厚生労働科学研究費補助金（免疫アレルギー疾患等予防・治療研究事業）
分担研究報告書

# 高齢者 HLA 不一致移植の安全性の検討

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**研究要旨**
高齢者はメタボリック・シンドロームの合併頻度が高いため、HLA 不一致移植においては、免疫抑制剤やステロイド剤の使用に伴い高血圧、高血糖、腎障害などの有害事象が高頻度に出現し、重篤化しやすい。既に研究者は患者の移植前併存疾患を総合的に評価する Comorbidity index が治療関連死亡の予測因子として有用である事を明らかにしたが、欧米の先行研究を参考としたため、肥満を評価するカットオフ値が Body mass index (BMI)30 と、高度の肥満の影響のみが評価されていた。わが国においては、むしろ軽度、中等度の肥満例が多いため、これらの影響を評価するため、より多数例について registry data を用いて検討した。3827 例の非血縁者間骨髄例の検討では、非再発死亡のリスクは BMI>30 の群で有意に増加することが再確認された。一方、急性移植片対宿主病の発症率ならびに感染症合併率は BMI<18, BMI 18-25, BMI 25-30, BMI>30 の四群比較で BMI 値と発症率に有意な正の増刊を認め、軽度ないしは中等度の肥満はこれらの合併症の予測因子となる事が確認された。

## A．研究目的

わが国の非血縁者間骨髄移植例を後方視的に検討し、移植前処置前の Body mass index (BMI) が治療結果に及ぼす影響を明らかにする。これによって、超過体重例の多い高齢者のミスマッチ移植を安全に実施する方法論を確立する。ミスマッチ移植が安全に実施できるようになれば、造血幹細胞移植の恩恵を受ける患者が飛躍的に増加する。特に造血器悪性腫瘍患者の多くが高齢者である事から、国民医療の向上に寄与するものと考える。

## B．研究方法

1998 年から 2005 年の間、日本骨髄移植推進財団 (JMDP) を介して非血縁者間骨髄移植が実施され、移植前処置前の身長と体重が報告されている 3827 例について、registry data を用いて後方視的に検討した。WHO の定義に従い、低 BMI (BMI < 18/kg/m$^2$) 群 (n = 295)、正 BMI (18 ≤ BMI < 25/kg/m$^2$) 群 (n = 2906)、超過体重 (25 ≤ BMI < 30/kg/m$^2$) 群 (n = 565)、肥満(30 < BMI/kg/m$^2$) 群 (n = 61)の四群に分類し、移植結果を群間比較した。
(倫理面の配慮)
本研究は「疫学研究に関する倫理指針」に基づいて計画され、骨髄移植推進財団のデータ資料管理委員会にデータ利用申請を行い、その倫理性、科学性、研究の必要性について審議、承認された後に実施された。

## C．研究結果

欧米の報告と大きく異り、わが国の非血縁者間骨髄移植例では BMI が 30 を超える肥満例は 61 例 (1.6%)と稀であった。一方低 BMI 群に分類された 295 例中では 50 歳以下の若年者が 80%を占めており、高齢者では超過体重傾向の患者の割合が高かった。累積非再発死亡率は低 BMI 群 29%、正 BMI 群 31%、超過体重群 32%、肥満群 40%と肥満群で高率であった（図 1）。原病再発率には差がみられず（図 2）、結果的に全生存割合では肥満群が他 3 群と比較して低下していた（図 3）。

図 1 累積非再発生存割合

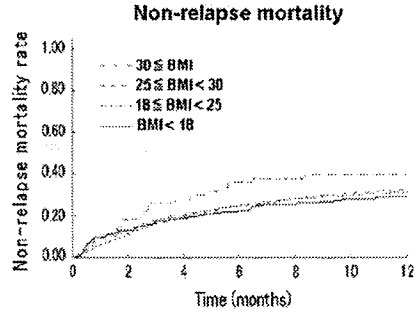


図 2 累積再発率

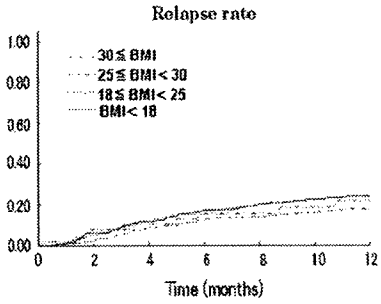
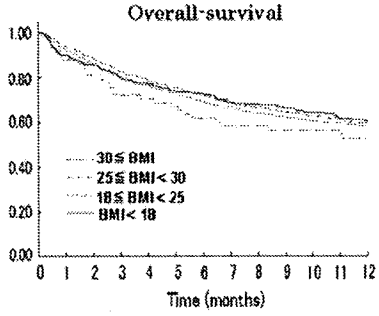



