**Table 1. Characteristics of patient, grafts, and GVHD prophylaxis**

| Case no. | Age, y | Previous treatment | Interval from diagnosis to UCBT, mo | Previous transfusion times (RBCs/platelet) | Disease status at UCBT | HLA match | HLA Ab (reactive to CB) | ABO group (R/D) | TNC $\times 10^7$/kg | CD34$^+$, $\times 10^5$/kg | GVHD prophylaxis |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 70 | CSA | 3 | 11/14 | SAA | 4/6 | NT | A/A | 4.00 | 1.23 | CSA |
| 2 | 20 | ATG + CSA | 78 | > 20/> 20 | VSAA | 4/6 | NT | B/O | 2.65 | 1.07 | CSA |
| 3 | 22 | ATG + CSA, PSL | 157 | > 20/> 20 | SAA | 4/6 | NT | A/O | 2.26 | 0.27 | Tac |
| 4 | 26 | ATG + CSA | 3 | > 20/> 20 | VSAA | 5/6 | NT | A/A | 2.65 | 0.70 | Tac |
| 5 | 59 | ATG + CSA | 8 | > 20/> 20 | SAA | 5/6 | Positive (no) | O/O | 2.15 | 1.52 | Tac + MMF |
| 6 | 49 | ATG + CSA, PSL | 12 | > 20/> 20 | VSAA | 3/6 | NT | A/A | 2.04 | 0.62 | Tac + MMF |
| 7 | 70 | None | 1 | 5/8 | Fulminant | 4/6 | Positive (yes) | A/O | 4.39 | 1.29 | Tac + MMF |
| 8 | 52 | None | 1 | 4/6 | Fulminant | 4/6 | NT | AB/A | 3.20 | 0.49 | Tac + MMF |
| 9 | 46 | ATG + CSA | 45 | > 20/> 20 | VSAA | 4/6 | Positive (no) | AB/O | 1.83 | 0.42 | Tac + MMF |
| 10 | 49 | ATG + CSA, PSL | 327 | > 20/> 20 | VSAA | 6/6 | Positive (no) | B/O | 2.34 | 0.82 | Tac + MMF |
| 11 | 65 | CSA | 6 | 16/> 20 | VSAA | 6/6 | Positive (no) | A/A | 3.31 | 0.56 | Tac + MMF |
| 12 | 31 | ATG + CSA, PSL | 215 | > 20/> 20 | SAA | 4/6 | Positive (no) | B/O | 2.09 | 1.26 | Tac + MMF |

RBC indicates red blood cell; CB, cord blood; R, recipient; D, donor; TNC, total nucleated cells; CSA, cyclosporine-A; ATG, antithymocyte globulin; PSL, prednisone; VSAA, very severe aplastic anemia; NT, not tested; Tac, tacrolimus; and MMF, mycophenolate mofetil.

42 days (range, 26-64 days), respectively. All patients who achieved engraftment had complete hematologic recovery and were free from transfusion, and they showed complete donor chimerism at the time of the first chimerism analysis (median, 14 days; range, 11-73 days). One patient developed primary GF and was later found to have antibody against mismatched HLA on donor cells. Another patient developed secondary GF 3 years after UCBT. Both patients underwent a second RI-UCBT and obtained rapid donor engraftment. The negative impact of multiple transfusions before transplantation was not detected (Tables 1-2). Among 11 evaluable patients, 2 developed grade I and 5 developed grade II acute GVHD. Of the 9 patients who survived longer than 100 days after transplantation, 3 developed limited type of chronic GVHD. No patients developed grade III-IV acute GVHD and extensive type of chronic GVHD. Two of the 12 patients died of idiopathic pneumonia syndrome, and the remaining 10 patients are alive, having survived for a median of 36 months (range, 14-91 months). The probability of overall survival at 3 years was 83.3% (Figure 1). The surviving patients had high Karnofsky performance status score with a median of 90% (range, 60%-100%).

The present study demonstrated that our RI conditioning regimen allows a sufficient sustained engraftment of UCB in adult SAA patients. The RI conditioning regimen was originally developed in our institute for UCBT for various hematologic malignancies.[9] Eleven of the 12 patients achieved primary engraftment, which compares favorably with previously reported engraftment rates of UCBT for SAA.[11-16] Our RI conditioning regimen would be more potent than the others to overcome immunologic barriers for engraftment. Cell dose has been known to significantly influence the rate of engraftment after UCBT.[14] In the present study, although the cell dose was not very large, sufficient engraftment was seen. Any significant relationship between cell dose (total nucleated cell, $\geq 2.5$ vs $< 2.5 \times 10^7$/kg; CD34$^+$, $\geq 0.8$ vs $< 0.8 \times 10^5$/kg) and engraftment kinetics were observed (data not shown). Thus, not just cell dose but other factors, such as the intensity of the conditioning regimen and posttransplantation immunosuppression, may be important to achieve better engraftment after UCBT for SAA patients. Interestingly, all 6 patients who were screened for HLA antibodies before transplantation had HLA antibodies, and the one case who had positive HLA antibodies against an HLA on a transplanted UCB unit was the only one who failed primary engraftment. Recently, Takanashi et al reported that, in large number of UCBT for various hematologic malignancies, the

**Table 2. Outcomes of 12 patients after reduced-intensity unrelated cord blood transplantation**

| Case no. | Days to ANC > 0.5 $\times 10^9$/L | Days to PC > 20 $\times 10^9$/L | % Donor chimerism (days tested, methods) | aGVHD | cGVHD | Discontinuation of IS (mo) | Complications | Survival (mo) |
|---|---|---|---|---|---|---|---|---|
| 1 | 12 | 52 | 100 (14, FISH) | Grade II (skin) | No | Yes (3) | Possible IPA | Alive (91) |
| 2 | 20 | 64 | > 90 (49, PCR-STR) | Grade II (skin) | Limited | Yes (2) | No | Alive (90) |
| 3 | 26 | 42 | 100 (26, FISH) | No | No | Yes (26) | Yes | Alive (69) |
| 4 | 18 | 53 | 100 (18, FISH) | No | No | Yes (5) | *Pneumocystis jirovecii*, late GF, rescued by second RI-UCBT | Alive (69) |
| 5 | 16 | 26 | 96.6 (14, FISH) | Grade I (skin) | Limited | Yes (14) | Norwalk virus colitis, EBV-PTLD | Alive (39) |
| 6 | 28 | 64 | 99.6 (11, FISH) | No | NE | No | IPS | Dead; IPS (3) |
| 7 | No | No | 48.8 (10, FISH), 4.3 (15, FISH) | NE | NE | NE | Primary GF, rescued by second RI-UCBT | Alive (32) |
| 8 | 18 | 28 | 99.2 (13, FISH) | Grade II (skin, gut) | No | Yes (7) | CMV colitis, EBV-PTLD | Alive (28) |
| 9 | 28 | 43 | > 90 (14, PCR-STR) | Grade I (skin) | NE | No | HSV pneumonia, IPS | Dead; IPS (3) |
| 10 | 15 | 27 | 99 (73, FISH) | No | Limited | No | No | Alive (22) |
| 11 | 15 | 27 | 100 (20, FISH) | Grade II (skin, gut) | No | No | No | Alive (22) |
| 12 | 13 | 28 | 100 (14, FISH) | Grade II (gut) | No | No | No | Alive (14) |

ANC indicates absolute neutrophil count; PC, platelet count; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; IS, immunosuppressant; FISH, fluorescence in situ hybridization; PCR-STR, PCR of short tandem repeat; NE, not evaluable; IPA, invasive pulmonary aspergillosis; EBV-PTLD, Epstein-Barr virus–associated posttransplantation lymphoproliferative disorder; and IPS, idiopathic pneumonia syndrome.

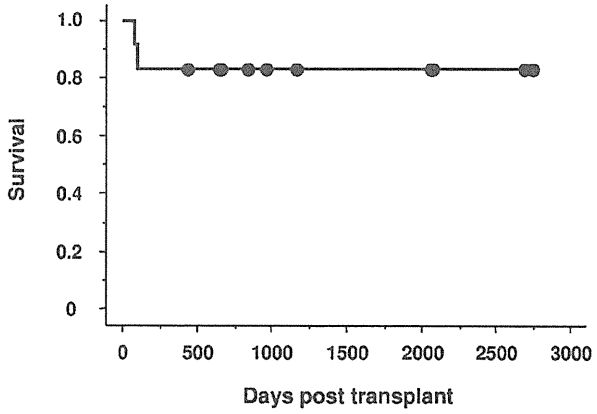


**Figure 1. Survival of 12 patients with SAA undergoing unrelated cord blood transplantation.**

patients with anti-HLA antibodies, when the specificity corresponding to mismatched antigen in UCB graft, showed significantly lower neutrophil or platelet recovery than those with antibody-negative or -positive but not corresponding to UCB graft.[17] Although the observations may differ from that of diverse populations and warrants further investigation, if possible, the use of a UCB unit with corresponding HLA antibodies in the recipient should be avoided.

Three-year survival in the studied patients was 83.3%. In addition to high rate of engraftment, the low risk of severe GVHD might contribute to high survival rate with good quality of life, and seems to be one of the important advantages of using a UCB unit for SAA patients. The other advantage of the use of UCB units is rapid availability. In the present study, 2 patients with fulminant type could be rescued by urgent hematopoietic stem cell transplantation using UCB units. More than 90% of recipients can find a suitable UCB unit in Japan; thus, UCB expands the chance to receive transplantation for those who need it urgently.

In conclusion, this retrospective study strongly suggests the feasibility and effectiveness of RI-UCBT for adult SAA patients. RI-UCBT may become a viable therapeutic option for those who lack suitable HLA-matched donors and who fail or relapse after immunosuppressive therapy. Although our results should be interpreted with caution because of the small number of patients and still short follow-up duration, we think that RI-UCBT with the conditioning regimen presented here deserves further evaluation in a prospective trial, hopefully in a multicenter setting.

## Acknowledgments

The authors thank data coordinators Kaori Kobayashi, Madoka Narita, Rumiko Tsuchihashi, and Naomi Yamada for their invaluable assistance as well as the physicians, nurses, pharmacists, and support personnel for their care of patients in this study.

This work was supported in part by the Japanese Ministry of Health, Labor, and Welfare (Research Grant for Allergic Disease and Immunology H20-015).

## Authorship

Contribution: H.Y. and D.K. performed transplantation, analyzed extracted data, and contributed to writing the paper; A.Y. reviewed histopathologic sections; H.Y. and N.M. performed statistical analysis; N.U., K. Izutsu, and S. Taniguchi reviewed study design and methods; and K. Ishiwata, H.A., S. Takagi, M.T., N.N., Y.A.-M., K.M., A.W., and S.M. performed transplantation and contributed to writing the paper.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Naoyuki Uchida, 2-2-2 Toranomon, Minato-ku, Tokyo 105-8470, Japan; e-mail: nuchida@toranomon.gr.jp.

## References

1. Marsh J. Making therapeutic decisions in adults with aplastic anemia. *Hematology Am Soc Hematol Educ Program.* 2006:78-85.
2. Marsh JC, Ball SE, Cavenagh J, et al. Guidelines for the diagnosis and management of aplastic anaemia. *Br J Haematol.* 2009;147(1):43-70.
3. Passweg JR, Perez WS, Eapen M, et al. Bone marrow transplants from mismatched related and unrelated donors for severe aplastic anemia. *Bone Marrow Transplant.* 2006;37(7):641-649.
4. Brunstein CG, Setubal DC, Wagner JE. Expanding the role of umbilical cord blood transplantation. *Br J Haematol.* 2007;137(1):20-35.
5. Kusumi E, Miyakoshi S, Murashige N, et al. Successful reduced-intensity stem cell transplantation (RIST) with mismatched cord blood in a 70-year-old patient with severe aplastic anemia (SAA). *Bone Marrow Transplant.* 2003;32(11):1111-1112.
6. Camitta BM, Thomas ED, Nathan DG, et al. Severe aplastic anemia: a prospective study of the effect of early marrow transplantation on acute mortality. *Blood.* 1976;48(1):63-70.
7. Pei R, Wang G, Tarsitani C, et al. Simultaneous HLA Class I and Class II antibodies screening with flow cytometry. *Hum Immunol.* 1998;59(5):313-322.
8. Takanashi M, Fujiwara K, Tanaka H, Satake M, Nakajima K. The impact of HLA antibodies on engraftment of unrelated cord blood transplants. *Transfusion.* 2008;48(4):791-793.
9. Miyakoshi S, Yuji K, Kami M, et al. Successful engraftment after reduced-intensity umbilical cord blood transplantation for adult patients with advanced hematological diseases. *Clin Cancer Res.* 2004;10(11):3586-3592.
10. Uchida N, Wake A, Takagi S, et al. Umbilical cord blood transplantation after reduced-intensity conditioning for elderly patients with hematologic diseases. *Biol Blood Marrow Transplant.* 2008;14(5):583-590.
11. Yoshimi A, Kojima S, Taniguchi S, et al. Unrelated cord blood transplantation for severe aplastic anemia. *Biol Blood Marrow Transplant.* 2008;14(9):1057-1063.
12. Mao P, Zhu Z, Wang H, et al. Sustained and stable hematopoietic donor-recipient mixed chimerism after unrelated cord blood transplantation for adult patients with severe aplastic anemia. *Eur J Haematol.* 2005;75(5):430-435.
13. Chan KW, McDonald L, Lim D, Grimley MS, Grayson G, Wall DA. Unrelated cord blood transplantation in children with idiopathic severe aplastic anemia. *Bone Marrow Transplant.* 2008;42(9):589-595.
14. Rubinstein P, Carrier C, Scaradavou A, et al. Outcomes among 562 recipients of placental-blood transplants from unrelated donors. *N Engl J Med.* 1998;339(22):1565-1577.
15. Lau FY, Wong R, Chui CH, Cheng G. Successful engraftment in two adult patients with severe aplastic anemia using nonmyeloablative conditioning followed by unrelated HLA-mismatched cord blood transplantation. *J Hematother Stem Cell Res.* 2001;10(2):309-311.
16. Schwinger W, Urban C, Lackner H, et al. Transplantation of related and unrelated umbilical cord blood stem cells in Austria: Austrian Working Party for Stem Cell Transplantation. Austrian Society of Hematology and Oncology. *Wien Klin Wochenschr.* 1999;111(9):348-353.
17. Takanashi M, Atsuta Y, Fujiwara K, et al. The impact of anti-HLA antibodies on unrelated cord blood transplantations. *Blood.* 2010;116(15):2839-2846.

