Effect of early posttransplantation tacrolimus concentration on the development of acute graft-versus-host disease after cord blood transplantation

Yukinori Nakamura, Yoshinori Tanaka, Mayumi Tanaka, Akiko Sugiyama, Yoshihiro Tokunaga, Yasuko Suehiro, Koumei Takeda, Toshiaki Yujiri, Yukio Tanizawa

Third Department of Internal Medicine, Yamaguchi University School of Medicine

We retrospectively evaluated the effect of blood tacrolimus concentration early after cord blood transplantation (CBT) on acute graft-versus-host disease (GVHD). Twenty-eight patients who underwent CBT and received continuous tacrolimus infusion were included. The mean concentration of tacrolimus during the second week (17.8±3.7 ng/mL in 0–1 versus 12.6±3.7 in II–IV; P<.01) after CBT was significantly associated with grade II–IV acute GVHD. On the receiver operator characteristic curves, a cutoff value of 15 ng/mL during the second week, provided the best balance between sensitivity and specificity. Multivariate analysis demonstrated that a mean tacrolimus concentration<15 ng/mL during the second week was a significant risk factor for grade II–IV acute GVHD (hazard ratio, 0.22; 95% confidence interval, 0.07–0.69; P<.01). Early post-CBT tacrolimus concentration has a significant impact on the development of grade II–IV acute GVHD. (Journal of Hematopoietic Cell Transplantation 4(3): 74–81, 2015.)

Introduction

Cord blood transplantation (CBT) represents an attractive alternative for patients with hematological disorders who lack matched-related or unrelated donors.1,2 Several investigators have reported transplant outcomes of CBT in adult patients, confirming successful restoration of hematopoiesis and further demonstrating favorable outcomes comparable with those of bone marrow transplantation (BMT) from unrelated donors.3–7 However, graft-versus-host disease (GVHD) prophylaxis has varied significantly in various studies, with the use of cyclosporine (CsA) alone, CsA plus corticosteroids, CsA plus methotrexate (MTX), tacrolimus plus MTX, tacrolimus plus mycophenolate mofetil (MMF), and others.3,6,8–12

Tacrolimus has been increasingly used for the prophylaxis and treatment of GVHD after allogeneic hematopoietic stem cell transplantation (HSCT). Tacrolimus possesses 100 times greater in vitro inhibitory activity against T cells than CsA, and its strong immunosuppressive effects have been documented particularly in the setting of HSCT from alternative donors.13,14 Several investigators have reported the transplant outcomes of CBT when tacrolimus was used for GVHD prophylaxis.8–11 Favorable outcomes have been reported; however, the optimal range of blood tacrolimus concentration early after CBT remains unclear. Several studies have evaluated whether the optimal range of blood tacrolimus concentration early after allogeneic HSCT, other than with CBT, prevents acute GVHD. Those studies clearly demonstrated that a higher blood tacrolimus concentration was associated with toxicity and transplant-related mortality (TRM), but failed to demonstrate any significant impact of blood tacrolimus concentration on acute GVHD incidence.15,16 On the basis of these findings, a target range of 10–20 ng/mL of tacrolimus was recommended. Recently, Mori et al.17 reported that the early posttransplantation blood tacrolimus concentration had a significant impact on the development of moderate to severe acute GVHD after BMT from unrelated donors. Their results have suggested that the in vivo efficacy of tacrolimus in pre-
venting acute GVHD is dose dependent as long as its concentration is strictly maintained within a less toxic therapeutic range.

Posttransplant immune disorders including acute GVHD are problematic in CBT recipients. These reactions and additional immunosuppression might increase the risk of infection and organ dysfunction, leading to a high TRM. It remains unclear whether the blood tacrolimus concentration early after CBT correlates with the incidence of acute GVHD. Moreover, the optimal range of blood tacrolimus concentration early after CBT is unknown. Therefore, in this study, we retrospectively evaluated the relationship between blood tacrolimus concentration by targeting the recommended range \(10-20\) ng/mL and the development of acute GVHD in adult patients who underwent single-unit CBT.