図 3 全生存期間




Grade II 以上の急性移植片対宿主病累積発症割合は低 BMI 群 42%、正 BMI 群 45%、超過体重群 48%、肥満群 58%と BMI と発症率に正の相関を認めた (p = 0.03) (図 4) 。また、全身性感染症の合併頻度も BMI と正の相関を認めた (図 5) 。

図 4 Grade II 以上の急性移植片対宿主病発症割合

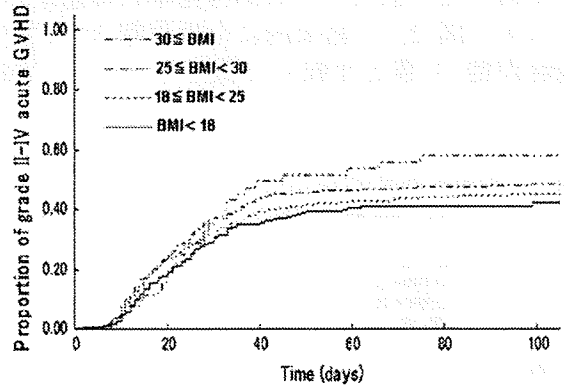
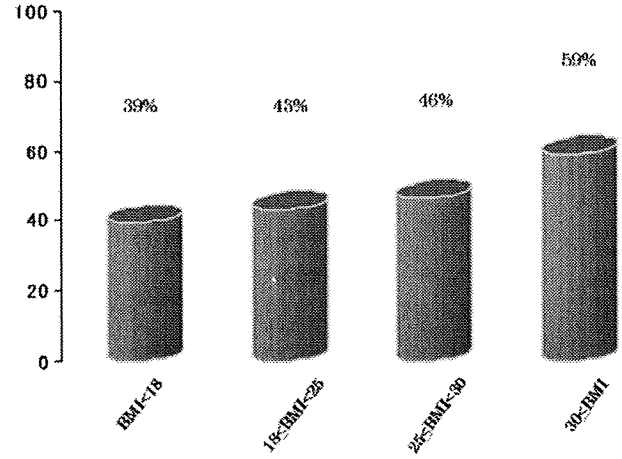



図 5 全身性感染症発症割合




## D．考察

欧米より、肥満は造血幹細胞移植後の非再発死亡のハイリスクである事を示唆するデータが報告されている。既に我々は、造血幹細胞移植患者の移植前合併症を評価する目的で欧米で開発された comorbidity index が、わが国の高齢者に対するミニ移植例でも、予測因子として有用である事を確認し、その過程で肥満 (BMI>30) もリスク因子となる事を明らかにしてきた。しかし、わが国では BMI が 30 を超える肥満例は稀であり、実際の臨床現場では BMI が 25 を超える超過体重の影響がより重要な検討課題である。今回の多数例の検討により、非再発死亡、全生存率、急性移植片対宿主病合併率、全身性感染症発症率と BMI には用量-反応関係を想定し得る正の相関がある事が確認され、肥満に限らず、超過体重も予後不良因子である事が明らかとなった。非血縁者間移植では HLA 一致血縁者間移植と比較し、免疫抑制剤やステロイドの使用量、使用頻度が高く、これらの薬剤の副作用である高血圧、高血糖、腎機能障害を生じやすく、超過体重はこの傾向に拍車をかけるものと推察される。また。高血糖は感染症のハイリスクとなり、感染症によって引き起こされる高サイトカイン血症が移植片対宿主病の発症を促進することも、既に我々は報告しており、超過体重による感染症リスクや移植片対宿主病発症リスクもメタボリックシンドロームを介した結果である事が推察される。従って、ミスマッチ移植では、より高用量の免疫抑制剤やステロイド剤が使用される可能性がある事から、移植患者の栄養管理と適切なメタボリックシンドローム対策は今後の極めて重要な課題であると思われる。