# Mycophenolate and Tacrolimus for Graft-Versus-Host Disease Prophylaxis for Elderly After Cord Blood Transplantation: A Matched Pair Comparison With Tacrolimus Alone

*Naoyuki Uchida,[1,5] Atsushi Wake,[1] Nobuaki Nakano,[1] Kazuya Ishiwata,[1] Shinsuke Takagi,[1] Masanori Tsuji,[1] Hisashi Yamamoto,[1] Daisuke Kato,[1] Naofumi Matsuno,[1] Kazuhiro Masuoka,[1] Hideki Araoka, MD,[2] Yuki Asano-Mori,[1] Koji Izutsu,[1] Shigeyoshi Makino,[3] Akiko Yoneyama,[2] and Shuichi Taniguchi[1,4]*

**Background.** The optimal graft-versus-host disease (GVHD) prophylaxis after umbilical cord blood transplantation has not been established. Our previous observation using single calcineurin inhibitors revealed a high incidence and severity of early immune-mediated complications, especially for older patients or those with poor performance status.
**Methods.** We conducted a single institute pilot study assessing the safety and effectiveness of mycophenolate mofetil (MMF) and tacrolimus (FK) combination as a GVHD prophylaxis for 29 patients (FK+MMF), and the results were compared with matched-pairs extracted from our historical database who received FK alone as GVHD prophylaxis (control).
**Results.** FK+MMF group showed superior engraftment rate compared with control group (cumulative incidence until day 60 posttransplant; 90%±0% vs. 69%±1%, $P=0.02$). A cumulative incidence of severe type preengraftment immune reactions was significantly decreased in FK+MMF group (16%±1%) compared with that of control group (52%±2%, $P=0.03$), and, remarkably, there was no nonrelapse mortality (NRM) observed up to day 30 posttransplant in FK+MMF group, whereas 21%±1% of NRM was observed in the control group. However, the incidences of acute and chronic GVHD, estimated overall and progression-free survivals were comparable between two groups.
**Conclusions.** MMF and FK in combination was well tolerated and decreased early NRM possibly by better control of preengraftment immune reactions. Subsequent NRM or disease progression needs to be overcome to further improve survival.

**Keywords:** Cord blood transplantation, GVHD prophylaxis, Mycophenolate mofetil, Tacrolimus, Elderly patients.

(*Transplantation* 2011;92: 366–371)

Although umbilical cord blood transplantation (UCBT) has been increasingly used as a curative treatment of hematological diseases, accompanying toxicity, especially early period posttransplant, has been a major problem (*1, 2*). Our previous observation indicated that elderly patients were more vulnerable to early toxicity posttransplant, with nonrelapse mortality (NRM) being a major cause of treatment failure (*3*). Early immune-mediated complications, termed preengraftment immune reactions (PIR), were significant factors that negatively affected overall survival (OS) (*3–5*).

Various immunosuppressive drugs have been used for graft-versus-host disease (GVHD) prophylaxis in UCBT, including mycophenolate mofetil (MMF), (*6–8*) methotrexate (MTX), (*9–11*) corticosteroids, (*11*) anti-thymocyte globulin, (*12, 13*), and sirolimus (*14*); mostly in combination with calcineurin inhibitors. So far, no available data indicate that one drug or combination is better than the other.

MMF is an inosine monophosphate dehydrogenase inhibitor that exerts its immunosuppressive effect by blocking the production of guanosine nucleotide synthesis through the

This work was supported by the Japanese Ministry of Health, Labor and Welfare grant H22-032.
The authors declare no competing financial interests.
[1] Department of Hematology, Toranomon Hospital, Minato-Ku, Tokyo, Japan.
[2] Department of Infectious Diseases, Toranomon Hospital, Minato-Ku, Tokyo, Japan.
[3] Department of Transfusion Medicine, Toranomon Hospital, Minato-Ku, Tokyo, Japan.
[4] Okinaka Memorial Institute for Medical Research, Minato-Ku, Tokyo, Japan.
[5] Address correspondence to: Naoyuki Uchida, M.D., Ph.D., Department of Hematology, Toranomon Hospital, 2-2-2 Toranomon, Minato-Ku, Tokyo 105-8470, Japan.
E-mail: nuchida@toranomon.gr.jp
N.U. performed research design, data analysis, and writing the manuscript; A.W., N.N., K.I., S.T., M.T., H.Y., D.K., N.M., K.M., H.A., Y.A.M., K.I., S.M., and A.Y. participated in the performance of the research; A.W. and S.T. contributed with data analysis and writing of the manuscript; and all authors reviewed the manuscript.

Received 1 March 2011. Revision requested 9 March 2011.
Accepted 10 May 2011.

ISSN 0041-1337/11/9203-366
DOI: 10.1097/TP.0b013e318223d7ac

de novo pathway (*15*). It has been extensively used in solid organ transplantations (*16*) and more recently, in hematopoietic cell transplantation (HCT) (*7, 17–19*). In HCT, less mucosal damage compared with MTX has been observed, (*19–21*) with a comparable incidence of GVHD, suggesting a potential advantage of MMF over MTX. It therefore seemed rational to incorporate MMF in reduced-intensity (RI) UCBT for patients at high risk for NRM. Since December 2005, MMF+tacrolimus (FK) combination was started to be used as GVHD prophylaxis in RI-UCBT as a pilot study for those who agreed to participate. The results were compared with that of those who performed RI-UCBT using FK alone extracted as matched pairs from our historical database.

## RESULTS

### Patients/Matched Controls

Table 1 shows the demography of the patient characteristics of two groups. A total of 89% of the control patients who had GVHD prophylaxis of FK alone were transplanted from 2004 to 2005, whereas 93% of the patients with FK+MMF were from 2006 to 2007 ($P<.0001$). The differences between the groups did not reach statistical significance in Eastern Cooperative Oncology Group (ECOG) performance status (PS), HCT-specific comorbidity index (HCT-CI) score, history of previous HCT, human leukocyte antigen (HLA) disparity to UCB, and conditioning regimen. The median FK concentrations (11.9±0.33 ng/mL in FK+MMF group vs. 12.6±0.47 ng/mL in control group, $P=0.46$) and the proportions of FK concentration more than or equal to 10 ng/mL during day 0 to the date of engraftment (72.4%±3.1% in FK+MMF group vs. 75.0%±4.0% in control group, $P=0.43$) were comparable in each group.

### Engraftment

Twenty-seven patients in FK+MMF group achieved neutrophil engraftment, and all except 1 showed complete donor chimerism. The cumulative incidence of primary engraftment until day 60 posttransplant was 90%±0%, whereas that of control group was 69%±1% ($P=0.02$). Median time to engraftment was 19 days after transplantation both in FK+MMF group (range, 13–32 days) and control group (range, 12–33 days). Among the two patients in FK+MMF group who failed to engraft, one experienced disease recurrence before day 28, and the other experienced rejection of donor cells and was later found to have anti-HLA antibodies against one of the antigens expressed on donor cells. One patient in FK+MMF group who showed mixed chimerism on neutrophil engraftment, when 87.2% of total bone marrow (BM) cells were of donor origin, experienced early BM relapse of leukemia on day 30 posttransplant. There were three patients in control group who experienced hemophagocytic syndrome (HPS) early after transplant and resulted in early death before engraftment, whereas there was no such cases observed in FK+MMF group. Platelet recovery more than $20\times10^9$/L was observed in 17 patients, with a cumulative incidence of 59%±1% at day 100 posttransplant (median, 40 days; range, 25–70 days), whereas in control group, the cumulative incidence was 52%±1% (median, 40 days; range, 26–62 days, $P=0.69$).

**TABLE 1.** Patient, treatment, and donor umbilical cord blood characteristics

| Characteristic | N (%) of patients FK+MMF | Control | P |
|---|---|---|---|
| Sex | | | 0.38 |
| Male | 21 (72) | 23 (79) | |
| Female | 8 (28) | 6 (21) | |
| Age (yr) | | | 0.67 |
| Median (range) | 62 (52–70) | 63 (56–69) | |
| Age distribution (yr) | | | |
| 51–55 | 5 (17) | 0 | |
| 56–60 | 4 (14) | 9 (31) | |
| 61–65 | 12 (41) | 13 (45) | |
| 66–70 | 8 (28) | 7 (24) | |
| Diagnosis | | | 0.11 |
| AML/MDS | 19 (66) | 16 (55) | |
| ALL | 2 (7) | 5 (17) | |
| ML | 5 (17) | 5 (17) | |
| CML | 0 | 3 (10) | |
| AA | 3 (10) | 0 | |
| ECOG performance status | | | 0.37 |
| 0 | 0 | 0 | |
| 1 | 22 (76) | 17 (59) | |
| 2 | 5 (17) | 9 (31) | |
| 3 | 2 (7) | 3 (10) | |
| HCT-CI | | | 0.25 |
| 0 | 9 (31) | 18 (62) | |
| 1 | 12 (41) | 7 (24) | |
| 2 | 1 (3) | 1 (3) | |
| ≥3 | 7 (24) | 3 (10) | |
| Disease status | | | 0.78 |
| Standard risk | 10 (34) | 9 (31) | |
| High risk | 19 (66) | 20 (69) | |
| History of prior HCT | | | 0.16 |
| None | 22 (76) | 26 (90) | |
| Autologous | 4 (14) | 3 (10) | |
| Allogeneic | 3 (10) | 0 | |
| Year of transplant | | | <0.0001 |
| 2004 | 0 | 11 (38) | |
| 2005 | 2 (7) | 12 (41) | |
| 2006 | 7 (24) | 6 (21) | |
| 2007 | 20 (69) | 4 (14) | |
| Conditioning regimen[a] | | | |
| Flu/Mel 140 | 8 (28) | 1 (3) | |
| Flu/Mel 80-140/TBI 2-8 | 13 (45) | 25 (86) | |
| Flu/Mel 80/Tespa 10 | 0 | 1 (3) | |
| Flu/Mel 80-140/Bu 8-16 | 4 (14) | 0 | |
| Flu/Bu 16 | 0 | 1 (3) | |
| Flu/Bu 8-16/TBI 2-4 | 3 (10) | 1 (3) | |
| Flu/Bu 8/VP-16 450 | 1 (3) | 0 | |
| HLA disparity to UCB | | | 0.22 |
| 0 antigen mismatch | 1 (3) | 1 (3) | |
| 1 antigen mismatch | 5 (17) | 1 (3) | |
| 2 antigen mismatch | 23 (79) | 27 (93) | |
| Total nucleated cell number | | | 0.66 |
| Median ($\times10^7$/kg) | 2.4 | 2.31 | |
| Range ($\times10^7$/kg) | 2.0–4.5 | 1.91–4.76 | |
| $CD34^+$ cell number | | | 0.15 |
| Median ($\times10^5$/kg) | 0.9 | 0.81 | |
| Range ($\times10^5$/kg) | 0.11–2.32 | 0.11–1.9 | |

[a] Units for each number are as follows: Mel (mg/m$^2$), TBI (Gy), Tespa (mg/kg), Bu doses: oral (1 dose=1 mg/kg) or iv (1 dose=0.8 mg/kg), and VP-16 (mg/m$^2$).

AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukemia; ML, malignant lymphoma; CML, chronic myeloid leukemia; AA, aplastic anemia; HCT-CI, hematopoietic cell transplantation-specific comorbidity index; Flu, fludarabine; Mel, melphalan; TBI, total body irradiation; Bu, busulfan; VP-16, etoposide; and UCB, umbilical cord blood; FK, tacrolimus; MMF, mycophenolate mofetil; ECOG, Eastern Cooperative Oncology Group; HLA, human leukocyte antigen.

**TABLE 2.** Incidence of PIR and GVHD

| | FK+MMF (N) | Control (N) |
|---|---|---|
| PIR (n=29) | | |
| No. of evaluable[a] | 29 | 28 |
| Yes | 22 | 23 |
| Severe type | 4 | 10 |
| Acute GVHD | | |
| No. of evaluable[b] | 27 | 20 |
| Grade I | 4 | 4 |
| Grade II | 7 | 2 |
| Grade III | 7 | 5 |
| Grade IV | 4 | 3 |
| Chronic GVHD | | |
| No. of evaluable[c] | 13 | 11 |
| Limited | 1 | 2 |
| Extensive | 1 | 2 |

[a] Those who showed clinical symptoms characteristic to PIR, and those who survived longer than 27 d posttransplant without PIR.

[b] Those who engrafted without disease progression.

[c] Those who survived beyond day 100 posttransplant without disease progression.

PIR, preengraftment immune reactions; GVHD, graft-versus-host disease; FK, tacrolimus; MMF, mycophenolate mofetil.

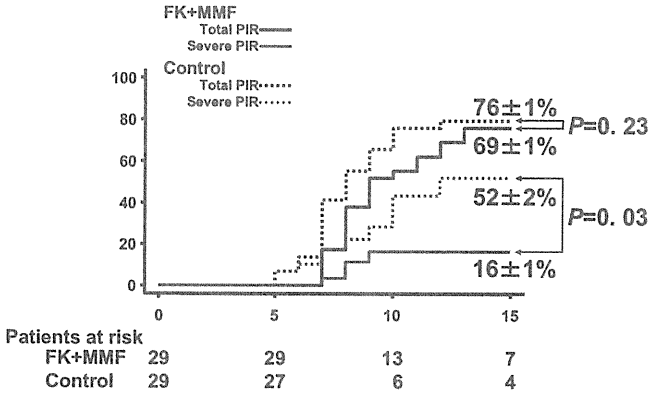


**FIGURE 1.** Cumulative incidences of preengraftment immune reactions (PIR) after RI-UCBT according to tacrolimus (FK)+mycophenolate mofetil (MMF) or FK alone graft-versus-host disease (GVHD) prophylaxis. The overall incidences of PIR in FK+MMF group (*black solid line*), in control group (*black dotted line*), and the incidences of severe type of PIR in FK+MMF group (*gray solid line*), and in control group (*gray dotted line*) were plotted. There was significant reduction of severe type of PIR in FK+MMF group compared with that in control group ($P$=0.03).

## PIR and GVHD

In FK+MMF group, 22 of 29 patients experienced clinical symptoms defined as PIR, whereas in control group, 23 of 28 evaluable patients did (Table 2). Cumulative incidences of PIR in both groups were comparable each other (76%±1% in control group and 69%±1% in FK+MMF group, $P$=0.23, Fig. 1) and were similar to that reported in our previous publication (*3*). However, the cumulative incidence of severe type of PIR, defined by the criteria described in materials and methods section, in the FK+MMF group was lower (16%±1%) than that of control group (52%±2%) with statistical significance ($P$=0.03, Fig. 1).

In FK+MMF group, 22 of 27 evaluable patients developed acute GVHD, and 18 of them were grade II and higher. In control group, 14 of 20 evaluable patients had acute GVHD, and 10 of them were grade II and higher (Table 2). Cumulative incidences of grade II and higher acute GVHD at day 100 posttransplant were 63%±1% in FK+MMF and 35%±1% in control group ($P$=0.09). Chronic GVHD was observed in two of 13 FK+MMF group and four of 11 control group patients who survived longer than 100 days posttransplant without disease progression (Table 2). Cumulative incidences of chronic GVHD at 2 years posttransplant were 7%±0% in FK+MMF and 16%±1% in control group ($P$=0.35).