**Patients and methods**

**Patients and donor characteristics**

This retrospective study included 28 adult patients with hematological malignancies who underwent single-unit CBT from an unrelated donor, receiving tacrolimus as part of the GVHD prophylaxis between October 2004 and January 2014 at Yamaguchi University Hospital. Patients who underwent CBT as the second allogeneic HSCT and those who failed to achieve neutrophil engraftment were excluded from this study. Patient and donor characteristics are shown in Table 1. Patients were qualified as being at standard risk if they were in the first or second complete remission (CR), if they were in the chronic phase of chronic myelogenous leukemia (CML), or if they had refractory anemia of myelodysplastic syndrome (MDS-RA). High-risk recipients were patients in the third CR, those in relapse, who had CML beyond the chronic phase, or those who had MDS but not MDS-RA. In brief, 13 patients were categorized as being at standard risk on the basis of a diagnosis of acute myeloid leukemia or acute lymphoblastic leukemia in the first or second CR, CML in a chronic phase, or MDS-RA. The remaining 15 patients were categorized as being at high risk. Human leucocyte antigen (HLA) \(^{-}A, \, A^{-}B, \, A^{-}DR\) antigens were typed by using standard serologic or low-resolution techniques. HLA-\(^{-}A, \, A^{-}B, \, A^{-}DR, \) and-\(DRB1\) alleles were typed by using high-resolution deoxyribonucleic acid techniques, as described previously. Cord blood units were obtained from the Japanese Cord Blood Bank Network. Cord blood grafts had at least 4 of 6 HLA-A, \(-B, -\, DR\) antigens that were matched to the recipient and had a cryopreserved cell dose of at least \(2.0 \times 10^7\) nucleated cells/kg of recipient body weight. An HLA mismatch in the graft-versus-host direction was defined as when recipient antigens or alleles were not shared by the donor; a mismatch in the host-versus-graft direction was defined as when donor antigens or alleles were not shared by the recipient. Fourteen patients received myeloablative conditioning including 12 Gy of total body irradiation, and 14 patients received a reduced-intensity regimen including fludarabine. This study was approved by the institutional review board of Yamaguchi University Hospital (No. H26–21). All procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 2008. Patients provided written informed consent for data exploitation.

| Table 1. Patients' characteristics |
|-----------------------------------|
| **Age (years)** | Median (range) | 50 (20–58) |
| **Sex** | | |
| Male | 12 |
| Female | 16 |
| **Diagnosis** | | |
| AML | 12 |
| ALL | 5 |
| MDS | 2 |
| CML | 2 |
| ML | 7 |
| **Disease status** | | |
| Standard risk | 13 |
| High risk | 15 |
| **Conditioning** | | |
| Myeloablative | 14 |
| Reduced intensity | 14 |
| **GVHD prophylaxis** | | |
| Tacrolimus+MTX | 27 |
| Tacrolimus+MMF | 1 |
| **HLA mismatched number (GVH)** | | |
| 0–1/6 | 8 |
| 2/6 | 20 |
| **Number of infused nucleated cells** | Median (range), \(\times 10^7/kg\) | 2.69 (2.10–4.86) |

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CML, chronic myelogenous leukemia; GVH, graft-versus-host direction; HLA, human leucocyte antigen; MDS, myelodysplastic syndrome; ML, malignant lymphoma; MMF, mycophenolate mofetil; MTX, methotrexate.
GVHD prophylaxis

Tacrolimus was administered daily starting from the day before the transplantation at a dose of 0.02 or 0.03 mg/kg via continuous intravenous infusion, and the dose was adjusted to maintain the whole-blood tacrolimus concentration between 10 and 20 ng/mL. The tacrolimus dose was adjusted by each physician according to the whole-blood tacrolimus concentration and adverse events such as renal dysfunction. The whole-blood tacrolimus concentration was measured 1–6 times a week by using an automated microparticle enzyme immunoassay. Tacrolimus was administered intravenously for at least 3 weeks after the transplantation without permanent discontinuation. Tacrolimus administration was switched from intravenous to oral when patients could reliably consume oral medication. MTX was administered intravenously at 5–15 mg/m²/day on day 1 and 5–10 mg/m²/day on days 3 and 6. In 1 patient, 15 mg/kg/day of oral MMF was used instead of MTX from day 1 because of pleural effusion. None of the patients received antithymocyte globulin for the prophylaxis of GVHD. The diagnosis of acute GVHD was based on clinical and pathological findings and graded according to the consensus criteria.19