## E．結論

高齢者等に対するミスマッチ移植を有効かつ安全に行うためには、補助療法としての栄養療法が治療成績の向上が期待できる事が示唆された。

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## G．知的財産権の出願・登録状況

なし

# Ⅲ．研究成果の刊行に関する一覧

# 研究成果の刊行に関する一覧表

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# Ⅳ. 研究成果の刊行物・別刷

Bone Marrow Transplantation (2009), 1–7

www.nature.com/bmt

ORIGINAL ARTICLE

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

S Kimura[1], K Oshima[1], S Okuda[1], K Sato, M Sato, K Terasako, H Nakasone, S Kako, R Yamazaki, Y Tanaka, A Tanihara, T Higuchi, J Nishida and Y Kanda

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

*Bone Marrow Transplantation* advance online publication, 9 November 2009; doi:10.1038/bmt.2009.316

**Keywords:** CsA; pharmacokinetics; bioavailability; drug interaction

## Introduction

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

## Patients and methods

*Patients*

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

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Received 22 June 2009; revised 17 September 2009; accepted 29 September 2009

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

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

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

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

## Results

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

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

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

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

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

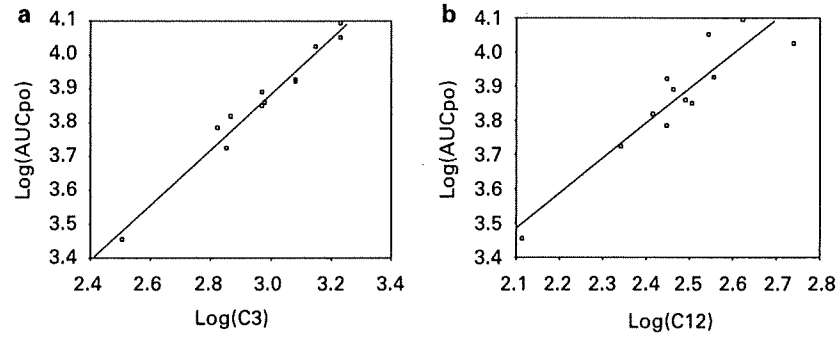


**Figure 1** Correlation between the AUC and the CsA peak (a: $C_3$) and trough (b: $C_{12}$) levels.

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

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

*Relationship between AUC and the CsA concentration at each measurement point*

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

*Influence of possible confounding factors on the bioavailability of Neoral*

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

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

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

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

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

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

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

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

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

*Clinical course after the change in the route of CsA administration*

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

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

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

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

**Table 5** Serial changes in laboratory data and blood pressure after the change in the route of CsA administration

| | *Mean (minimum–maximum)* | | | |
|---|---|---|---|---|
| | *Serum creatinine (mg per 100 ml)* | *ALT (IU/l)* | *Total bilirubin (mg per 100 ml)* | *Blood pressure level (mm Hg)* |
| Day 0 | 0.87 (0.60–1.43) | 64.4 (16–182) | 0.63 (0.24–1.06) | Systolic 130 (114–173)<br>Diastolic 82 (63–103) |
| Day 3 | 0.86 (0.32–1.63) | 50.1 (10–106) | 0.62 (0.27–1.47) | Systolic 124 (109–150)<br>Diastolic 79 (51–103) |
| Day 7 | 0.92 (0.69–1.31) | 44.6 (10–103) | 0.61 (0.30–1.17) | Systolic 122 (109–132)<br>Diastolic 80 (51–103) |
| Day 14 | 0.83 (0.67–1.29) | 65.8 (10–300) | 0.64 (0.27–0.96) | Systolic 121 (113–135)<br>Diastolic 76 (68–89) |

Abbreviation: ALT = alanine aminotransferase.

weeks after the conversion. The AUC of CsA was rather lower after conversion, and thus CsA was not considered to be the causative agent of liver dysfunction. Otherwise, no notable changes in laboratory and clinical data were observed (Table 5).