**TABLE 3.** Causes of death

| | FK+MMF, N (%) | Control, N (%) |
|---|---|---|
| NRM | 9 (45) | 11 (65) |
| GVHD | 5 (25) | 3 (18) |
| IPS | 4 (20) | 1 (6) |
| Infection | 0 | 5 (29) |
| CNS complication | 0 | 2 (12) |
| Relapse/disease progression | 11 (55) | 6 (35) |
| Total | 20 | 17 |

FK, tacrolimus; MMF, mycophenolate mofetil; NRM, nonrelapse mortality; GVHD, graft-versus-host disease; IPS, idiopathic pneumonia syndrome; CNS, central nervous system.

## Survival, Disease Progression, and NRM

At the time of analysis, 9 FK+MMF group patients survived for a median of 980 days (range, 145–1430 days) after transplantation, whereas 12 control group patients were alive for a median of 1073 days (range, 49–2071 days). The Kaplan-Meier estimates of OS and progression-free survival (PFS) at 2 year posttransplant in FK+MMF group were 33%±9% and 21%±8%, whereas those in control group were 45%±10% and 34%±9%, respectively. The differences were not statistically significant ($P$=0.83 for OS, and $P$=0.75 for PFS).

Thirteen patients in FK+MMF group showed progression of the underlying disease at a median of 84 days (range, 19–344 days) after transplantation, and 11 of these patients died of the disease (Table 3). In control group, 9 patients did so at a median of 126 days (range, 12–1084 days) and 6 died of the disease. The cumulative incidences of disease progression at 2 years were 46%±1% in FK+MMF group and 29%±1% in control group, respectively ($P$=0.29).

Nine in FK+MMF group died of nonrelapse causes, whereas in control group patients, 11 NRM were observed (Table 3). GVHD and noninfectious pulmonary complications were observed in both groups as cause of death. None of the FK+MMF group died from infections as a sole reason of death, whereas five of the control group did. There was no death before day 30 posttransplant in FK+MMF group, whereas six in control group did. The cumulative incidences of NRM at day 30, 100, 365 were 0%±0%, 21%±1%, 28%±1% in FK+MMF group, and 21%±1%, 35%±1%,

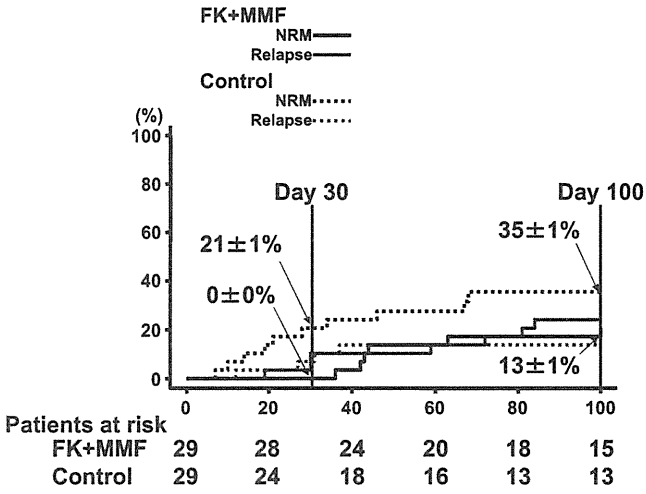


**FIGURE 2.** Day 100 nonrelapse mortality (NRM) and disease progression. Cumulative incidence estimates of NRM (*black line*) and disease progression (*gray line*) up to day 100 posttransplant for tacrolimus (FK)+mycophenolate mofetil (MMF) group (*solid line*) and control group (*dotted line*) were plotted. There were no NRM within 30 days posttransplant in FK+MMF group, whereas 21%±1% NRM were estimated in control group ($P$=0.01).

39%±1% in control group, respectively ($P$=0.01, $P$=0.17, $P$=0.29, Fig. 2).

## DISCUSSION

The most remarkable observation in this study was that higher rate of neutrophil recovery and no early deaths before day 30 posttransplant were observed in FK+MMF group despite the patients' poor conditions before transplant, that is, all were older than 50 years and 69% of them had some comorbidities. Although the incidence of PIR in FK+MMF group was comparable with control group, the severity of PIR was less and thus did not result in severe organ damage early after transplant. There was no death directly caused by infections in FK+MMF group. We have reported higher incidence of HPS after RI-UCBT, which has been reasoned to be the delayed engraftment or graft failure (*22*). Interestingly, majority of the suffered BM cells were donor cell dominant, indicating HPS was mediated by donor-derived immune cells. Moreover, we have reported HLA mismatch in GVH direction, not in host-versus-graft direction, affected negatively to successful engraftment (*23*). All these facts fit well to the idea that hyperimmune reactions caused by donor cord blood (CB) cells may play crucial role in high rate of early NRM. Because there were no case of HPS in FK+MMF group, MMF may have promoted engraftment by sufficiently suppressing immune reactions of CB cells and preventing development of hemophagocytosis, which may also have reduced the incidence of severe infections. The presence of this type of hyperimmune reactions after UCBT has recently been recognized by others (*24*). The differences in incidence of PIR may have been affected by agents included in pretransplant conditioning, such as antithymocyte globulin, or by GVHD prophylaxis including corticosteroids or intravenous MMF.

Despite the present observation that the combination of MMF and FK succeeded in reducing early NRM, the incidence and severity of GVHD was not altered. Because most of the patients in the present study had advanced disease status, MMF was discontinued or started to be tapered on the day of neutrophil engraftment, which may have been responsible for this results. Much longer administration of MMF has been used in the setting of matched unrelated BM/peripheral blood (PB) transplantation (*7*). In addition, MMF was administered at 15 mg/kg twice daily in this study, which is the common dosing schedule in the settings of solid organ transplant (*16*). Several recent reports from Minnesota and Seattle considered 15 mg/kg three times daily as more appropriate based on pharmacokinetic data obtained from HCT recipients (*7, 17, 25*). A serum concentration measurement of mycophenolic acid, which was not assessed in this study, is needed to determine the optimal dosing of MMF.

Although NRM early after UCBT was significantly reduced in FK+MMF group, OS and PFS at 2 year posttransplant were still comparable with those of control group. Fifty-five percent of the deaths were from disease relapse or progression. Although MMF may have a beneficial effect on early survival after transplant by reducing severe immune reactions, it may increase the risk of disease progression for those who have active disease with a high risk of disease recurrence. According to previous publications, relapse rate is comparable in CB and unrelated BM/PB recipients despite lower incidences of chronic GVHD in CB recipients (*26, 27*), early immune reactions may have impact on reducing disease relapse. Because this is a relatively small sized, retrospective study, the presence of uncontrolled bias cannot be excluded. Prospectively conducted larger studies are warranted to further confirm the results.

In conclusion, MMF, used in combination with FK as GHVD prophylaxis in elderly patients with advanced hematologic diseases with or without comorbidities, may reduce early mortality posttransplant by regulating severe PIR and thus protecting patients from severe organ damage or HPS. An optimal dosing schedule of MMF needs to be determined prospectively using more homogenous populations.

## MATERIALS AND METHODS

### Patients

The initial pilot study included patients aged 51 years and older who underwent RI-UCBT using MMF+FK combination as GVHD prophylaxis at our institute from December 2005 through December 2007. Patients were eligible for this study if they had any hematologic malignancies at high risk for relapse or severe aplastic anemia refractory to standard immunosuppressive therapy and were unable to find suitable related or unrelated BM/PB donors within reasonable periods relative to their disease conditions. Patients with acute leukemia could be at first remission but at high risk for relapse due to adverse cytogenetic abnormalities, have a previous hematologic disorder, or be at any status beyond first remission. Patients with myelodysplastic syndrome (MDS) had to be refractory anemia (RA) with excess of blasts or chronic myelomonocytic leukemia, or have RA with transfusion dependency or severe neutropenia. Malignant lymphoma (ML) patients had to be beyond first remission. Patients who had end-stage cardiac dysfunction (left ventricular ejection fraction <35%), pulmonary dysfunction ($SpO_2$ <90% in room air), or active serious infection at the time of transplantation were not eligible. All patients gave written informed consent. Twenty-nine patients were enrolled and subjected to the matched pair analysis as below.

## Selection of Matched Controls and Matching Variables

A matched-pair control group (GVHD prophylaxis with FK alone) for 29 patients who used MMF+FK combination was obtained by selecting one of the most recently transplanted control patients from our historical RICBT database from 2004 to 2007 after excluding those who met exclusion criteria of the pilot study described earlier. Controls were individually matched to cases on a 1:1 ratio. Matching was attempted for the following criteria applied in the order listed: age at transplantation (51–60, 61–70 years), disease risk (standard risk vs. high risk, acute leukemia, chronic myeloid leukemia, or ML in complete remission, MDS RA, aplastic anemia patients were categorized as standard risk, and all the others were as high risk), ECOG PS (PS 0–1, 2–3), pretransplant conditioning (busulfan containing vs. others), number of serological HLA mismatch (0–1, 2), HCT-CI (0–1, ≥2), total nucleated cell dose infused (≤2.3, >$2.3\times10^7$/kg), and $CD34^+$ cell dose infused (≤0.8, >$0.8\times10^5$/kg). To avoid any potential selection bias, matching was blinded, and only the patient's initials and pretreatment variables were known. This retrospective analysis was approved by the institutional review board.

One hundred percent matching was achieved for age group; 97% for disease risk (high risk, 66% of FK+MMF patients vs. 69% of control patients; $P=0.78$); 83% for ECOG PS (≥2 score, 32% of FK+MMF patients vs. 41% of FK alone patients; $P=0.16$); 72% for HCT-CI (≥2 score, 28% of FK+MMF patients vs. 14% of control patients; $P=0.19$); and number of serological HLA mismatch (2 antigens, 79% of FK+MMF patients vs. 93% of control patients; $P=0.13$); 86% for pretransplant conditioning (inclusion of busulfan, 24% of FK+MMF patients vs. 7% of control patients; $P=0.07$); 69% for total nucleated cell dose (≤$2.3\times10^7$/kg, 41% of FK+MMF patients vs. 45% of control patients; $P=0.79$); and 62% for $CD34^+$ cell dose (≤$0.8\times10^5$/kg, 45% of FK+MMF patients vs. 48% of control patients; $P=0.79$). Characteristics of the studied patients in both groups were shown in Table 1. Patients' comorbidity was assessed by a previously reported scoring system (*28*).

## Donor Selection

UCB units were obtained from the Japanese Cord Blood Bank Network. HLA-A, -B, and -DR antigens were identified by serologic typing. UCB grafts had at least four of six HLA-A, -B, and -DR antigens that were matched to the recipient and had a cryopreserved cell dose of at least $1.9\times10^7$ nucleated cells per kg of recipient body weight.

## Conditioning Regimens and Postgrafting Immunosuppression

Pretransplant conditionings were primarily RI regimens including 125 to 180 mg/m$^2$ of fludarabine (25 mg/m$^2$ for 5 days or 30 mg/m$^2$ for 6 days). Antithymocyte globulin was not incorporated. Granulocyte colony-stimulating factor (G-CSF) was started on day 1 posttransplant. Detailed information is shown in Table 1. Immunosuppressive therapy with FK (0.03 mg/kg continuous infusion, aiming for 12 to 17 ng/mL by at least three times a week measurement) with or without MMF (15 mg/kg twice daily) were started on day −1. MMF was discontinued or started to taper down on the day of neutrophil engraftment in the absence of active GVHD.

## Definition of Engraftment, Preengraftment Immune Reactions, and Endpoints

Engraftment was defined as absolute neutrophil count more than $0.5\times10^9$/L for 3 consecutive days. Chimerism was assessed using fluorescent in situ hybridization in sex-mismatched donor-recipient pairs, or polymerase chain reaction for a variable number of tandem repeats with donor cells detected at a sensitivity of 10% in sex-matched pairs. Whole blood or BM cells were assessed at the time of granulocyte engraftment. Complete donor-type chimerism was defined when donor cells consisted of more than 90% of analyzed cells. PIR was characterized by the presence of at least three of the following symptoms with no direct consequences of infection or adverse effects of medication six or more days before engraftment, as described previously (*4*, *5*): a high fever (>38.5°C), skin eruptions, body weight gain greater than 5% of baseline, or peripheral edema. Those who had all four symptoms and at least two of the following criteria indicating severe organ damage were classified as severe type; (1) $SpO_2$ less than 92% or pleural/pericardial effusions present; (2) serum creatinine level more than or equal to 3 times of baseline; (3) total bilirubin level more than 3 mg/dL or asparatate aminotransferase/alanine aminotransferase levels more than three times of upper limit of normal; and (4) development of hemophagocytosis in BM.

The main parameters analyzed between groups were as follows: (1) cumulative incidences of neutrophil or platelet engraftment; (2) cumulative incidences of NRM and relapse; (3) incidences of PIR, acute and chronic GVHD; and (4) overall and progression-free survival (OS and PFS). The analysis was performed as of April, 2010. OS was calculated from the day of transplantation until death from any cause or last follow-up. PFS was calculated from the day of transplantation until relapse, second transplantation due to engraftment failure, or death from any cause or last follow-up. NRM was defined as death in the absence of disease progression. Deaths occurring after disease progression were categorized as relapse regardless of the cause of death. Infection was considered the cause of death when bacterial, viral, or fungal infection was determined to be the proximate cause of death in patients who had not relapsed. Patients underwent BM aspiration at the time of engraftment or if clinically indicated. Relapse for acute myeloid leukemia, acute lymphoblastic leukemia, MDS, or chronic myeloid leukemia was determined by flow cytometric, morphologic, or cytogenetic evidence of malignant or dysplastic cells with clonal markers similar to those observed before transplantation. Relapse for ML was defined as progressive adenopathy or BM involvement. Acute and chronic GVHD were defined and graded by standard criteria (*29*). Relapse and NRM rates were estimated using cumulative incidence analysis and were considered competing risks (*30*). Similarly, in the analysis of PIR rates, death due to other causes or relapse leading to early withdrawal of immune suppression were considered competing risks.

## Statistical Methods

Chi-square test was used to compare patient characteristics between two groups in matched-pair analysis. For continuous variables, Mann-Whitney nonparametric test was used. The probabilities of OS and PFS were estimated and plotted using the Kaplan-Meier method (*31*). Cumulative incidence curves were drawn using Gray's method (*32*). The level of significance in all cases was set at $P$ less than 0.05. The effect of various categoric variables on survival probabilities was studied with the log-rank test. A Cox proportional hazard model with limited variables because of small sample was used to determine the significance of multiple variables in determining these outcomes. All analyses were carried out using StatView statistical software for Kaplan-Meier curve, and S-PLUS software (Mathsoft, Seattle, WA) for cumulative incidence curve.