Statistical analysis

The mean blood tacrolimus concentrations were calculated for the first, second, and third weeks after CBT, and they were treated as fixed covariates. The relationship between the acute GVHD grade and tacrolimus concentrations was evaluated by using the Jonckheere-Terpstra test. Dichotomous and continuous variables in the 2 groups were compared by using Fisher’s exact test and the Mann-Whitney U-test, respectively. Receiver operator characteristic (ROC) curves were constructed by using the mean tacrolimus concentrations as predictors of the incidence of acute GVHD. The area under the ROC curve was calculated as an overall performance indicator of the concentration. The cutoff values derived from ROC curves were evaluated in terms of the sensitivity and specificity. The cumulative incidence of acute GVHD was calculated by using the Gray test, considering death without acute GVHD or relapse as a competing risk.20 Factors that showed at least borderline significance (P<.10) in univariate analyses were included in the multivariate analyses by using the Fine and Gray proportional hazards model, and then stepwisely deleted from the model. Factors with P values less than .05 were considered significant. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). More precisely, it is a modified version of R commander designed to add statistical functions frequently used in biostatistics.21

Results

Relationship between the incidence of acute GVHD grade and tacrolimus concentration

Among the 28 patients, 3 (10.7%) developed grade I, 11 (39.3%) developed grade II, 4 (14.3%) developed grade III, and no patients developed grade IV acute GVHD (Table 2). The cumulative incidence of grade II–IV acute GVHD was 53.6% (95% confidence interval [CI], 33.2–70.2%). All 15 patients who developed grade II–IV acute GVHD did so in the third week or later after CBT. The mean tacrolimus concentrations during the first, second, and third weeks after CBT was 10.8–25.0 ng/mL (median, 15.8 ng/mL), 7.3–24.2 ng/mL (median, 15.7 ng/mL), and 8.1–21.3 ng/mL (median, 16.6 ng/mL), respectively. In relation to the acute GVHD grade, there was a significant trend of lower mean tacrolimus concentration in the second week (P<.01), but not in the first and third weeks after CBT (Table 2). The comparative analysis of the tacrolimus concentration each week during the first 3 weeks after CBT indicated that the mean tacrolimus concentrations in the second week was significantly lower in patients with grade II–IV acute GVHD than in those with grade 0–I acute GVHD (Table 3). No significant difference was observed in these analyses when comparing the concentrations in the first and third weeks after CBT.

Factors affecting the development of acute GVHD

To evaluate the predictive value of the mean blood tacrolimus concentrations during the second week after CBT for grade II–IV acute GVHD, we performed ROC analyses and evaluated the area under the curve (AUC). The AUC of the second week was 0.85 (95% CI, 0.71–0.99), suggesting that the mean tacrolimus concentrations during the second week after CBT could be used to predict grade II–IV acute GVHD. During the second week, the sensitivity and specificity were 73% and 85%, respectively, with a cutoff concentration of 15 ng/mL. Patients receiving tacrolimus were dichotomized into 2 groups according to the ROC analyses being lower or higher than 15 ng/mL at the second week. In addition to the tacrolimus concentration in the second week after CBT, the effects
Effect of tacrolimus concentration after CBT

of patient age, patient sex, donor sex, HLA compatibility in graft-versus-host direction, disease status, conditioning regimen, and planned MTX doses on the incidence of grade II–IV acute GVHD were evaluated (Table 4). Using univariate analysis, planned MTX doses lower than 35 mg/m² and blood tacrolimus concentrations lower than 15 ng/mL in the second week were identified as significant factors for developing grade II–IV acute GVHD (Table 4). Using multivariate analysis, a significant factor for developing grade II–IV acute GVHD included mean blood tacrolimus concentration in the