Four patients had developed grade I acute GVHD of the skin before the change in the route of CsA administration. During the 2 weeks after the switch, 3 of the 4 patients had persistent grade I skin GVHD, whereas GVHD was improved in 1 patient. Among the eight patients who did not have acute GVHD at the switch, one patient developed grade I acute GVHD of the skin, which was well controlled by topical steroid, and the other seven patients did not develop acute GVHD during the observation period. No clinically significant changes in vital or biological parameters occurred in the study patients. One patient (No. 9) developed nausea soon after conversion. An excessive increase in the CsA concentration was considered to be the cause of nausea and this symptom was improved after the dose of Neoral was reduced.

## Discussion

Neoral is a microemulsion formulation of CsA that has improved bioavailability and reduced variability in pharmacokinetic parameters within and between patients compared with a conventional CsA formulation (Sandimmun).[4] Its bioavailability has been reported to be 0.38 (38%) in healthy volunteers.[13] However, allogeneic HSCT patients have complications that could influence the CsA pharmacokinetics, such as damaged gastrointestinal mucosa and multiple drug interactions. The results of this study showed that the median value of the bioavailability of Neoral was 0.685 (range, 0.45–1.04). Detailed analyses revealed that the oral administration of VRCZ strongly affected the bioavailability of Neoral (0.87 vs 0.54). Therefore, although the switch from intravenous to oral administration of CsA at a ratio of 1:2 seemed to be appropriate in most patients, a lower conversion ratio such as 1:1.1 or 1:1.2 may be better in patients taking oral VRCZ.

The drug interactions between CsA and azole antifungal agents including FLCZ, ITCZ, and VRCZ have been well recognized.[14] Azole antifungal agents are metabolized through the cytochrome P450-3A (CYP3A4) enzyme system, interfere with the metabolism of CsA, and thereby increase the exposure to CsA. Therefore, careful monitoring of the blood CsA concentration is recommended when these agents are added during CsA administration. On the other hand, there are considerable differences among azole antifungals with regard to their ability to inhibit CYP3A4.[14] Interestingly, the concomitant use of oral VRCZ significantly increased the bioavailability of Neoral. We confirmed that VRCZ was started at least 7 days before the switch from intravenous to oral administration of CsA and was continued at the same dose after the switch. Therefore, the drug interaction between CsA and VRCZ seemed to be stronger during oral administration than during the intravenous infusion of CsA. We hypothesized that this stronger interaction can be explained by the presence of the P450 enzyme system in the gastrointestinal mucosa. The CYP3A4 isoenzymes are the most abundant isoforms of CYP and it has been postulated that CsA is also metabolized in the intestine by gut CYP3A4 isoenzymes.[15] The administration of VRCZ might have inhibited the gut metabolism of CsA and increased the bioavailability of CsA. However, a prospective controlled study is required to confirm this hypothesis.

ITCZ, another strong inhibitor of CYP3A4, did not increase the bioavailability of Neoral. As the ratio of $AUC_{IV}/DOSE_{IV}$ was higher not only in patients taking VRCZ but also in patients taking ITCZ compared with other patients (median 47.5, 55, and 41), ITCZ might have inhibited liver CYP3A4 similar to VRCZ, but inhibited gut CYP3A4 less strongly than VRCZ. This might have been affected by the different bioavailable dose of these agents, as the bioavailability of ITCZ is lower than that of VRCZ, in addition to the fact that the dose of ITCZ was lower than that of VRCZ (200 vs 400 mg/day).

With regard to the route of VRCZ, it was exclusively administered orally in this study. Therefore, we could not conclude whether the intravenous administration of VRCZ would similarly affect the bioavailability of CsA. In earlier reports, the extent of drug interaction between CsA and azole antifungals varied according to the route of administration and the dose or kind of antifungal agent. Numerous reports have shown a significant interaction (>84%) between oral FLCZ with a dose of 200 mg/day or greater and oral CsA.[16,17] On the other hand, Osowski *et al.*[18] evaluated the drug interaction between intravenous FLCZ at 400 mg/day and intravenous CsA in HSCT recipients and there was a statistically significant but smaller increase (21%) in the serum CsA concentration.