## ACKNOWLEDGMENTS

*The authors thank data coordinators Kaori Kobayashi, Madoka Narita, Rumiko Tsuchihashi, and Naomi Yamada for their invaluable assistance. The authors also thank all physicians, nurses, pharmacists, and support personnel for their care of patients in this study.*

## REFERENCES

1. Brunstein CG, Wagner JE. Cord blood transplantation for adults. *Vox Sang* 2006; 91: 195.
2. Schoemans H, Theunissen K, Maertens J, et al. Adult umbilical cord blood transplantation: A comprehensive review. *Bone Marrow Transplant* 2006; 38: 83.
3. Uchida N, Wake A, Takagi S, et al. Umbilical cord blood transplantation after reduced-intensity conditioning for elderly patients with hematologic diseases. *Biol Blood Marrow Transplant* 2008; 14: 583.
4. Miyakoshi S, Yuji K, Kami M, et al. Successful engraftment after reduced-intensity umbilical cord blood transplantation for adult patients with advanced hematological diseases. *Clin Cancer Res* 2004; 10: 3586.
5. Kishi Y, Kami M, Miyakoshi S, et al. Early immune reaction after reduced-intensity cord-blood transplantation for adult patients. *Transplantation* 2005; 80: 34.
6. Brunstein CG, Barker JN, Weisdorf DJ, et al. Umbilical cord blood transplantation after nonmyeloablative conditioning: Impact on trans-

plantation outcomes in 110 adults with hematologic disease. *Blood* 2007; 110: 3064.
7. Giaccone L, McCune JS, Maris MB, et al. Pharmacodynamics of mycophenolate mofetil after nonmyeloablative conditioning and unrelated donor hematopoietic cell transplantation. *Blood* 2005; 106: 4381.
8. Osunkwo I, Bessmertny O, Harrison L, et al. A pilot study of tacrolimus and mycophenolate mofetil graft-versus-host disease prophylaxis in childhood and adolescent allogeneic stem cell transplant recipients. *Biol Blood Marrow Transplant* 2004; 10: 246.
9. Takahashi S, Ooi J, Tomonari A, et al. Comparative single-institute analysis of cord blood transplantation from unrelated donors with bone marrow or peripheral blood stem-cell transplants from related donors in adult patients with hematologic malignancies after myeloablative conditioning regimen. *Blood* 2007; 109: 1322.
10. Narimatsu H, Terakura S, Matsuo K, et al. Short-term methotrexate could reduce early immune reactions and improve outcomes in umbilical cord blood transplantation for adults. *Bone Marrow Transplant* 2007; 39: 31.
11. Wagner JE, Barker JN, DeFor TE, et al. Transplantation of unrelated donor umbilical cord blood in 102 patients with malignant and nonmalignant diseases: Influence of CD34 cell dose and HLA disparity on treatment-related mortality and survival. *Blood* 2002; 100: 1611.
12. Sanz GF, Saavedra S, Planelles D, et al. Standardized, unrelated donor cord blood transplantation in adults with hematologic malignancies. *Blood* 2001; 98: 2332.
13. Lekakis L, Giralt S, Couriel D, et al. Phase II study of unrelated cord blood transplantation for adults with high-risk hematologic malignancies. *Bone Marrow Transplant* 2006; 38: 421.
14. Culter CM, Stevenson K, Kim HT, et al. Double umbilical cord blood transplantation with reduced intensity conditioning and sirolimus-based GVHD prophylaxis. *Bone Marrow Transplant* 2011; 46: 659.
15. Lipsky JJ. Mycophenolate mofetil. *Lancet* 1996; 348: 1357.
16. Bardsley-Elliot A, Noble S, Foster RH. Mycophenolate mofetil: A review of its use in the management of solid organ transplantation. *BioDrugs* 1999; 12: 363.
17. Jacobson P, Rogosheske J, Barker JN, et al. Relationship of mycophenolic acid exposure to clinical outcome after hematopoietic cell transplantation. *Clin Pharmacol Ther* 2005; 78: 486.
18. Ballen KK, Spitzer TR, Yeap BY, et al. Double unrelated reduced-intensity umbilical cord blood transplantation in adults. *Biol Blood Marrow Transplant* 2007; 13: 82.
19. Bolwell B, Sobecks R, Pohlman B, et al. A prospective randomized trial comparing cyclosporine and short course methotrexate with cyclosporine and mycophenolate mofetil for GVHD prophylaxis in myeloablative allogeneic bone marrow transplantation. *Bone Marrow Transplant* 2004; 34: 621.
20. Cutler C, Li S, Kim HT, et al. Mucositis after allogeneic hematopoietic stem cell transplantation: A cohort study of methotrexate- and non-methotrexate-containing graft-versus-host disease prophylaxis regimens. *Biol Blood Marrow Transplant* 2005; 11: 383.
21. Pohlreich D, Vitek A, Maalouf J, et al. Decreased risk of acute gastrointestinal toxicity when substituting methotrexate with mycophenolate mofetil in the prevention of graft-versus-host disease in stem cell transplantation following myeloablative conditioning regimens. *Bone Marrow Transplant* 2006; 37: 235.
22. 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.
23. Matsuno N, Wake A, Uchida N, et al. Impact of HLA disparity in the graft-versus-host direction on engraftment in adult patients receiving reduced-intensity cord blood transplantation. *Blood* 2009; 114: 1689.
24. Patel KJ, Rice RD, Hawke R, et al. Pre-engraftment syndrome after double-unit cord blood transplantation: A distinct syndrome not associated with acute graft-versus-host disease. *Biol Blood Marrow Transplant* 2010; 16: 435.
25. Nash RA, Johnston L, Parker P, et al. A phase I/II study of mycophenolate mofetil in combination with cyclosporine for prophylaxis of acute graft-versus-host disease after myeloablative conditioning and allogeneic hematopoietic cell transplantation. *Biol Blood Marrow Transplant* 2005; 11: 495.
26. Barker JN, Davies SM, DeFor T, et al. Survival after transplantation of unrelated donor umbilical cord blood is comparable to that of human leukocyte antigen-matched unrelated donor bone marrow: Results of a matched-pair analysis. *Blood* 2001; 97: 2957.
27. 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.
28. Sorror ML, Giralt S, Sandmaier BM, et al. Hematopoietic cell transplantation specific comorbidity index as an outcome predictor for patients with acute myeloid leukemia in first remission: Combined FHCRC and MDACC experiences. *Blood* 2007; 110: 4606.
29. Sullivan KM. Graft-versus-host-disease [ed. 4]. Cambridge, MA: Blackwell Science 1999.
30. Gooley TA, Leisenring W, Crowley J, et al. Estimation of failure probabilities in the presence of competing risks: New representations of old estimators. *Stat Med* 1999; 18: 695.
31. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 1958; 53: 457.
32. Gray RJ. A class of k-sample tests for comparing the cumulative incidence of a competing risk. *Ann Statistics* 1988; 116: 1141.

Bone Marrow Transplantation (2011) 46, 619–620

www.nature.com/bmt

## LETTER TO THE EDITOR

# What is the upper age limit for performing allo-SCT? Cord blood transplantation for an 82-year-old patient with AML

*Bone Marrow Transplantation* (2011) **46,** 619–620; doi:10.1038/bmt.2010.159; published online 28 June 2010

Since morbidity and mortality associated with hematologic malignant diseases in elderly patients is higher than that in younger patients,[1] elderly patients are less likely to be candidates for allo-SCT, due to the facts that they are more likely to have comorbid organ conditions, either clinically or subclinically, which results in a higher rate of procedure-related mortality,[2] and that they are less likely to have HLA-matched related donors available, as siblings also tend to be elderly.

The development of reduced-intensity (RI) conditioning for transplants, which results in less toxicity and depends largely on GVL effects rather than high-dose therapy to eliminate leukemic cells, has been shown to allow elderly patients to undergo allo-SCT.[3–5] The use of umbilical cord blood transplantation (UCBT) for adults has been increasing due to the potential advantage of rapid availability and the lower risk of GVHD, thus permitting less stringent HLA matching.[4,5] RI-UCBT for adults, mostly elderly patients, has been increasingly reported and shown to be applicable.[6,7] However, there has been no clear description on the upper age limit of receiving allo-SCT, and it varies among institutes at this moment. We report here an 82-year-old man with refractory AML who had successfully treated with RI-UCBT.

The patient was diagnosed as AML (M5b) with adverse risk karyotype (46, XY, −7, +8) and complicated with disseminated intravascular coagulation (DIC). Although DIC was resolved soon after remission induction therapy consisted of idarubicin and cytarabine, and the patient achieved hematological remission, the disease subsequently progressed with lung infiltration and systemic skin tumor formation (Figure 1a). Immunohistochemical analysis of skin tumor showed positive for CD45, myeloperoxidase, and CD68 consistent with leukemic cell infiltration. Skin and lung infiltration was refractory to following high-dose Ara-C-containing chemotherapy. At 4 months after diagnosis of AML, following careful discussion and consent among the patient, his family and transplant staff, he received an RI-UCBT using two antigen- and three allele-mismatched CB in August 2007. His Eastern Cooperative Oncology Group (ECOG) performance status was 2, and HCT-CI score was 1. The preparative regimen consisted of i.v. fludarabine 25 mg/m$^2$ daily for 5 days (total dose 125 mg/m$^2$), i.v. melphalan 40 mg/m$^2$ daily for 2 days (total dose 80 mg/m$^2$) and 4 Gy of TBI fractionated by 2. GVHD prophylaxis consisted of tacrolimus by continuous infusion and 15 mg/kg twice daily of oral mycophenolate mofetil from day −1. CB unit contained $2.5 \times 10^7$ per kg of total nucleated cells and $0.98 \times 10^5$ per kg of CD34+ cells before cryopreservation. G-CSF 300 μg/m$^2$ was administered from day 1 until neutrophil engraftment. On day 14, the patient developed erythema, fever (39 °C) and diarrhea, and was diagnosed as having preengraftment immune reactions (PIR).[8] The symptoms disappeared immediately after initiation of methylprednisolone 0.5 mg/kg for 3 days. There was no episode of bacterial infection during neutropenia. ANC recovered to $0.5 \times 10^9$ per liter on day 25, and platelet count reached $2.0 \times 10^9$ per liter on day 64. Complete donor-cell chimerism was confirmed on day 27 by BM analysis using short tandem repeat-PCR method. Human herpesvirus-6 limbic encephalitis developed on day 17, which was successfully managed with foscarnet. The regimen-related toxicities observed were mucositis (grade 2), nausea (grade 2), renal dysfunction (grade 2) and diarrhea (grade 1), according to the National Cancer Institute Common Toxicity Criteria version 3.0. Acute GVHD of grade III (gut: stage 2) on day 46 was observed, but successfully managed with oral beclomethasone dipropionate. He finally achieved CR in BM, and his lung lesion and skin tumors also disappeared (Figure 1b). He was discharged from hospital on day 123 after RI-UCBT. To our surprise, his level of performance status got improved thereafter, almost as score 1 measured by ECOG PS scoring system, and returned to his work in 1 month after discharge. In the meantime, chronic GVHD of limited type developed, which was managed without treatment. One year after RI-UCBT, unfortunately, his disease relapsed and he died from disease progression 1 month later.

This remarkable case told us two important issues. First, some, may be not all, patients older than 80 years still can tolerate RI-UCBT. TRM has been shown to be correlated with several factors including age, or more comprehensively, the number of coexisting comorbidities.[9] According to our previous report, those older than 54 years showed cumulative incidence of TRM reaching to approximately 50%, and most of TRM occurred early period post-UCBT.[10] This patient had also faced life-threatening events, such as PIR or viral encephalitis, and was successfully managed by corticosteroid and foscarnet. In allo-SCT settings, there are always several factors that cannot be modulated intentionally, and there may have been good coincidences for him to reach this successful outcome. Nevertheless, this case strongly claims higher age should not be the single determinant of not performing allo-SCT. Second, the most powerful antileukemic activity was observed with RI-UCBT. Although, the patient had finally disease relapse, it was obvious that only RI-UCBT sufficiently suppressed leukemic cells and gave him a

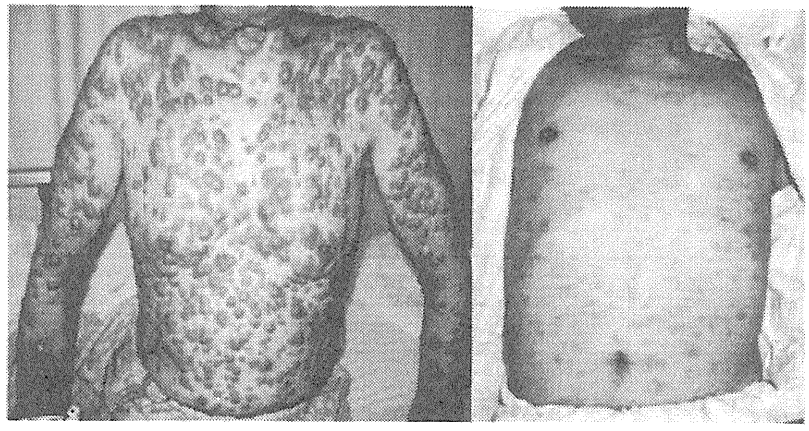

**Figure 1** Skin tumors covering whole body of the patient just before RI-UCBT (a). Skin tumors of the patient had disappeared in 90 days after RI-UCBT (b).

sustained CR so that he had enough time to return to his job. Although CB has been shown to have functionally immature immune cells, it showed its extremely powerful anti-leukemic activity even from the early period post transplant, as the patient's skin lesion had never disappeared during induction chemotherapy including high-dose Ara-C.

Whether the clinical course of this case can be applicable to all aged patients or this is exceptional case needs to be investigated carefully. The indication of allo-SCT for those who are elderly has to be determined individually with extremely careful and repeated discussion with patients, families and transplant staff. Nevertheless, the indication of allo-SCT should not be determined by age as a sole factor. Otherwise, elderly patients may lose chance of cure or good disease control, by not performing toxic yet powerful treatment, such as transplant.

## Conflict of interest

The authors declare no conflict of interest.