### Table 2. Relationship between grade of acute graft-versus-host disease and tacrolimus concentration at each week after cord blood transplantation

| Grade | n  | First week | Second week | Third week |
|-------|----|------------|-------------|------------|
| 0     | 10 | 16.3±3.9   | 17.6±4.1*   | 16.5±3.0   |
| I     | 3  | 18.8±1.6   | 18.7±1.1*   | 18.2±0.4   |
| II    | 11 | 15.5±3.4   | 13.2±3.6*   | 14.0±4.9   |
| III   | 4  | 13.9±4.2   | 10.9±3.7*   | 13.1±4.4   |

*Significant trend of decrease in relation to grade of acute graft-versus-host disease by the Jonckheere-Terpstra test (P<.01).

### Table 3. Relationship between grades of acute graft-versus-host disease and tacrolimus concentration at each week after cord blood transplantation

| Grades | n  | First week | Second week | Third week |
|--------|----|------------|-------------|------------|
| 0 to I | 13 | 16.9±3.6   | 17.8±3.7    | 16.9±2.7   |
| II to IV | 15 | 15.1±3.5   | 12.6±3.7*   | 13.8±4.6   |

*Statistically significant compared with grades 0 to I (P<.01).

### Table 4. Univariate and multivariate analyses for factors affecting the incidence of grade II–IV acute graft-versus-host disease

| Factors                      | Incidence of acute GVHD (%) | P value | Hazard ratio | 95% CI | P value |
|------------------------------|----------------------------|---------|--------------|--------|---------|
| Patient age (years)          | <50                        | 69.2    |              |        | .14     |
|                              | ≥50                        | 40.0    |              |        |         |
| Patient sex                  | Male                       | 58.3    |              |        | .69     |
|                              | Female                     | 50.0    |              |        | .87     |
| Donor sex                    | Male                       | 55.0    |              |        |         |
|                              | Female                     | 50.0    |              |        |         |
| HLA mismatched number (GVH)  | 0–1                        | 50.0    |              |        | .76     |
|                              | 2                          | 55.0    |              |        |         |
| Disease status               | Standard                   | 61.5    |              |        | .60     |
|                              | High                       | 46.7    |              |        |         |
| Conditioning                 | Myeloablative              | 50.0    |              |        | .68     |
|                              | Reduced intensity          | 57.1    |              |        |         |
| MTX doses (mg/m²)            | <35                        | 81.8    |              |        | .81     |
|                              | ≥35                        | 35.3    |              |        | .01     |
| Tacrolimus conc. (ng/mL)     | <15                        | 84.6    |              |        | 1.00    |
|                              | ≥15                        | 26.7    | <.01         | 0.22   | 0.07–0.69 | <.01 |

CI, confidence interval; conc., concentration; GVH, graft-versus-host direction; HLA, human leucocyte antigen; MTX, methotrexate
second week after CBT (Table 4). Figure 1 shows the cumulative incidence of grade II–IV acute GVHD when patients were grouped by using a tacrolimus concentration of 15 ng/mL as a cutoff value in the second week after CBT. The incidence of grade II–IV acute GVHD was significantly higher in patients with a concentration of tacrolimus lower than 15 ng/mL (84.6%; range, 43.6–96.7%) compared with patients with a concentration higher than 15 ng/mL (26.7%; range, 7.7–50.5%; \( P = .005 \)). Hepatic dysfunction and concomitant administration of other agents interacting with tacrolimus, especially voriconazole and itraconazole, could alter the blood tacrolimus concentrations. To clarify the reason for the difference in tacrolimus concentration, the incidence of hepatic dysfunction and the concomitant administration of voriconazole or itraconazole were compared in patients with lower or higher than 15 ng/mL of tacrolimus concentration at the second week after CBT, however, no significant difference was observed. During the second week, the actual total dose of tacrolimus administered was higher in patients with a concentration of tacrolimus higher than 15 ng/mL compared with that of patients with a concentration lower than 15 ng/mL (\( P = .02 \)), however, the actual total dose of tacrolimus had no significant impact on the development of grade II–IV acute GVHD (date not shown).