Mihara *et al.*[19] reported that the mean steady-state whole-blood level of CsA significantly increased after the route of FLCZ administration was switched from intravenous to oral. These data suggest that the drug interaction between CsA and FLCZ was stronger when FLCZ was administered orally. With regard to other azole antifungal agents, not only oral but also intravenous administration of ITCZ significantly affected the blood concentration of CsA.[20–22] Concerning the interaction between VRCZ and CsA, Mori *et al.*[23] reported that the administration of VRCZ to patients receiving CsA resulted in a significant increase in the concentration/dose ratio of CsA, but the route of VRCZ administration did not affect the changes in the concentration/dose ratio. If we consider these findings together, it may be reasonable to suggest that the interaction between azole antifungal agents and CsA is stronger when the antifungals are given orally, but the difference becomes unclear with ITCZ and VRCZ, as the interactions of these agents are stronger than that of FLCZ and can be detected even when they are given intravenously. Therefore, when we interpret pharmacokinetic data of CsA, we must be cautious not only about concomitantly used agents but also the route of administration of both CsA and the other drugs. For example, Parquet *et al.* reported that a ratio of 1:2 in the switch from intravenous to oral administration was appropriate,[5] whereas a 1:1 ratio seemed to be appropriate in the study by McGuire *et al.*[6] In the former study, oral FLCZ was used concomitantly and thus their conclusion was consistent with our data. In the latter study, information on the use of antifungal agents was not described, and thus the data were difficult to interpret.

When we switch the route of CsA administration from continuous infusion to twice-daily oral administration, the target blood concentration should also be changed. Nakamura *et al.*[12] reported that the CsA blood concentration during continuous infusion was estimated to be 2.55 times the trough level during twice-daily oral administration of Neoral to obtain an equal AUC of CsA in kidney transplant patients. In this study, we concluded that the CsA concentration during continuous infusion should be doubled compared with the trough concentration during twice-daily oral administration in allogeneic HSCT recipients. Although the calculation method was different, the conclusion was consistent (mean 2.01) when we applied their methods. Although the reason for the difference between these studies remains unclear, it may have been due to the differences in the use of concomitant drugs or the status of the gastrointestinal tract.

In conclusion, when switching CsA from continuous infusion to oral administration, concomitant medications that could affect the bioavailability of CsA, especially azole antifungal agents, should be taken into account. Although a 1:2 ratio on switching may be appropriate in most patients, a lower conversion ratio is recommended in patients taking oral VRCZ.

## Conflict of interest

The authors declare no conflict of interest.

## Acknowledgements

This research was supported in part by grants from the Ministry of Health, Labor and Welfare of Japan.

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Bone Marrow Transplantation (2009), 1–8

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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.**

*Bone Marrow Transplantation* advance online publication, 7 December 2009; doi:10.1038/bmt.2009.337

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

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Received 1 July 2009; revised 1 October 2009; accepted 12 October 2009

## 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, $1.5 \times 10^5$ 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