K Masuoka[1], N Uchida[1], K Ishiwata[1], S Takagi[1], M Tsuji[1], H Yamamoto[1], S Seo[1], N Matsuno[1], A Wake[1], S Makino[2], A Yoneyama[3] and S Taniguchi[1]
[1]*Department of Hematology, Toranomon Hospital, Tokyo, Japan;*
[2]*Department of Transfusion Medicine, Toranomon Hospital, Tokyo, Japan and*
[3]*Department of Infectious Diseases, Toranomon Hospital, Tokyo, Japan*
*E-mail: masuoka@mishuku.gr.jp*

## References

1 Deschler B, Lubbert M. Acute myeloid leukemia: epidemiology and etiology. *Cancer* 2006; **107**: 2099–2107.
2 Ringden O, Horowitz MM, Gale RP, Biggs JC, Gajewski J, Rimm AA *et al.* Outcome after allogeneic bone marrow transplant for leukemia in older adults. *JAMA* 1993; **270**: 57–60.
3 Slavin S, Nagler A, Naparstek E, Kapelushnik Y, Aker M, Cividalli G *et al.* Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. *Blood* 1998; **91**: 756–763.
4 Laughlin MJ, Eapen M, Rubinstein P, Wagner JE, Zhang MJ, Champlin RE *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.
5 Rocha V, Labopin M, Sanz G, Arcese W, Schwerdtfeger R, Bosi A *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.
6 Barker JN, Weisdorf DJ, DeFor TE, Blazar BR, Miller JS, Wagner JE. Rapid and complete donor chimerism in adult recipients of unrelated donor umbilical cord blood transplantation after reduced-intensity conditioning. *Blood* 2003; **102**: 1915–1919.
7 Miyakoshi S, Yuji K, Kami M, Kusumi E, Kishi Y, Kobayashi K *et al.* Successful engraftment after reduced-intensity umbilical cord blood transplantation for adult patients with advanced hematological diseases. *Clin Cancer Res* 2004; **10**: 3586–3592.
8 Kishi Y, Kami M, Miyakoshi S, Kanda Y, Murashige N, Teshima T *et al.* Early immune reaction after reduced-intensity cord-blood transplantation for adult patients. *Transplantation* 2005; **80**: 34–40.
9 Sorror ML, Maris MB, Storb R, Baron F, Sandmaier BM, Maloney DG *et al.* Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. *Blood* 2005; **106**: 2912–2919.
10 Uchida N, Wake A, Takagi S, Yamamoto H, Kato D, Matsuhashi Y *et al.* Umbilical cord blood transplantation after reduced-intensity conditioning for elderly patients with hematologic diseases. *Biol Blood Marrow Transplant* 2008; **14**: 583–590.

Leukemia (2011) 1-3

www.nature.com/leu

**Letter to the Editor**

# Expansion of NK cells from cord blood with antileukemic activity using GMP-compliant substances without feeder cells

*Leukemia* advance online publication, 6 December 2011; doi:10.1038/leu.2011.345

Neonatal cord blood (CB) cells have been demonstrated to contain a high percentage of natural killer (NK) cells, but the NK cells are immature, with a low level of cytolytic activity. However, expression levels of perforin and granzyme have been reported to be high in CB NK cells, and it has been suggested that CB NK cells are phenotypically and functionally mature. It has been suggested that CB is a source of stem cells that is as safe and effective as bone marrow or mobilized peripheral blood.[1,2] Also, there are a number of progenitor cell populations in CB that can be differentiated to NK cells. Therefore, CB is a useful source to expand NK cells for adoptive immunotherapy, particularly against malignant cells that express a low level of human leukocyte antigen class I molecules. Resting CB NK cells rapidly respond to cytokine stimulation by increasing cytolytic activity. Adoptive transfer of allogeneic NK cells is a potential immunotherapy to induce a graft-versus-leukemia (GVL) effect, without causing a graft-versus-host disease (GVHD).[3] In this study, we tried to expand NK cells from CB with antileukemic activity using good manufacturing practice (GMP)-compliant substances without feeder cells. We used tacrolimus (FK506) and low molecular weight heparin (dalteparin sodium) for expansion of NK cells. Kim *et al.*[4] showed impaired interleukin (IL)-2 signaling and a reduction in activating receptors in NK cells by tacrolimus. However, we have reported that tacrolimus enhances the cytolytic activity of inhibitory NK cell receptor (CD94/NKG2A)-expressing CD8T cells.[5] Also, Wang *et al.*[6] showed that a related compound (cyclosporine A) has essentially no effect on cytolytic activity of NK cells. There is conflicting data on the effect of calcineurin inhibitors on NK cell. Anyways, tacrolimus can inhibit T-cell proliferation. Heparin was reported to bind to several types of cytokines and to activate them, and also to have an important role in the expansion of hematopoietic progenitor cells.[7] Also, heparin sulfate and its related compounds were recognized by natural cytotoxicity receptors such as NKp30, NKp44 and NKp46, and soluble heparin enhanced the secretion of interferon-γ by NK cells.[8] Spanholtz *et al.*[9] reported efficient expansion of NK cells from CB $CD34^+$ cells, using low molecular weight heparin-based media containing various cytokines. Therefore, we tried to use tacrolimus to inhibit T-cell proliferation and dalteparin to support NK-cell proliferation during NK-cell expansion using IL-2, IL-15 and OKT3 *in vitro*.

Umbilical CB cells (Hokkaido Cord Blood Bank, Sapporo, Japan; $1 \times 10^6$ per ml) were cultured with IL-15 (10 ng/ml; PeproTech Inc., Rocky Hill, NJ, USA), IL-2 (5 ng/ml; R&D Systems, Minneapolis, MN, USA) and anti-CD3 monoclonal antibody (mAb) (OKT3, 10–1000 ng/ml, Janssen Pharmaceutical Company, Tokyo, Japan), with or without tacrolimus (0.02–0.1 ng/ml, Fujisawa, Osaka, Japan) and dalteparin sodium (Fragmin, 5–10 U/ml, Pfizer Japan, Tokyo, Japan) in culture medium stem cell growth medium (SCGM) (CeeGenix, Freiburg, Germany), which was produced under GMP, with 5% human AB serum in 24-well plates or T25 flasks without feeder cells. Cell cultures were split approximately one-second to one-fourth after 3–4 days of culture, and fresh medium, cytokines and reagents were added. After 3 weeks culture of umbilical CB cells ($1 \times 10^6$ per ml) with IL-15, IL-2 and anti-CD3 mAb without feeder cells, $CD56^+CD3^-$ NK cells had increased by more than 1000-fold with about 50% purity. Furthermore, addition of dalteparin sodium and tacrolimus efficiently augmented NK cell expansion (1700-fold expansion with 72.8% purity). Also, NK cell proportion was the highest (72.8%) after expansion with both dalteparin sodium and tacrolimus compared with expansion with cytokines only, dalteparin sodium only and tacrolimus only (Table 1, means ± s.d.s, $n = 5$). The proportion of $CD56^+CD3^-$ NK cells increased after more than 7 days of culture, and the proportion of CD56-expressing cells increased up to 90% after 3 weeks of culture (Figure 1a, bars indicate means ± s.d.s, $n = 5$). Finally, we could obtain about $40 \times 10^6$ NK cells from $1 \times 10^6$ unmanipulated CB cells under GMP-conditioned medium with 5% human AB serum without feeder cells. Furthermore, this method has also enabled to expand NK cells from adult peripheral blood mononuclear cells (PBMCs; preliminary data not shown).

These expanded NK cells expressed stimulatory NK cell receptor NKG2D and intracellular cytotoxic molecule granzyme (Figure 1b, bars indicate means ± s.d.s, $n = 5$). Also, the expanded $CD16^+CD56^+$ NK cells expressed high levels of inhibitory NK receptors, but significantly higher levels of stimulatory NK cell receptors including NKG2C, NKG2D, NKp30, NKp46 and especially, NKp44, than the levels of these receptors on $CD16^+CD56^+$ NK cells in resting CB before culture were noted (Figure 1c, $P < 0.01$, bars indicate means ± s.d.s, $n = 10$).

The cytolytic activities of expanded NK cells were tested against $^{51}Cr$-labeled K562 human leukemic cell lines, patients' leukemic cells and allogeneic phytohemagglutin (PHA) blasts ($5 \times 10^3$), using standard 4-h $^{51}Cr$ release assays. The expanded NK cells had

**Table 1.** Expansion of NK cells from CB samples

| | *Pre* | *IL2+15* | *+Tacrolimus* | *+Fragmin* | *+T+F* |
|---|---|---|---|---|---|
| % | 2.8 ± 0.8 | 48.3 ± 6.5 | 50.4 ± 3.7 | 65.0 ± 10.8[b] | 72.8 ± 9.6[a,c,d] |
| Absolute number | 0.028 ± 0.008 | 36.1 ± 10.4 | 34.7 ± 16.2 | 49.9 ± 17.7[e] | 43.5 ± 14.3 |
| Fold expansion | 1 | 1422 ± 316 | 1360 ± 581 | 1989 ± 678[e] | 1706 ± 389 |

Abbreviations: CB, cord blood; IL, interleukin; NK, natural killer. Values in the upper column indicate the percentage of $CD56^+CD3^-$ cells after 3-weeks expansion of CB cells with indicated factors (%). Significant differences were found in the values compared with the values for culture with only IL-2 and IL-15. Significant differences were also found in the values compared with the values for culture with 0.02 ng/ml of tacrolimus and 5 IU/ml of fragmin. Values in the middle and lower columns indicate the calculated absolute $CD56^+CD3^-$ cell number and fold expansion of NK cells after 3-weeks expansion of $1 \times 10^6$ CB cells. Significant difference was found only in the value for culture with IL-2, IL-15 and 0.02 ng/ml of tacrolimus, compared with the values for culture with IL-2, IL-15 and 5 IU/ml of fragmin (means ± s.d.s, $n = 5$). [a]$P < 0.01$, [b]$P < 0.05$, [c]$P < 0.01$, [d]$P < 0.05$ and [e]$P < 0.05$.

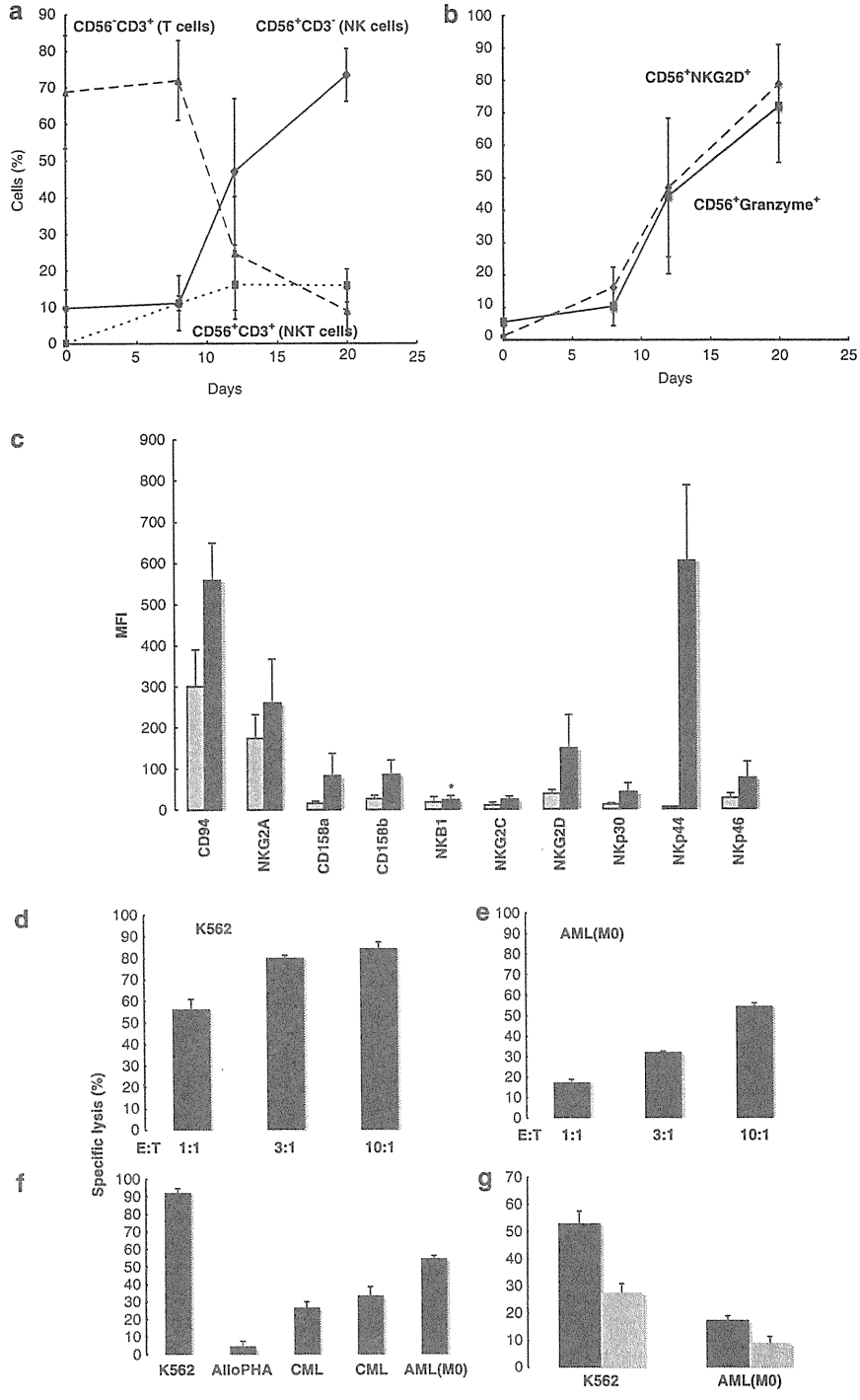


**Figure 1.** Time course profile of expanded cells during culture without feeder cells. $CD56^+CD3^-$ (NK cells), $CD56^-CD3^+$ (T cells) and $CD56^+CD3^+$ cells (NKT cells; **a**) and $CD56^+NKG2D^+$ cells and $CD56^+$granzyme$^+$ cells (**b**); bars indicate means ± s.d.s, $n=5$. Mean fluorescence intensity (MFI) of NK receptors on $CD16^+CD56^+$ NK cells in CB before expansion (gray bars) and after expansion (black bars). There were significant differences between before and after expansion except for NKB1 *($P<0.01$, $n=10$; **c**). Cytolytic activities of expanded NK cells against K562 cells (**d**) and AML (M0) patient's leukemic cells (**e**). Data presented are means ± s.d.s (effector/target ratios of 1:1, 3:1 and 10:1). Cytolytic activities against K562 cells, allogeneic third-party PHA blasts and patients's leukemic cells from chronic myeloid leukemia and AML (M0; E:T ratio of 10:1; **f**). Inhibitory effect of anti-NKG2D monoclonal antibody (20 μg/ml, gray bars) against cytolytic activities of expanded NK cells (E:T ratio is 1:1; **g**).

a very high level of cytolytic activity against the K562 leukemic cell line, with specific lysis of more than 50% under the condition of an effector:target ratio (E:T ratio) of 1:1 and more than 80% under the condition of an E:T ratio of 3:1 (Figure 1d). Also, the expanded NK cells could attack patients' primary acute myeloid leukemia (AML; M0) and chronic myeloid leukemia (CP) leukemic cells, but could not attack allogeneic third-party PHA blasts (Figures 1e and f). Anti-NKG2D mAb (1D11, 20 μg/ml, Serotec, Oxford, UK)

suppressed the cytolytic activity against K562 cells and also patients' primary leukemic cells (Figure 1g). Therefore, the cytolytic activity of these expanded NK cells depended at least partially on NKG2D-activating receptor.