Effects of tacrolimus concentration on renal function

In all patients, the serum creatinine level was measured every day during the first 4 weeks post-CBT. Among the 28 patients, this level increased 2-fold or more during the first 4 weeks after CBT as compared with that before CBT in 2 patients (7.1%). There was no significant correlation between the blood tacrolimus concentration in the second week after CBT and an increase in serum creatinine (\( P = .48 \)). No patients developed posterior reversible encephalopathy syndrome.

Discussion

The results of this study clearly demonstrate the importance of early blood tacrolimus concentration after single-unit CBT, especially during the second week, on the development of grade II–IV acute GVHD. In this study, all patients developed grade II–IV acute GVHD in the third week or later after CBT. The blood tacrolimus concentration during the second week was measured after initial dose adjustments had been made, but before acute GVHD occurred. Therefore, it may be important for preventing acute GVHD. In this study, the cumulative incidence of grade II–IV acute GVHD was similar to that in previous studies that evaluated the outcome of single-unit
CBT by using a calcineurin inhibitor including tacrolimus for GVHD prophylaxis. The results of our study indicate that an early posttransplantation blood tacrolimus concentration of 15 ng/mL constituted the dividing line between patients with grade 0–1 and those with grade II–IV acute GVHD after CBT. In the majority of studies comparing the outcomes of CBT with those of BMT from unrelated donors, the risk of acute GVHD was reported to be lower after CBT than after BMT from unrelated donors. These results implied that the recommended targeted blood tacrolimus concentration could be lower in patients after CBT than in those after BMT from unrelated donors. However, in a recent report, Mori et al. evaluated the relationship between blood tacrolimus concentration and the development of acute GVHD in adult patients who underwent BMT from unrelated donors. Their study also indicated that the dividing line should be 15 ng/mL, and thus, 15–20 ng/mL might be the recommended early posttransplantation concentration for efficacious prophylaxis of moderate to severe acute GVHD after BMT from unrelated donors. In both their study and ours, almost all patients received tacrolimus with short-term MTX for GVHD prophylaxis, and the target range of the blood tacrolimus concentration was set at 10–20 ng/mL. These results suggest that the target range of blood tacrolimus concentration for efficacious prophylaxis of moderate to severe acute GVHD may be equivalent in adult patients who undergo CBT or BMT from unrelated donors. Previous studies comparing the outcome of CBT with those of BMT from unrelated donors were retrospective, and they had differences in patient characteristics, donor-recipient HLA disparities, and GVHD prophylaxis among the types of stem cell sources; therefore, a larger-sized, randomized trial is needed to clarify the risk of acute GVHD in patients after CBT compared with unrelated donors.

Renal toxicity is one of the most common adverse effects of tacrolimus. In our study, only 7% of the patients experienced renal impairment. In addition, there was no significant correlation between the concentration of tacrolimus and an increase in the serum creatinine level. Strict dose adjustment of tacrolimus and efforts such as hydration and dose adjustment of other nephrotoxic drugs concurrently administered were made to correct renal impairments. Therefore, our study suggests that tacrolimus could be safely administered at a concentration of 10–20 ng/mL as previously reported.

In CBT recipients, especially in those who receive a reduced-intensity conditioning regimen, posttransplant immune disorders including pre-engraftment immune reactions (PIR) and hemophagocytic syndrome (HPS) are problematic. These complications are caused by hyperimmune reactions. PIR could trigger GVHD, and HPS has been considered to cause delayed engraftment or graft failure. Therefore, these factors could negatively affect transplant outcomes. Previous studies reported that intensification of GVHD prophylaxis, such as tacrolimus instead of CsA and the combination of tacrolimus and MMF, could suppress post-CBT immune reactions, including PIR, HPS, and GVHD, and decrease TRM, improving the prognosis after CBT. Additional immunosuppression such as steroids for treating moderate to severe GVHD may increase the risk of infection, especially after CBT. Intense GVHD prophylaxis probably reduces steroid use and hence, the risk of severe infection. Higher blood tacrolimus concentration could suppress severe immune reactions including GVHD and could reduce the incidence of TRM. On the other hand, intense GVHD prophylaxis might hamper the graft-versus-leukemia (GVL) effect, as they are closely associated with GVHD. Several studies have reported that higher donor-recipient HLA disparity in single-unit CBT for adult patients with leukemia decreased the risk of relapse, which translated to a survival benefit. These reports indicated that a GVL effect could be present in patients after CBT. It is possible that the concentration of tacrolimus influenced the transplant outcome differently according to disease status before the transplantation; therefore, the optimal range of blood tacrolimus concentration early after CBT should be determined by balancing for GVHD prophylaxis and the effect of GVL.