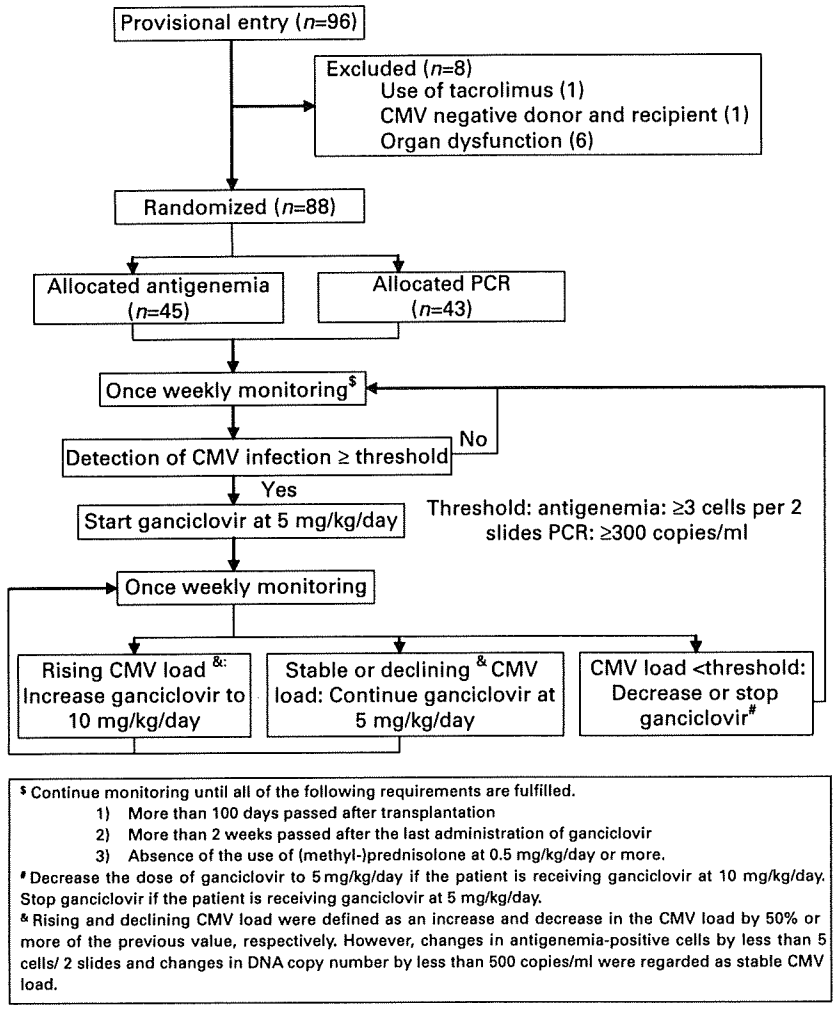


**Figure 1** Design of the study.

PCR group (27 of 43 patients, 62.8%) ($P=0.0050$, Table 2). The probability of starting GCV was significantly higher in the antigenemia group than in the PCR group (73.3 vs 44.2%, $P=0.0089$, Figure 2). The results of PCR in the antigenemia group and those of the antigenemia assay in the PCR group were disclosed after the completion of the study. A good correlation was seen between the results of PCR and the antigenemia assay ($P<0.0001$, $r^2=0.38$, Figure 3). Of the 33 patients who received GCV in the antigenemia group, PCR and the antigenemia assay reached the threshold simultaneously in five patients and PCR reached the threshold before starting GCV in only four patients (Figures 4a and 5a). In the other 24 patients, the CMV DNA copy number was persistently below the threshold until GCV was started. On the other hand, in 11 of 19 patients who received GCV in the PCR group, the results of the antigenemia assay reached the threshold earlier in 11 patients and simultaneously in 7 patients (Figures 4b and 5b). The results of the antigenemia assay were persistently below the threshold until GCV was started in only one patient. The median number of antigenemia-positive cells at the start of GCV was 5 (range: 3–102) and 47 (range: 0–2921) in the antigenemia and PCR groups, respectively (Figure 6a, $P=0.0051$). The median CMV DNA copy number was negative (range: 0–4400) and 750 (range: 310–13000) in the antigenemia and PCR groups, respectively (Figure 6b, $P<0.0001$).

Among the 52 patients who received preemptive therapy with GCV at 5 mg/kg/day, only 13 and 7 patients in the antigenemia and PCR groups, respectively, experienced a rising CMV load and required dose-escalation to 10 mg/kg/day, suggesting that the initiation of GCV at 5 mg/kg was appropriate.

**Table 1** Patient characteristics

| | *Antigenemia (n=45)* | *PCR (n=43)* | P-*value* |
|---|---|---|---|
| *Pre-transplantation factors* | | | |
| Median age (range) | 41 (20–55) | 40 (20–53) | 0.82 |
| Sex (male/female) | 25/20 | 24/19 | >0.99 |
| HLA mismatch | 7 (16%) | 9 (21%) | 0.59 |
| *Background disease* | | | |
| AML | 17 | 18 | |
| ALL | 12 | 12 | |
| CML | 6 | 3 | |
| MDS | 5 | 7 | |
| Others | 5 | 3 | 0.57 |
| *Donor/recipient CMV status* | | | |
| Pos./Pos. | 28 | 26 | |
| Pos./Neg. | 5 | 4 | |
| Neg./Pos. | 8 | 6 | 0.74 |
| *Conditioning regimen* | | | |
| TBI | 39 | 36 | |
| Non-TBI | 6 | 7 | 0.77 |
| *GVHD prophylaxis* | | | |
| CYA–MTX | 25 | 25 | |
| TAC–MTX | 16 | 16 | 0.59 |

Abbreviations: MDS = myelodysplastic syndrome; Neg. = negative; Pos. = positive; TAC = tacrolimus.