Allogeneic NK cells have been reported to have a strong GVL effect after haploidentical hematopoietic stem cell transplantation (HSCT) in patients with advanced AML, without causing GVHD.[3] Adoptive transfer of allogeneic NK cells may be a promising immunotherapy. However, expansion of NK cells seems to be difficult compared with expansion of T cells. About 20-fold expansion of NK cells was achieved by culture of PBMCs with cytokines for a short time and co-culture with K562 cells that had been transfected with, and expressed membrane-bound IL-15 and 4-1BBL. These expanded NK cells using artificial feeder cells had cytolytic activity against human AML cells and also pediatric solid tumors such as Ewing sarcoma and rhabdomyosarcoma. On the other hand, there have been several reports of NK cell expansion from PBMCs without using feeder cells. Alici *et al.*[10] reported the possibility of expanding NK cells without feeder cells from PBMCs of multiple myeloma patients with significant cytolytic activity against primary autologous multiple myeloma cells. It is more beneficial for clinical use if it is not necessary to use feeder cells for efficient expansion of NK cells *in vitro*. Also, Ayello *et al.*[11] reported a 20-fold expansion of NK cells from CB cells with depletion of adherent monocytes by *ex vivo* culture with IL-2, IL-7 and IL-12 for 7 days. Therefore, NK cells can be expanded from not only PBMCs, but also CB.

Clinical-scale NK cell purification has so far been performed by donor leukapheresis followed by CD3 depletion with or without CD56 enrichment. There have been several clinical reports on adoptive transfer of allogeneic NK cells for hematological malignancies. Passweg *et al.*[12] first reported the feasibility of allogeneic NK cell purification and infusion in five myeloid malignant patients after haploidentical HSCT. Miller *et al.*[13] reported that haploidentical NK cell infusions after cyclophosphamide and fludarabine treatment resulted in expansion of donor NK cells and induction of complete hematological remission in 5 of 19 AML patients with poor prognosis. A recent pilot study showed good results in pediatric patients who received haploidentical NK cells to consolidate chemotherapy for AML patients. Also, Nguyen *et al.*[14] reported a persistent and massive expansion of infused alloreactive NK cells in an AML patient who had relapsed after haploidentical HSCT. Yoon *et al.*[15] reported patients who underwent human leukocyte antigen-mismatched HSCT and subsequently received donor NK cells that were generated from CD34+ cells from donor leukapheresis products by *ex vivo* culture for more than 6 weeks ($9.3 \times 10^6$ per kg, CD122/CD56$^+$ 64%, CD3$^+$ 1%). There were no signs of acute toxicity in 14 adult patients infused with these cells 6–7 weeks after transplantation, with one patient developing acute GVHD and five patients developing chronic GVHD. Therefore, clinical-grade allogeneic NK cell infusion is safe and feasible. However, many issues remain to be resolved, including selection of donor, NK cell selection and expansion procedure, type of conditioning regimen, survival and expansion of NK cells in the recipient after infusion, their localization and finally, the clinical effect of NK cell infusion.

In this study, we could obtain about $40 \times 10^6$ NK cells from $1 \times 10^6$ unmanipulated CB cells using tacrolimus and dalteparin sodium without feeder cells (more than 1000-fold expansion with more than 70% purity). Thus, alloreactive Killer cell immunoglobulin-like receptor (KIR)-mismatched expanded NK cells from CB can be used for adoptive NK cell immunotherapy to induce a strong GVL/tumor effect without severe GVHD for patients who do not have KIR-mismatched related donors.

## CONFLICT OF INTEREST

A patent application for the composition for expanding NK cells and the use of them has been filed with Junji Tanaka as a sole inventor.

## ACKNOWLEDGEMENTS

We thank Ms M Yamane, Ms M Mayanagi and Ms Y Ishimaru for their technical assistance.

J Tanaka, J Sugita, S Shiratori, A Shigematu, S Asanuma, K Fujimoto, M Nishio, T Kondo and M Imamura
*Department of Hematology and Oncology, Hokkaido University Graduate School of Medicine, Sapporo, Japan*
*E-mail: jutanaka@med.hokudai.ac.jp*

## REFERENCES

1 Rocha V, Labopin M, Sanz G, Arcese W, Schwerdtfeger R, Bosi A *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.
2 Eapen M, Rubinstein P, Zhang MJ, Stevens C, Kurtzberg J, Scaradavou A *et al.* Outcomes of transplantation of unrelated donor umbilical cord blood and bone marrow in children with acute leukaemia: a comparison study. *Lancet* 2007; **369**: 1947–1954.
3 Ruggeri L, Capanni M, Urbani E, Perruccio K, Shlomchik WD, Tosti A *et al.* Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. *Science* 2002; **295**: 2097–2100.
4 Kim TJ, Kim N, Kang HJ, Kim EO, Kim ST, Ahn HS *et al.* FK506 causes cellular and functional defects in human natural killer cells. *J Leukoc Biol* 2010; **88**: 1089–1097.
5 Tanaka J, Toubai T, Iwao N, Tsutsumi Y, Kato N, Miura Y *et al.* The immunosuppressive agent FK506 enhances the cytolytic activity of inhibitory natural killer cell receptor (CD94/NKG2A)-expressing CD8 T cells. *Transplantation* 2005; **80**: 1813–1815.
6 Wang H, Grzywacz B, Sukovich D, McCullar V, Cao Q, Lee AB *et al.* The unexpected effect of cyclosporin A on CD56+CD16- and CD56+CD16+ natural killer cell subpopulations. *Blood* 2007; **110**: 1530–1539.
7 Gupta P, Oegema Jr TR, Brazil JJ, Dudek AZ, Slungaard A, Verfaillie CM. Human LTC-IC can be maintained for at least 5 weeks *in vitro* when interleukin-3 and a single chemokine are combined with O-sulfated heparan sulfates: requirement for optimal binding interactions of heparan sulfate with early-acting cytokines and matrix proteins. *Blood* 2000; **95**: 147–155.
8 Hecht ML, Rosental B, Horlacher T, Hershkovitz O, De Paz JL, Noti C *et al.* Natural cytotoxicity receptors NKp30, NKp44 and NKp46 bind to different heparan sulfate/heparin sequences. *J Proteome Res* 2009; **8**: 712–720.
9 Spanholtz J, Tordoir M, Eissens D, Preijers F, van der Meer A, Joosten I *et al.* High log-scale expansion of functional human natural killer cells from umbilical cord blood CD34-positive cells for adoptive cancer immunotherapy. *PLoS One* 2010; **5**: e9221.
10 Alici E, Sutlu T, Björkstrand B, Gilljam M, Stellan B, Nahi H *et al.* Autologous antitumor activity by NK cells expanded from myeloma patients using GMP-compliant components. *Blood* 2008; **111**: 3155–3162.
11 Ayello J, van de Ven C, Cairo E, Hochberg J, Baxi L, Satwani P *et al.* Characterization of natural killer and natural killer-like T cells derived from *ex vivo* expanded and activated cord blood mononuclear cells: implications for adoptive cellular immunotherapy. *Exp Hematol* 2009; **37**: 1216–1229.
12 Passweg JR, Tichelli A, Meyer-Monard S, Heim D, Stern M, Kühne T *et al.* Purified donor NK-lymphocyte infusion to consolidate engraftment after haploidentical stem cell transplantation. *Leukemia* 2004; **18**: 1835–1838.
13 Miller JS, Soignier Y, Panoskaltsis-Mortari A, McNearney SA, Yun GH, Fautsch SK *et al.* Successful adoptive transfer and *in vivo* expansion of human haploidentical NK cells in patients with cancer. *Blood* 2005; **105**: 3051–3057.
14 Nguyen S, Béziat V, Norol F, Uzunov M, Trebeden-Negre H, Azar N *et al.* Infusion of allogeneic natural killer cells in a patient with acute myeloid leukemia in relapse after haploidentical hematopoietic stem cell transplantation. *Transfusion* 2011; **51**: 1769–1778.
15 Yoon SR, Lee YS, Yang SH, Ahn KH, Lee JH, Lee JH *et al.* Generation of donor natural killer cells from CD34(+) progenitor cells and subsequent infusion after HLA-mismatched allogeneic hematopoietic cell transplantation: a feasibility study. *Bone Marrow Transplant* 2010; **45**: 1038–1046.

Bone Marrow Transplantation (2011), 1–7

www.nature.com/bmt

ORIGINAL ARTICLE

# Efficacy of folinic acid in preventing oral mucositis in allogeneic hematopoietic stem cell transplant patients receiving MTX as prophylaxis for GVHD

J Sugita[1], T Matsushita[2], H Kashiwazaki[2], M Kosugi[1], S Takahashi[1], K Wakasa[1], S Shiratori[1], M Ibata[1], Y Shono[1], A Shigematsu[1], M Obara[1], K Fujimoto[1], T Endo[1], M Nishio[1], T Kondo[1], S Hashino[1], J Tanaka[1], M Asaka[3] and M Imamura[1]

[1]*Stem Cell Transplantation Center, Hokkaido University Hospital, Sapporo, Japan;* [2]*Center for Dental Clinics, Hokkaido University Hospital, Sapporo, Japan and* [3]*Department of Gastroenterology and Hematology, Hokkaido University Graduate School of Medicine, Sapporo, Japan*

**As the safety of folinic acid administration and its efficacy for reducing the toxicity of MTX remain controversial, we assessed the effect of folinic acid administration after MTX treatment for GVHD prophylaxis on the incidence of oral mucositis and acute GVHD. We retrospectively analyzed data for 118 patients who had undergone allogeneic hematopoietic SCT and had received MTX for GVHD prophylaxis. Multivariate analysis showed that systemic folinic acid administration significantly reduced the incidence of severe oral mucositis (odds ratio (OR) = 0.13, 95% confidence interval (CI) 0.04–0.73, $P = 0.014$). There was also a tendency for a lower incidence of severe oral mucositis in patients who received folinic acid mouthwash (OR = 0.39, 95%CI 0.15–1.00, $P = 0.051$). No significant difference was observed in the incidence of acute GVHD between patients who received systemic folinic acid administration and those who did not ($P = 0.88$). Systemic folinic acid administration and mouthwash appear to be useful for reducing the incidence of severe oral mucositis in patients who have received allogeneic hematopoietic SCT using MTX as GVHD prophylaxis.**

*Bone Marrow Transplantation* advance online publication, 21 March 2011; doi:10.1038/bmt.2011.53

**Keywords:** oral mucositis; folinic acid; SCT

## Introduction

Oral mucositis is one of the most common complications associated with allogeneic hematopoietic SCT, occuring in 60–90% of patients who have received SCT.[1–3] Oral mucositis is associated with severe pain, which can lead to anorexia and dehydration. A large population of patients with severe oral mucositis require total parenteral nutrition and opioid analgesics.[4] Severe oral mucositis is associated with not only severe pain but also poor clinical and economic outcomes.[5]

Oral mucositis is caused mainly by the toxicity associated with chemotherapy and TBI as a conditioning regimen; however, it is also associated with the use of MTX for GVHD prophylaxis.[6,7] Although several studies have shown that folinic acid administration reduced the toxicity of MTX,[8–10] the efficacy and safety of folinic acid administration remain controversial. Ruutu *et al.*[11] reported that folinic acid was administered after MTX in 37 (45.7%) of 81 European Group for Blood and Marrow Transplantation (EBMT) centers, and Bhurani *et al.*[12] reported that folinic acid was administered after MTX in 8 (44.4%) of 8 centers in Australia and New Zealand. More than half of the centers surveyed in those studies did not use systemic folinic acid administration because of the lack of support for its efficacy or because of the risk of acute GVHD being induced by folinic acid.

Therefore, this study was performed to assess the effects of systemic folinic acid administration after MTX for GVHD prophylaxis on the incidence of oral mucositis and acute GVHD.

## Patients and methods

We retrospectively analyzed data for 141 consecutive patients who had undergone allogeneic hematopoietic SCT and had received MTX for GVHD prophylaxis between March 2006 and December 2009 in Stem Cell Transplantation Center of Hokkaido University Hospital. We excluded seven patients whose data were insufficient. Furthermore, we excluded 16 patients who failed to achieve engraftment because we hypothesized that duration of neutropenia was a risk factor for the development of

Correspondence: Dr J Sugita, Department of Hematology and Oncology, Hokkaido University Graduate School of Medicine, N15 W7, Kita-Ku, Sapporo 060-8638, Japan.
E-mail: sugitaj@med.hokudai.ac.jp
Received 26 July 2010; revised 10 January 2011; accepted 28 January 2011

mucositis, and that engraftment failure might be an extremely strong risk factor for the development of mucositis. Therefore, data for 118 patients were analyzed in this study. The study protocol was approved by the review board of Hokkaido University Graduate School of Medicine.

*Conditioning regimens and transplantation procedures*
Most of the conventional conditioning regimens consisted of TBI (12 Gy in six fractions) plus CY (60 mg/kg once daily i.v. for 2 days, total dose of 120 mg/kg) ± VP-16 (etoposide) (15 mg/kg once daily i.v. for 2 days, total dose of 30 mg/kg),[13] and most of the reduced-intensity conditioning regimens consisted of fludarabine (30 mg/m$^2$ once daily i.v. for 6 days, total dose of 180 mg/m$^2$) plus oral BU (4 mg/kg p.o. in divided doses daily for 2 days, total dose of 8 mg/kg) or i.v. BU (3.2 mg/kg i.v. in divided doses daily for 2 days, total dose of 6.4 mg/kg) plus low-dose TBI (4 Gy in two fractions).[14] CsA (3 mg/kg) or tacrolimus (FK, 0.03 mg/kg) and short-course MTX were used for GVHD prophylaxis. MTX was given at a dose of 15 or 10 mg/m$^2$ on day 1 and at a dose of 10 or 7 mg/m$^2$ on day 3 and day 6.

*Supportive care and infection prophylaxis*
Granulocyte CSF was administered from day 5 until engraftment. Levofloxacin was administered for prevention of bacterial infections until engraftment, and an antifungal (fluconazole, itraconazole or micafungin) was administered for prevention of fungal infections. Oral acyclovir was given from day −7 to day 35 for prevention of HSV infection.

*Systemic folinic acid administration and mouthwash*
Folinic acid was given i.v. at the same dose as that used for each administration of MTX at 12, 18 and 24 h after administration of MTX on days 1 and 3, and at 24, 30 and 36 h after administration of MTX on day 6. Folinic acid mouthwash (13.0% folinic acid) was given four times a day from day 1 to day 7. Systemic folinic acid administration and folinic acid mouthwash were given according to physicians' discretion. They were given to the patients who were considered by physicians to be at high risk for severe oral mucositis. For example, conventional conditioning regimens, female gender and higher doses of MTX were considered as high risk for severe oral mucositis.

*Grading of oral mucositis*
Oral mucositis was graded by the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 3.0. The criteria for oral mucositis were as follows: Grade 0, none; Grade 1, erythema of the mucosa; Grade 2, patchy ulcerations of pseudomembranes; Grade 3, confluent ulcerations or pseudomembranes, bleeding with minor trauma; Grade 4, tissue necrosis, significant spontaneous bleeding, life-threatening consequences. Severe oral mucositis was defined as grade 3 or 4 oral mucositis.

