods after logarithmic transformation.

Results

Incidence of CMV reactivation and the use of GCV

A total of 96 patients were enrolled in the study between January 2002 and March 2007. Among these patients, eight patients were excluded because of the use of tacrolimus as GVHD prophylaxis in one, negative CMV Ab in both the donor and recipient in one and organ dysfunction after the conditioning regimen in six. Therefore, a total of 88 patients were randomized into the antigenemia group ($n=45$) or the PCR group ($n=43$) (Figure 1). There were no differences in age, sex, background disease, CMV serostatus, conditioning regimen or GVHD prophylaxis between the two groups (Table 1). In addition, the incidence of grade II–IV acute GVHD was similar (42 vs 47%, $P=0.67$).

Cytomegalovirus reactivation, defined as a detection of CMV at any level, was more frequently observed in the antigenemia group (40 of 45 patients, 88.9%) than in the

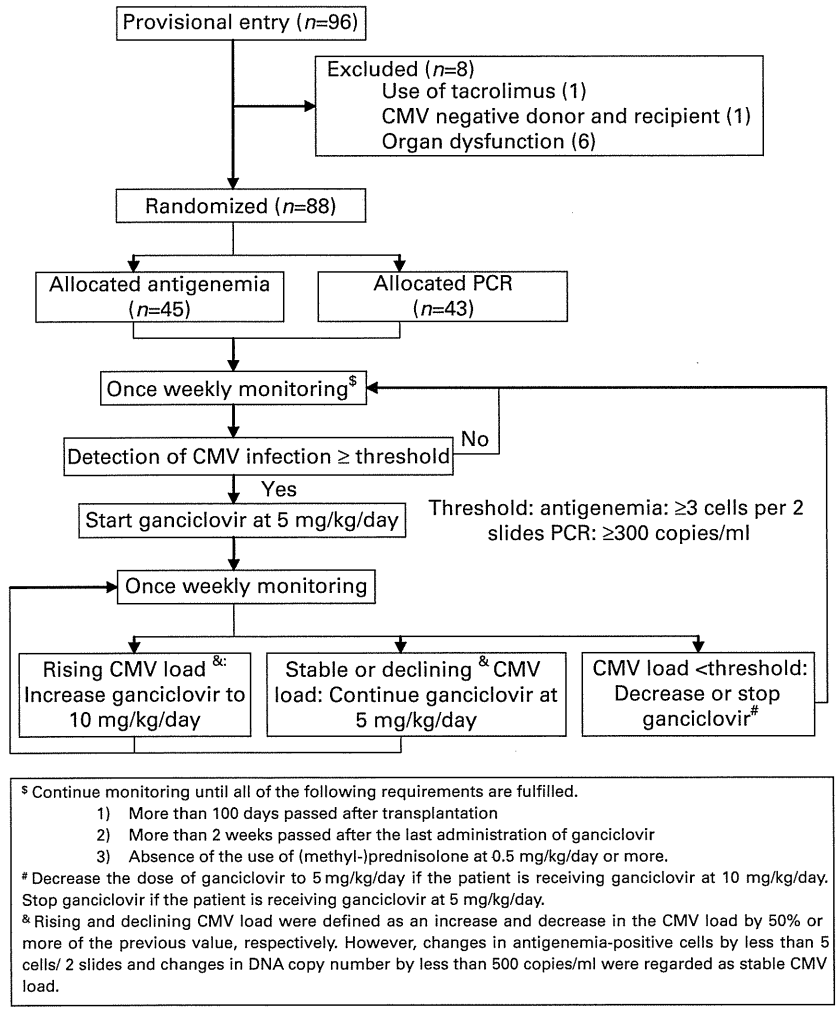

Figure 1 Design of the study.