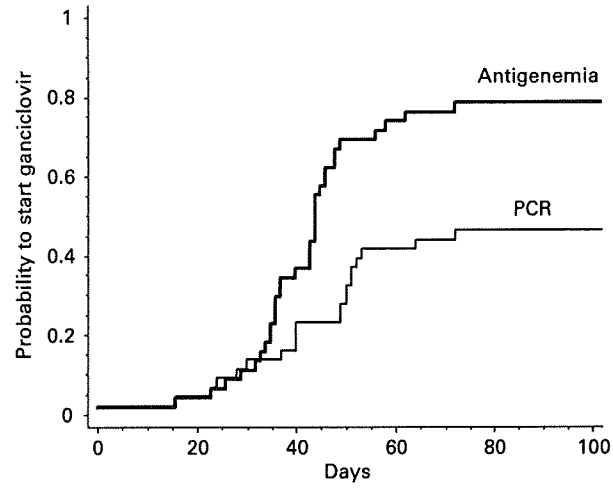


**Figure 2** Days to start ganciclovir after transplantation.

**Table 2** CMV-related events after engraftment

| | *Antigenemia (n=45)* | *PCR (n=43)* | P-*value* |
|---|---|---|---|
| CMV reactivation[a] | 40 | 27 | 0.0050 |
| *Start ganciclovir* | 33 | 19 | 0.0089 |
| Duration of ganciclovir (days) | 23.2 ± 19.4 | 20.8 ± 14.2 | 0.64 |
| Total dose of ganciclovir (mg/kg) | 140.8 ± 129.7 | 118.4 ± 91.2 | 0.51 |
| Dose escalation to level II | 13 | 7 | >0.99 |
| Neutropenia <500 per μl | 5 | 3 | >0.99 |
| Stop ganciclovir because of neutropenia | 1 | 0 | >0.99 |
| Increase in serum creatinine[b] | 8 | 0 | 0.039 |
| *CMV disease* | | | |
| Early (before day 100) | 1 | 2 | 0.61 |
| Late (after day 100) | 0 | 1[c] | 0.48 |

[a]Detection of antigenemia or DNA at any level.
[b]Increase in serum creatinine level by 0.5 mg per 100 ml or more from the baseline level.
[c]The patient developed early CMV disease, which was improved by ganciclovir. However, intestinal symptoms recurred after day 100 and CMV colitis was suspected because of positive antigenemia, although it was not confirmed by biopsy.

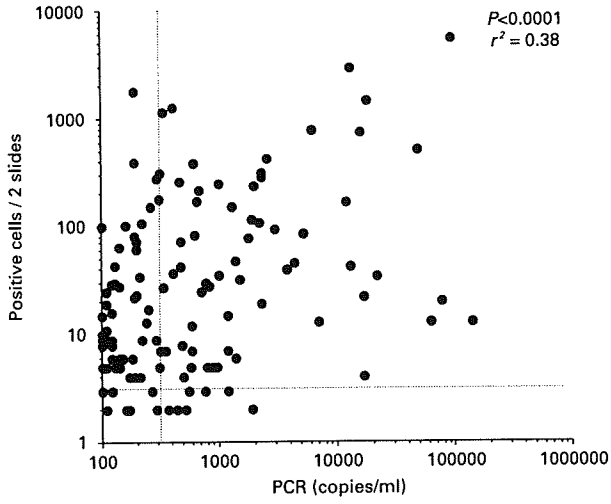


**Figure 3** Correlation between the number of positive cells in the antigenemia assay and copy number by PCR.

*CMV diseases*

Early CMV disease was diagnosed in 1 of the 45 patients (2.2%) in the antigenemia group and 2 of the 43 patients (4.7%) in the PCR group ($P=0.61$). These patients exclusively developed CMV colitis. Another patient in the PCR group showed characteristic retinal changes and was presumptively treated with GCV, although CMV infection was not detected in either the aqueous humor or the peripheral blood. The 95% confidence interval for the difference in the success rate was −10.1 to 5.2%, and thus was just outside the predefined lower limit of −10%. However, as shown in Table 3, the development of CMV disease in the PCR group could not be avoided even if these patients were assigned to the antigenemia group, as either the antigenemia assay and PCR reached the threshold simultaneously (UPN32) or the antigenemia assay did not reach the threshold before the diagnosis of CMV disease (UPN35). All of these patients were successfully treated with GCV or foscarnet, although one patient (UPN35) showed the recurrence of colitis after day 100. None of the other patients developed late CMV disease.