The incidence and severity of oral mucositis were evaluated daily by physicians and nurses. Dentists and dental hygienists evaluated oral mucositis at least once per week. The grading of oral mucositis was assigned at the time of evaluation.

*Evaluation of GVHD*
Acute GVHD was graded according to the consensus criteria.[15]

*Statistical analysis*
Univariate analyses were performed using the $\chi^2$-test and Fisher's exact test, as appropriate. Factors with a $P$-value of 0.2 or less in the univariate analyses were included in the multivariate analysis. Stepwise multivariate logistic regression models were used to analyze the influence of selected variables on the risk of severe oral mucositis. Cumulative incidence of acute GVHD was calculated using the Gray method,[16] considering death without acute GVHD or relapse as competing events. Similarly, in the analysis of relapse incidence, death resulting from other causes was considered as a competing risk. In the analysis of non-relapse mortality, relapse was considered as a competing risk. JMP software version 8.0.2 (SAS Institute, Cary, NC, USA) was used for most of the statistical analyses. Analysis of cumulative incidences was carried out with the package 'comprsk' of the R statistical software 2.10.1 (R Foundation for Statistical Computing, Vienna, Austria; available at http://www.r-project.org/). All $P$-values were two sided, and differences were considered to be statistically significant when $P<0.05$.

## Results

The patient characteristics are shown in Table 1. Systemic folinic acid administration was given to 29 patients. The systemic folinic acid administration group had significantly higher proportions of female patients ($P=0.03$), patients who received higher doses of MTX ($P=0.0002$) and patients who received folinic acid mouthwash ($P<0.0001$). The mean duration of neutropenia in all patients was 18.3 days. No significant difference was observed in the duration of neutropenia between patients who received systemic folinic acid administration and those who did not (17.3 days vs 18.6 days, $P=0.53$). There was a difference over time. Systemic folinic acid administration was not given to any patients in 2006–2007. In 2008–2009, 29 (42.0%) of 69 patients received systemic folinic acid administration. Other characteristics in the two groups were the same.

Oral mucositis was observed in 91 (77.1%) of the patients (Table 2), and severe oral mucositis (NCI-CTCAE Grade 3 or Grade 4) was observed in 37 (31.4%) of the patients. The incidence of oral mucositis was significantly lower in patients who received systemic folinic acid administration than in patients who did not receive systemic folinic acid administration (58.6 vs 83.2%, $P=0.0063$), and the incidence of severe oral mucositis was also significantly lower in patients who received systemic folinic acid administration than in patients who did not receive systemic folinic acid administration (10.3 vs 38.2%, $P=0.005$).

**Table 1** Patient characteristics

| | *Total* (n = *118*) | *Folinic acid administration* | | P-*values* |
|---|---|---|---|---|
| | | *Yes* (n = *29*) | *No* (n = *89*) | |
| *Age (years)* | | | | 0.17 |
| Median | 47 | 41 | 48 | |
| Range | 17–68 | 18–66 | 17–68 | |
| *Gender* | | | | 0.03 |
| Male | 61 | 10 | 51 | |
| Female | 57 | 19 | 38 | |
| *Disease* | | | | 0.48 |
| AML | 44 | 12 | 32 | |
| ALL | 23 | 9 | 14 | |
| MDS | 9 | 0 | 9 | |
| CML | 5 | 0 | 5 | |
| HL | 2 | 0 | 2 | |
| NHL | 23 | 5 | 18 | |
| ATLL | 3 | 1 | 2 | |
| MM | 4 | 1 | 3 | |
| AA | 4 | 1 | 3 | |
| MF | 1 | 0 | 1 | |
| *Disease status at transplantation* | | | | 0.23 |
| CR | 66 | 18 | 48 | |
| Non-CR | 37 | 10 | 27 | |
| Chronic phase/stable disease | 15 | 1 | 14 | |
| *Conditioning* | | | | 0.07 |
| CST | 56 | 18 | 38 | |
| VP/CY/TBI | 25 | 7 | 18 | |
| CY/TBI | 21 | 9 | 12 | |
| RIST | 62 | 11 | 51 | |
| Flu/BU/TBI | 51 | 7 | 44 | |
| *GVHD prophylaxis* | | | | 0.42 |
| CsA + MTX | 44 | 9 | 35 | |
| FK + MTX | 74 | 20 | 54 | |
| *Doses of MTX* | | | | 0.0002 |
| 15-10-10 (mg/m$^2$) | 72 | 27 | 45 | |
| 10-10-10 (mg/m$^2$) | 41 | 1 | 40 | |
| 10-7-7 (mg/m$^2$) | 5 | 1 | 4 | |
| *Stem cell source* | | | | 0.21 |
| Related BM | 14 | 4 | 10 | |
| Related PBSC | 13 | 5 | 8 | |
| Unrelated BM | 82 | 16 | 66 | |
| Unrelated CB | 9 | 4 | 5 | |
| *Duration of neutropenia (<500/μL)* | | | | 0.57 |
| ⩾21 days | 28 | 8 | 20 | |
| <21 days | 90 | 21 | 69 | |
| *Folinic acid mouthwash* | | | | <0.0001 |
| Yes | 60 | 25 | 35 | |
| No | 58 | 4 | 54 | |

Abbreviations: AA = aplastic anemia; ATLL = adult T-cell leukemia/lymphoma; CB = cord blood; CST = conventional SCT; FK = tacrolimus; Flu = fludarabine; HL = Hodgkin lymphoma; MDS = myelodysplastic syndrome; MF = myelofibrosis; MM = multiple myeloma; NHL = non-Hodgkin lymphoma; VP16 = etoposide.

Table 3 shows clinical factors and results of univariate analysis of clinical factors associated with the incidence of severe oral mucositis. Severe oral mucositis was significantly associated with VP/CY/TBI ($P = 0.048$) and duration of neutropenia (<500/μL) ($P = 0.0047$). Systemic folinic acid administration and folinic acid mouthwash reduced the incidence of severe oral mucositis ($P = 0.0038$ and $P = 0.0017$, respectively). Age, gender, disease status at transplantation, GVHD prophylaxis, stem cell source and dose of MTX did not correlate with severe oral mucositis. In multivariate analysis, duration of neutropenia was significantly associated with severe oral mucositis (odds ratio (OR) = 4.78, 95% confidence interval (CI) 1.77–13.9, $P = 0.0019$), and systemic folinic acid administration significantly reduced the incidence of severe oral mucositis (OR = 0.13, 95%CI 0.04–0.73, $P = 0.014$) (Table 4). There was a tendency for a higher incidence of severe oral mucositis in patients who received VP/CY/TBI (OR = 2.42, 95%CI 0.86–6.99, $P = 0.095$), and there was a tendency for a lower incidence of severe oral mucositis in patients who received folinic acid mouthwash (OR = 0.39, 95%CI 0.15–1.00, $P = 0.051$).

No significant difference was observed in the incidence of acute GVHD on day 100 after transplantation between patients who received systemic folinic acid administration and those who did not (acute GVHD grade 1–4, 71.3 vs 68.5%, $P = 0.88$; acute GVHD grade 2–4, 49.9 vs 40.4%, $P = 0.36$; acute GVHD grade 3–4, 6.0 vs 11.2%, $P = 0.51$) (Figure 1). There was no difference in the incidence of severe oral mucositis between patients who developed acute GVHD and those who did not (GVHD grade 1–4; 29.6%, grade 0; 31.25%, $P = 0.87$). There was no difference in the incidence of severe oral mucositis between patients who had severe acute GVHD (grade 3–4) and those who did not (GVHD grade 3–4; 16.7%, grade 1–2; 31.9%, $P = 0.47$).

No significant difference was observed in the incidences of relapse and non-relapse mortality after transplantation between patients who received systemic folinic acid administration and those who did not (relapse, 7.4 vs 22.8%, $P = 0.19$; non-relapse mortality, 7.8 vs 12.1%, $P = 0.71$) (Figure 2).

Table 5 shows the effects of systemic folinic acid administration and/or mouthwash. Use of i.v. opioid analgesics and duration of inability to eat were significantly reduced in patients who received systemic folinic acid administration and/or mouthwash compared with those in patients who received neither systemic folinic acid administration nor folinic acid mouthwash. There was no difference in the duration of total parenteral nutrition between patients who received systemic folinic acid administration and/or mouthwash and patients who received neither systemic folinic acid administration nor folinic acid mouthwash.

## Discussion

The efficacy and safety of folinic acid administration have been controversial so far. Less than half of the centers surveyed have used folinic acid administration.[11,12] Therefore, we retrospectively analyzed data for 118 patients who had undergone allogeneic hematopoietic SCT and had received MTX for GVHD prophylaxis.

**Table 2** Incidence of oral mucositis

| *Total* | *Grades of oral mucositis* | | | | |
|---|---|---|---|---|---|
| | *0* | *1* | *2* | *3* | *4* |
| | 27 (22.9%) | 26 (22.0%) | 28 (23.7%) | 36 (30.5%) | 1 (0.85%) |
| *Folinic acid administration* | | | | | |
| Yes ($n=29$) | 12 (41.4%) | 9 (31.0%) | 5 (17.2%) | 3 (10.3%) | 0 (0.0%) |
| No ($n=89$) | 15 (16.9%) | 17 (19.1%) | 23 (25.8%) | 33 (37.1%) | 1 (1.1%) |

**Table 3** Univariate analysis of severe oral mucositis

| | n | *Severe oral mucositis (%)* | *OR* | *94%CI* | P-*values* |
|---|---|---|---|---|---|
| *Age* | | | | | |
| ⩾50 | 48 | 16 (33.3%) | 1.17 | 0.53–2.56 | 0.70 |
| <50 | 70 | 21 (30.0%) | 1 | | |
| *Gender* | | | | | |
| Male | 61 | 16 (26.2%) | 1 | | |
| Female | 57 | 21 (36.8%) | 1.64 | 0.75–3.64 | 0.22 |
| *Disease status at transplantation* | | | | | |
| CR | 66 | 24 (36.4%) | 1.54 | 0.65–3.84 | 0.33 |
| Non-CR | 37 | 10 (27.0%) | 1 | | |
| *Conditioning* | | | | | |
| CST | 56 | 18 (32.1%) | 1.07 | 0.49–2.34 | 0.86 |
| RIST | 62 | 19 (30.6%) | 1 | | |
| VP/CY/TBI | 25 | 12 (48.0%) | 2.51 | 1.01–6.28 | 0.048 |
| Non-(VP/CY/TBI) | 93 | 25 (26.9%) | 1 | | |
| *GVHD prophylaxis* | | | | | |
| CsA + MTX | 44 | 12 (27.3%) | 0.74 | 0.32–1.65 | 0.46 |
| FK + MTX | 74 | 25 (33.8%) | 1 | | |
| *Doses of MTX* | | | | | |
| 15-10-10 | 72 | 18 (25.0%) | 1.33 | 0.18–27.0 | 0.80 |
| 10-10-10 | 41 | 18 (43.9%) | 3.13 | 0.42–64.1 | 0.29 |
| 10-7-7 | 5 | 1 (28.0%) | 1 | | |
| *Stem cell source* | | | | | |
| Related BM | 14 | 3 (21.4%) | 1 | | |
| Related PBSC | 13 | 4 (30.8%) | 1.62 | 0.29–10.2 | 0.58 |
| Unrelated BM | 82 | 27 (32.9%) | 1.80 | 0.51–8.44 | 0.38 |
| Unrelated CB | 9 | 3 (33.3%) | 1.83 | 0.27–12.9 | 0.53 |
| *Duration of neutropenia (<500/μL)* | | | | | |
| ⩾21 days | 28 | 15 (53.6%) | 3.57 | 1.48–8.78 | 0.0047 |
| <21 days | 90 | 22 (24.4%) | 1 | | |
| *Folinic acid administration* | | | | | |
| Yes | 29 | 3 (10.3%) | 0.20 | 0.04–0.62 | 0.0038 |
| No | 89 | 34 (38.2%) | 1 | | |
| *Folinic acid mouthwash* | | | | | |
| Yes | 60 | 11 (18.3%) | 0.28 | 0.12-0.62 | 0.0017 |
| No | 58 | 26 (44.8%) | 1 | | |

Abbreviations: CB = cord blood; CI = confidence interval; CST = conventional SCT; FK = tacrolimus; OR = odds ratio; VP16 = etoposide.

Multivariate analysis showed that systemic folinic acid administration significantly reduced the incidence of severe oral mucositis (OR = 0.13, 95%CI 0.04–0.73, $P=0.014$). Furthermore, use of opioid analgesics and duration of inability to eat were significantly reduced in patients who received systemic folinic acid administration.

**Table 4** Multivariate analysis of severe oral mucositis

| | *OR* | *95%CI* | P-*values* |
|---|---|---|---|
| *Conditioning* | | | |
| VP/CY/TBI | 2.42 | 0.86–6.99 | 0.095 |
| Non-(VP/CY/TBI) | 1 | | |
| *Duration of neutropenia (<500/μL)* | | | |
| ⩾21 days | 4.78 | 1.77–13.9 | 0.0019 |
| <21 days | 1 | | |
| *Folinic acid administration* | | | |
| Yes | 0.13 | 0.04–0.73 | 0.014 |
| No | 1 | | |
| *Folinic acid mouthwash* | | | |
| Yes | 0.39 | 0.15–1.00 | 0.051 |
| No | 1 | | |

The group of patients who received systemic folinic acid administration had significantly higher proportions of female patients ($P=0.03$) and patients who received higher doses of MTX ($P=0.0002$). Although gender did not correlate with severe oral mucositis in our study, several studies have shown that female gender is one of the risk factors for oral mucositis.[17,18] In our retrospective study, systemic folinic acid administration was performed according to physicians'discretion. Therefore, it is likely that systemic folinic acid administration was used for patients who were considered by physicians to be at high risk for severe oral mucositis.