In conclusion, the blood tacrolimus concentration early after transplantation significantly affected the incidence of grade II–IV acute GVHD; the dividing line was 15 ng/mL in adult patients who underwent single-unit CBT. This report could contribute to the establishment of an optimal range of blood tacrolimus concentration early after CBT. Because this is a relatively small-sized retrospective study, the presence of uncontrolled bias cannot be excluded. Larger prospective studies are warranted to evaluate the optimal range of blood tacrolimus concentration for GVHD prophylaxis and transplant outcome in CBT recipients.

Acknowledgment

We express our gratitude to all the participants, physicians, and associated co-medical workers.
Conflict of interest disclosure

Yukio Tanizawa is a recipient of a non-restricted research grant and honorarium for the lectures from Astellas Pharma Inc. The other authors state that they have no conflict of interest.

Author contributions

Yukinori Nakamura designed the study, collected data, planned and performed statistical analyses, provided clinical care of patients, and wrote the manuscript; Yoshinori Tanaka, Mayumi Tanaka, Akiko Sugiyama, Yoshihiro Tokunaga, Yasuko Suehiro, and Koumei Takeda collected data and provided clinical care of patients; Toshiaki Yujiri and Yukio Tanizawa revised the manuscript; All authors approved the manuscript.

References

1. Ooi J. Cord blood transplantation in adults. Bone Marrow Transplant. 2009; 44: 661–666.
2. Ballen KK, Gluckman E, Broxmeyer HE. Umbilical cord blood transplantation: the first 25 years and beyond. Blood. 2013; 122: 491–498.
3. Takahashi S, Iseki T, Ooi J, et al. Single-institute comparative analysis of unrelated bone marrow transplantation and cord blood transplantation for adult patients with hematologic malignancies. Blood. 2004; 104: 3813–3820.
4. Rocha V, Labopin M, Sanz G, et al. Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia. N Engl J Med. 2004; 351: 2276–2285.
5. Laughlin MJ, Eapen M, Rubinstein P, et al. Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. N Engl J Med. 2004; 351: 2265–2275.
6. Atsuta Y, Suzuki R, Nagamura-Inoue T, et al. Disease-specific analyses of unrelated cord blood transplantation compared with unrelated bone marrow transplantation in adult patients with acute leukemia. Blood. 2009; 113: 1631–1638.
7. Eapen M, Rocha V, Sanz G, et al. Effect of graft source on unrelated donor haemopoietic stem-cell transplantation in adults with acute leukaemia: a retrospective analysis. Lancet Oncol. 2010; 11: 653–660.
8. Mori T, Aisa Y, Nakazato T, et al.Tacrolimus and methotrexate for the prophylaxis of graft-versus-host disease after unrelated donor cord blood transplantation for adult patients with hematologic malignancies. Transplant Proc. 2007; 39: 1615–1619.
9. Yamada MF, Miyamura K, Fujiwara T, et al. Myeloablative cord blood transplantation for adults with hematological malignancies using tacrolimus and short-term methotrexate for graft-versus-host disease prophylaxis: single-institution analysis. Transplant Proc. 2008; 40: 3637–3642.
10. Miyakoshi S, Kami M, Tanimoto T, et al. Tacrolimus as prophylaxis for acute graft-versus-host disease in reduced intensity cord blood transplantation for adult patients with advanced hematologic diseases. Transplantation. 2007; 84: 316–322.
11. Uchida N, Wake A, Nakano N, et al. Mycophenolate and tacrolimus for graft-versus-host disease prophylaxis for elderly after cord blood transplantation: a matched pair comparison with tacrolimus alone. Transplantation. 2011; 92: 366–371.