*Adverse events during preemptive therapy*

The mean duration of preemptive therapy with GCV and the mean total dose of GCV was 23.2 ± 19.4 days and 140.8 ± 129.7 mg/kg in the antigenemia group and 20.8 ± 14.2 days and 118.4 ± 91.2 mg/kg in the PCR group ($P=0.64$ and $P=0.51$), respectively. Neutropenia with a neutrophil count of $<500$ per μl was observed in 5 of the 33 patients in the antigenemia group and 3 of the 19 patients in the PCR group ($P>0.99$). Only one patient in the antigenemia group required a discontinuation of GCV because of neutropenia. The total dose of GCV was higher in patients who developed neutropenia, but this difference was not statistically significant (163.8 ± 82.5 vs 126.9 ± 121.4, $P=0.42$).

An increase in the serum creatinine level by at least 0.5 mg per 100 ml was observed in 8 of the 33 patients in the antigenemia group and in none of the 19 patients in the PCR group ($P=0.039$). The total dose of GCV was significantly higher in patients who developed renal impairment (255.0 ± 198.0 vs 106.0 ± 45.5, $P=0.0004$).

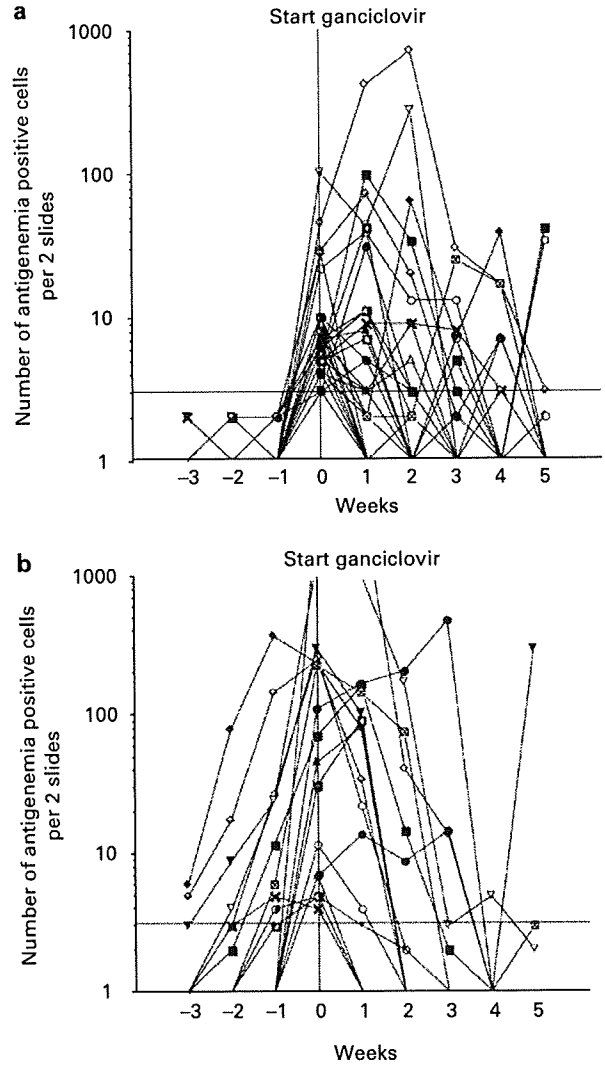


**Figure 4** Serial changes in the number of antigenemia-positive cells in patients who received preemptive therapy in the antigenemia group (**a**) and in the PCR group (**b**). Week 0 represents the day ganciclovir was started.

## Discussion

In this randomized controlled trial, we compared plasma real-time PCR with a cutoff at 300 copies per ml and an antigenemia assay with a cutoff at three positive cells per two slides as a trigger for deciding when to start preemptive therapy with GCV after unrelated BMT. GCV was used significantly less frequently in the PCR group. A comparison of the number of antigenemia-positive cells and the CMV DNA copy number at the start of GCV treatment clearly revealed that plasma PCR was significantly less sensitive than the antigenemia assay, at least with the current cutoff values. Although the 95% confidence