There are data that provide a rationale for using MTX and folinic acid in combination for GVHD prophylaxis.[19] Gratwohl *et al.*[20] reported that systemic folinic acid administration 6 h after each administration of MTX reduced the toxicity of MTX and maintained the effect of MTX on prevention of GVHD in dogs, and that MTX at concentrations above $10^{-6}$ M completely abrogated thymidine uptake in lymphocytes with stimulation for 6 h *in vitro*.[21]

In pediatrics, European Group for Blood and Marrow Transplantation Working Party Paediatric Diseases and International BFM Study Group-Subcommittee Bone Marrow Transplantation recommended that folinic acid (15 mg/m$^2$ per day) should be given 24 h after MTX.[22] However, there are no recommendations or guidelines in adult transplant groups for the use of folinic acid following MTX. Therefore, systemic folinic acid administration was given at various doses and schedules, starting 6–24 h after MTX administration.[11,23] We used folinic acid i.v. at the same dose as that used for each administration of MTX at

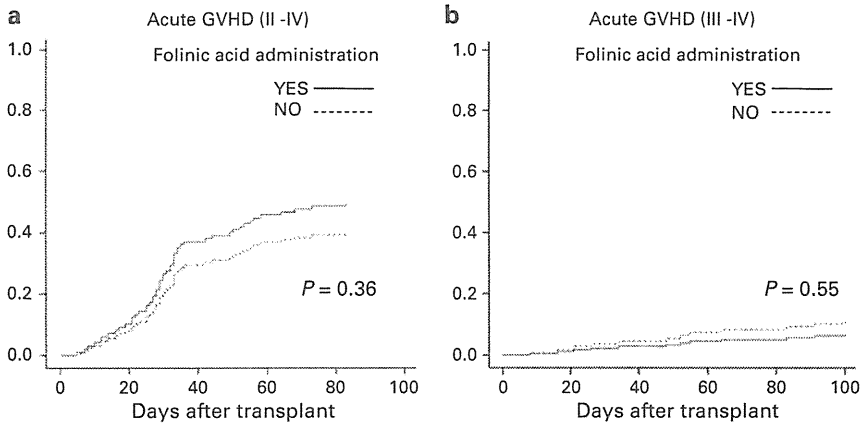


**Figure 1** Cumulative incidence of grade II–IV acute GVHD (**a**) and grade III–IV acute GVHD (**b**) grouped according to the use of folinic acid administration.

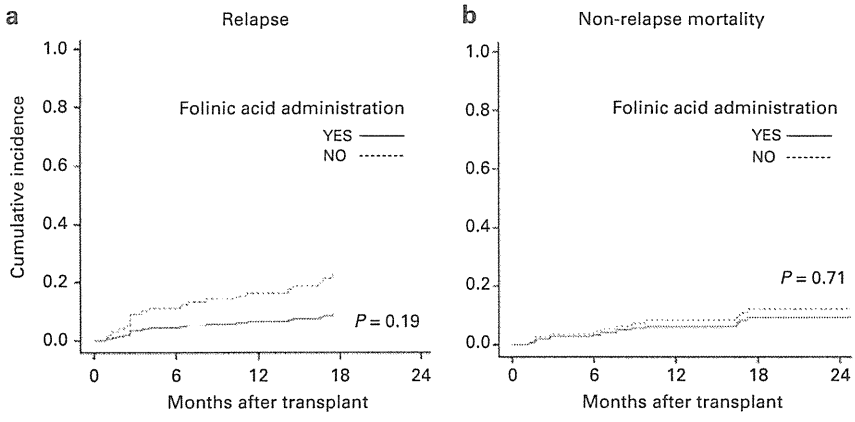


**Figure 2** Cumulative incidence of relapse (**a**) and non-relapse mortality (**b**) grouped according to the use of folinic acid administration.

**Table 5** Effect of folinic acid administration and mouthwash

| | *Without folinic acid (n = 54)* | *Folinic acid mouthwash without administration (n = 35)* | *Folinic acid administration with or without mouthwash (n = 29)* |
|---|---|---|---|
| Severe oral mucositis (grade 3–4) | 25 (46.3%) | 9 (25.7%), $P = 0.053$ | 3 (10.3%), $P = 0.0008$ |
| Use of opioid analgesics | 32 (59.3%) | 11 (31.4%), $P = 0.010$ | 10 (34.5%), $P = 0.038$ |
| Duration of inability to eat (days; mean (range)) | 25.6 (0–53) | 12.6 (0–55), $P < 0.0001$ | 8.5 (0–30), $P < 0.0001$ |
| Duration of total parenteral nutrition (days; mean (range)) | 41.8 (0–272) | 26.0 (0–96), $P = 0.054$ | 31.6 (0–90), $P = 0.32$ |

12, 18 and 24 h after administration of MTX on days 1 and 3, and at 24, 30 and 36 h after administration of MTX on day 6. We decided the dose and timing of systemic folinic acid administration according to the dose and timing of systemic folinic acid administration after high-dose MTX. Although there was no significant difference in the incidence and severity of acute GVHD in our study, our dose of folinic acid, which is about three times higher than that of the pediatric recommendation, might be in excess of those required. Further studies are needed to establish the optimal dose and timing of systemic folinic acid administration.

Although there is no evidence to support the use of folinic acid mouthwash for prevention of mucositis, folinic acid mouthwashes have been given to patients who received MTX administration in some centers.[24–26] In our study, multivariate analysis showed that there was a tendency for a lower incidence of severe oral mucositis in patients who received folinic acid mouthwash (OR = 0.39, 95%CI 0.15–1.00, $P = 0.051$). Not only systemic folinic acid administration but also folinic acid mouthwash significantly reduced the use of opioid analgesics and the duration of inability to eat. Therefore, it is likely that folinic acid mouthwash had a positive effect on the prevention of severe oral mucositis.

In multivariate analysis, duration of neutropenia (more than 21 days) was significantly associated with severe oral mucositis (OR = 4.78, 95%CI 1.77–13.9, $P = 0.0019$). The mean duration of neutropenia was 18.3 days in our study. Therefore, the cutoff point of duration of neutropenia appeared to be 3 weeks (21 days). The use of folinic acid did not reduce the duration of neutropenia in our study (17.3 days vs 18.6 days, $P = 0.53$). It is important to reduce the duration of neutropenia to prevent severe oral mucositis.

Hoyt *et al.*[27] reported that etoposide induces more severe mucositis than dose CY when added to TBI. In our study, there was a tendency for a higher incidence of severe oral mucositis in patients who received VP/CY/TBI (OR = 2.42, 95%CI 0.86–6.99, $P = 0.095$). A VP/CY/TBI regimen may increase the incidence of severe oral mucositis compared with the effects of other conditioning regimens. In patients with a high risk of severe oral mucositis, use of MTX for GVHD prophylaxis may cause more severe oral mucositis. Therefore, systemic folinic acid administration may be useful to reduce the incidence of severe oral mucositis in patients who have received a VP/CY/TBI regimen.

Although Takahashi *et al.*[1] reported that the severity of oral mucositis was reduced in reduced intensity stem cell transplantation (RIST) patients compared with that in conventional stem cell transplantation (CST) patients, no significant difference was observed in the incidence of severe oral mucositis between patients who received CST and those who received RIST in our study. Several studies have shown that severe oral mucositis was correlated with TBI.[28] One reason for no significant difference being found in our study might be the use of TBI in most RIST patients.

Although Sonis *et al.*[5] reported that oral mucositis is associated with significantly worse economic outcomes, there was no difference in the duration of total parenteral nutrition in our study. We were not able to show the cost effectiveness of the use of folinic acid. Further studies are needed to clarify the cost effectiveness.

In this retrospective study, systemic folinic acid administration and mouthwash appear to be useful for reducing the incidence of severe oral mucositis in those patients who were considered by physicians to be at high risk for severe oral mucositis. Further prospective controlled studies are needed to assess the efficacy of systemic folinic acid administration and mouthwash.

## Conflict of interest

The authors declare no conflict of interest.

## Acknowledgements

We thank Ms M Yamane, Ms M Mayanagi and Ms Y Ishimaru for their technical assistance.

## References

1 Takahashi K, Soga Y, Murayama Y, Udagawa M, Nishimoto H, Sugiura Y *et al.* Oral mucositis in patients receiving reduced-intensity regimens for allogeneic hematopoietic cell transplantation: comparison with conventional regimen. *Support Care Cancer* 2010; **18**: 115–119.
2 Vokurka S, Steinerova K, Karas M, Koza V. Characteristics and risk factors of oral mucositis after allogeneic stem cell transplantation with FLU/MEL conditioning regimen in context with BU/CY2. *Bone Marrow Transplant* 2009; **44**: 601–605.
3 Langner S, Staber P, Schub N, Gramatzki M, Grothe W, Behre G *et al.* Palifermin reduces incidence and severity of oral mucositis in allogeneic stem-cell transplant recipients. *Bone Marrow Transplant* 2008; **42**: 275–279.
4 Sonis ST, Elting LS, Keefe D, Peterson DE, Schubert M, Hauer-Jensen M *et al.* Perspectives on cancer therapy-induced mucosal injury: pathogenesis, measurement, epidemiology, and consequences for patients. *Cancer* 2004; **100**: 1995–2025.
5 Sonis ST, Oster G, Fuchs H, Bellm L, Bradford WZ, Edelsberg J *et al.* Oral mucositis and the clinical and economic outcomes of hematopoietic stem-cell transplantation. *J Clin Oncol* 2001; **19**: 2201–2205.
6 Cutler C, Li S, Kim H, Laglenne P, Szeto K, Hoffmeister L *et al.* Mucositis after allogeneic hematopoietic stem cell transplantation: a cohort study of methotrexate-and non-methotrexate-containing graft-versus-host disease prophylaxis regimens. *Biol Blood Marrow Transplant* 2005; **11**: 383–388.
7 Bolwell B, Sobecks R, Pohlman B, Andresen S, Rybicki L, Kuczkowski E *et al.* A prospective randomized trial comparing cyclosporine and short course methotrexate with cyclosporine and mycophenolate mofetil for GVHD prophylaxis in myeloablative allogeneic bone marrow transplantation. *Bone Marrow Transplant* 2004; **34**: 621–625.
8 Torres A, Martinez F, Gomez P, Herrera C, Rojas R, Gomez-Villagran JL *et al.* Cyclosporin A versus methotrexate, followed by rescue with folinic acid as prophylaxis of acute graft-versus-host disease after bone marrow transplantation. *Blut* 1989; **58**: 63–68.
9 Nevill TJ, Tirgan MH, Deeg HJ, Klingemann HG, Reece DE, Shepherd JD *et al.* Influence of post-methotrexate folinic acid rescue on regimen-related toxicity and graft-versus-host disease after allogeneic bone marrow transplantation. *Bone Marrow Transplant* 1992; **9**: 349–354.
10 Russell JA, Woodman RC, Poon MC, Jones AR, Ruether BA. Addition of low-dose folinic acid to a methotrexate/cyclosporin A regimen for prevention of acute graft-versus-host disease. *Bone Marrow Transplant* 1994; **14**: 397–401.
11 Ruutu T, Niederwieser D, Gratwohl A, Apperley JF. A survey of the prophylaxis and treatment of acute GVHD in Europe: a report of the European Group for Blood and Marrow, Transplantation (EBMT). Chronic Leukaemia Working Party of the EBMT. *Bone Marrow Transplant* 1997; **19**: 759–764.
12 Bhurani D, Schifter M, Kerridge I. Folinic acid administration following MTX as prophylaxis for GVHD in allogeneic HSCT centres in Australia and New Zealand. *Bone Marrow Transplant* 2008; **42**: 547–550.
13 Shigematsu A, Kondo T, Yamamoto S, Sugita J, Onozawa M, Kahata K *et al.* Excellent outcome of allogeneic hematopoietic stem cell transplantation using a conditioning regimen with medium-dose VP-16, cyclophosphamide and total-body irradiation for adult patients with acute lymphoblastic leukemia. *Biol Blood Marrow Transplant* 2008; **14**: 568–575.
14 Takahata M, Hashino S, Okada K, Onozawa M, Kahata K, Sugita J *et al.* Reduced intensity conditioning regimen with fludarabine, busulfan, and low-dose TBI (Flu-BU2-TBI): clinical efficacy in high-risk patients. *Am J Hematol* 2010; **85**: 243–248.

15 Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J *et al.* 1994 Consensus conference on acute GVHD grading. *Bone Marrow Transplant* 1995; **15**: 825–828.
16 Gray RJ. A class of K-sample tests for comparing the cumulative incidence of a competing risk. *Ann stat* 1988; **16**: 1141–1154.
17 Vokurka S, Bystrická E, Koza V, Scudlová J, Pavlicová V, Valentová D *et al.* Higher incidence of chemotherapy induced oral mucositis in females: a supplement of multivariate analysis to a randomized multicentre study. *Support Care Cancer* 2006; **14**: 974–976.
18 Vokurka S, Bystrická E, Koza V, Scudlová J, Pavlicová V, Valentová D *et al.* The comparative effects of povidone-iodine and normal saline mouthwashes on oral mucositis in patients after high-dose chemotherapy and APBSCT—results of a randomized multicentre study. *Support Care Cancer* 2005; **13**: 554–558.
19 Gratwohl A. Folinic acid administration following MTX as prophylaxis for GVHD. *Bone Marrow Transplant* 2009; **44**: 257.
20 Gratwohl AA, Bull MI, Graw RG, Norton L, Knutsen T. Methotrexate and citrovorum factor after histoincompatible allogeneic bone marrow transplants in dogs. *Acta Haematol* 1978; **60**: 233–242.
21 Gratwhol A, Deisseroth A. Suppression of humoral immune response with methotrexate and citroforum factor. In: International Society of Chemotherapy (eds) *Current Chemotherapy*. American Society for Microbiology: Washington, DC, USA, 1978, pp 1262–1263.
22 Peters C, Minkov M, Gadner H, Klingebiel T, Vossen J, Locatelli F *et al.* Statement of current majority practices in graft-versus-host disease prophylaxis and treatment in children. *Bone Marrow Transplant* 2000; **26**: 405–411.
23 Gori E, Arpinati M, Bonifazi F, Errico A, Mega A, Alberani F *et al.* Cryotherapy in the prevention of oral mucositis in patients receiving low-dose methotrexate following myeloablative allogeneic stem cell transplantation: a prospective randomized study of the Gruppo Italiano Trapianto di Midollo Osseo nurses group. *Bone Marrow Transplant* 2007; **39**: 347–352.
24 Stark AN, Jackson G, Carey PJ, Arfeen S, Proctor SJ. Severe renal toxicity due to intermediate-dose methotrexate. *Cancer Chemother Pharmacol* 1989; **24**: 243–245.
25 van den Bongard HJ, Mathjt RA, Boogerd W, Schornagel JH, Soesan M, Schellens JH *et al.* Successful rescue with leucovorin and thymidine in a patient with high-dose methotrexate induced acute renal failure. *Cancer Chemother Pharmacol* 2001; **47**: 537–540.
26 Craig JV, Gibson F, Glenny AM, On behalf of Children's Cancer and Leukaemia Group and Paediatric Oncology Nurses Forum (CCLG–PONF) Mouth Care Group. Audit to monitor the uptake of national mouth care guidelines for children and young people being treated for cancer. *Support Care Cancer* 2010 (e-pub ahead of print 31 July 2010; doi: 10.1007/s00520-010-0953-3).
27 Hoyt R, Ritchie DS, Wirth A, Szer J, Grigg AP. Etoposide induces more severe mucositis than CY when added to TBI as conditioning in allograft recipients receiving CsA and MTX. *Bone Marrow Transplant* 2010; **45**: 1457–1462.
28 Robien K, Schubert M, Bruemmer B, Lloid M, Potter J, Ulrich C. Predictors of oral mucositis in patients receiving hematopoietic cell transplants for chronic myelogenous leukemia. *J Clin Oncol* 2004; **22**: 1268.