12. Mori T, Tanaka M, Kobayashi T, et al. Prospective multicenter study of single-unit cord blood transplantation with myeloablative conditioning for adult patients with high-risk hematologic malignancies. Biol Blood Marrow Transplant. 2013; 19: 486–491.
13. Nash RA, Antin JH, Karanes C, et al. Phase 3 study comparing methotrexate and tacrolimus with methotrexate and cyclosporine for prophylaxis of acute graft-versus-host disease after marrow transplantation from unrelated donors. Blood. 2000; 96: 2062–2068.
14. Hiraoka A, Ohashi Y, Okamoto S, et al. Phase III study comparing tacrolimus (FK506) with cyclosporine for graft-versus-host disease prophylaxis after allogeneic bone marrow transplantation. Bone Marrow Transplant. 2001; 28: 181–185.
15. Wingard JR, Nash RA, Przepiorka D, et al. Relationship of tacrolimus (FK506) whole blood concentrations and efficacy and safety after HLA-identical sibling bone marrow transplantation. Biol Blood Marrow Transplant. 1998; 4: 157–163.
16. Przepiorka D, Nash RA, Wingard JR, et al. Relationship of tacrolimus whole blood levels to efficacy and safety outcomes after unrelated donor marrow transplantation. Biol Blood Marrow Transplant. 1999; 5: 94–97.
17. Mori T, Kato J, Shimizu T, et al. Effect of early posttransplantation tacrolimus concentration on the development of acute graft-versus-host disease after allogeneic hematopoietic stem cell transplantation from unrelated donors. Biol Blood Marrow Transplant. 2012; 18: 229–234.
18. Morishima Y, Sasazuki T, Inoko H, et al. The clinical significance of human leukocyte antigen (HLA) allele compatibility in patients receiving a marrow transplant from serologically HLA-A, HLA-B, and HLA-DR matched unrelated donors. Blood. 2002; 99: 4200–4206.
19. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. Bone Marrow Transplant. 1995; 15: 825–828.
20. Gooley TA, Leisenring W, Crowley J, Storer BE. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. Stat Med. 1999; 18: 695–706.
21. Kanda Y. Investigation of the freely available easy-to-use software ‘EZR’ for medical statistics. Bone Marrow Transplant. 2013; 48: 452–458.
22. Jacobson P, Uberti J, Davis W, Ratanatharathorn V. Tacrolimus:
a new agent for the prevention of graft-versus-host disease in hematopoietic stem cell transplantation. Bone Marrow Transplant. 1998; 22: 217–225.

23. Mori T, Aisa Y, Kato J, et al. Drug interaction between voriconazole and calcineurin inhibitors in allogeneic hematopoietic stem cell transplant recipients. Bone Marrow Transplant. 2009; 44: 371–374.

24. Mori T, Aisa Y, Kato J, et al. Drug interaction between oral solution itraconazole and calcineurin inhibitors in allogeneic hematopoietic stem cell transplantation recipients: an association with bioavailability of oral solution itraconazole. Int J Hematol. 2009; 90: 103–107.

25. Takagi S, Masuoka K, Uchida N, et al. High incidence of haemophagocytic syndrome following umbilical cord blood transplantation for adults. Br J Haematol. 2009; 147: 543–553.

26. Horowitz MM, Gale RP, Sondel PM, et al. Graft-versus-leukemia reactions after bone marrow transplantation. Blood. 1990; 75: 555–562.

27. Atsuta Y, Kanda J, Takanashi M, et al. Different effects of HLA disparity on transplant outcomes after single-unit cord blood transplantation between pediatric and adult patients with leukemia. Haematologica. 2013; 98: 814–822.

28. Sanz J, Jaramillo FJ, Planelles D, et al. Impact on outcomes of human leukocyte antigen matching by allele-level typing in adults with acute myeloid leukemia undergoing umbilical cord blood transplantation. Biol Blood Marrow Transplant. 2014; 20: 106–110.