

Brief report

Successful engraftment after reduced-intensity umbilical cord blood transplantation for myelofibrosis

Shinsuke Takagi,¹ Yasunori Ota,² Naoyuki Uchida,¹ Koichi Takahashi,¹ Kazuya Ishiwata,¹ Masanori Tsuji,¹ Hisashi Yamamoto,¹ Yuki Asano-Mori,¹ Naofumi Matsuno,¹ Kazuhiro Masuoka,³ Atsushi Wake,¹ Shigesaburo Miyakoshi,⁴ Kenichi Ohashi,² and Shuichi Taniguchi^{1,5}

Departments of ¹Hematology and ²Pathology, Toranomon Hospital, Tokyo; ³Department of Hematology, Mishuku Hospital, Tokyo; ⁴Department of Hematology, Tokyo Metropolitan Geriatric Hospital, Tokyo; and ⁵Okinaka Memorial Institute for Medical Research, Tokyo, Japan

Although allogeneic hematopoietic stem cell transplantation has recently been applied to patients with myelofibrosis with reproducible engraftment and resolution of marrow fibrosis, no data describe the outcomes of umbilical cord blood transplantation. We describe 14 patients with primary (n = 1) and secondary myelofibrosis (n = 13) who underwent reduced-

intensity umbilical cord blood transplantation. Conditioning regimens included fludarabine and graft-versus-host disease prophylaxis composed cyclosporine/tacrolimus alone (n = 6) or a combination of tacrolimus and mycophenolate mofetil (n = 8). Thirteen patients achieved neutrophil engraftment at a median of 23 days. The cumulative incidence of neutrophil

and platelet engraftment was 92.9% at day 60 and 42.9% at day 100, respectively. Posttransplantation chimerism analysis showed full donor type in all patients at a median of 14 days. The use of umbilical cord blood could be feasible even for patients with severe marrow fibrosis, from the viewpoint of donor cell engraftment. (Blood. 2010;116(4):649-652)

Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is considered the only curative therapy for primary myelofibrosis (MF) and MF secondary to hematologic malignancies.¹ Myeloablative conditioning regimens are associated with high rates of transplantation-related mortality (TRM), especially among elderly patients.²⁻⁴ Recent reports indicate that reduced-intensity conditioning (RIC) regimens can improve outcomes in such patients.⁵⁻⁸ These reports also confirm the safety and effectiveness of bone marrow (BM) and mobilized peripheral blood stem cells (PBSCs) from matched related or unrelated donors as stem cell sources. In contrast, the feasibility of umbilical cord blood transplantation (CBT) for MF is unknown.

CBT is a valuable alternative to allo-HSCT for treating patients with hematologic diseases who do not have matched related or unrelated donors and who need urgent transplantation.⁹⁻¹² On the other hand, engraftment delay or failure is one of the most critical issues that can arise after CBT. The limited doses of total nucleated cells and CD34⁺ cells in umbilical cord blood and a human leukocyte antigen (HLA) disparity influence the kinetics of hematopoietic recovery.¹³⁻¹⁵ Considering these disadvantages of CBT, delayed engraftment or engraftment failure is a great concern for MF patients who undergo CBT.¹⁶ The goal of this study is to evaluate the feasibility of reduced-intensity CBT (RI-CBT) for MF.

regimen. Marrow fibrosis was assessed on silver-stained BM trephine biopsies and classified into 4 grades according to the World Health Organization classification.¹⁷ All the patients were incurable using conventional approaches and lacked an HLA-identical sibling or a suitable unrelated donor from the Japan Marrow Donor Program. Cord blood units serologically matching more than or equal to 4 of 6 HLA antigens and containing at least 1.8×10^7 nucleated cells/kg of recipient body weight before freezing were obtained from the Japan Cord Blood Bank Network. Conditioning regimens were determined at the discretion of each physician according to the patients' disease, disease status, and history of prior therapy. Information about baseline demographics, clinical characteristics, transplantation, and its outcome were collected from medical records. Assessment of engraftment, chimerism (one or more times a week), pre-engraftment immune reactions, graft-versus-host disease (GVHD), and supportive care during transplantation were performed as previously reported.¹⁸⁻²⁰ Cumulative incidences were estimated for neutrophil and platelet engraftment. Overall survival was estimated using the Kaplan-Meier method, taking the interval from date of transplantation to death or last contact.²¹ The Institutional Review Board of Toranomon Hospital approved the study, and written informed consent was provided by all patients to use their records in accordance with the Declaration of Helsinki.

Results and discussion

Fourteen MF patients (median age, 57.5 years; range, 46-72 years) were extracted. Table 1 shows the clinical characteristics of the patients. They had primary MF (n = 1), leukemic transformation from MF secondary to polycythemia vera or essential thrombocytosis (n = 2), or MF secondary to acute myeloid leukemia (AML; n = 11; AML with multilineage dysplasia in all patients except for one with de novo AML). All but one patient had the highest-grade

Methods

The records of all patients who underwent RI-CBT at Toranomon Hospital from August 2003 and December 2008 were reviewed to identify patients who had histologically confirmed MF before starting the conditioning

payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

© 2010 by The American Society of Hematology

Submitted November 10, 2009; accepted April 19, 2010. Prepublished online as *Blood* First Edition paper, May 3, 2010; DOI 10.1182/blood-2009-11-252601.

The publication costs of this article were defrayed in part by page charge

Table 1. Patient characteristics

Patient no.	Age, y/sex	Diagnosis	Disease status	Time from diagnosis to transplantation, d	Pretransplantation MF grade	Splenomegaly	Cytogenetics
1	55/M	AML/MF/ET	PIF	1732	3	Yes	Normal
2	53/M	PMF	Untreated	307	3	Yes	NA
3	61/M	AML/MDS	PIF	116	3	Yes	Complex*
4	51/F	AML/MDS	PIF	740	3	Yes	Normal
5	61/F	AML/MDS	PIF	227	3	Yes	NA
6	55/M	AML/MDS	Untreated	299	3	Yes	Complex
7	46/M	AML/MDS	Untreated	600	3	Yes	NA
8	58/M	AML/MDS	Untreated	544	3	Yes	Complex
9	67/F	AML/MF/PV	Untreated	150	3	Yes	t(3;3)(q21;q26), -7
10	53/M	De novo AML	PIF	111	3	No	Complex
11	57/F	AML/MDS	Untreated	352	3	Yes	Complex with t(9;22)(q34;q11)
12	62/M	AML/MDS	Untreated	147	3	Yes	add(1)(p32), -7
13	72/F	AML/MDS	PIF	329	2	No	Complex with t(9;22)(q34;q11)
14	66/M	AML/MDS	Untreated	92	3	No	Normal

AML indicate acute myeloid leukemia; MF, myelofibrosis; ET, essential thrombocythemia; PIF, primary induction failure; PMF, primary myelofibrosis; AML/MDS, acute myeloid leukemia with multilineage dysplasia; NA, not available; and PV, polycythemia vera.

*Complex karyotype was defined as 3 or more abnormalities at pretransplantation evaluation.

MF. The median time from diagnosis to transplantation was 303 days (range, 92-1732 days). Table 2 shows the transplantation characteristics. All received purine analog–based conditioning regimens composing fludarabine phosphate (125-180 mg/m²), melphalan (80-140 mg/m²), or intravenous busulfan (12.8 mg/kg) and 0 to 8 Gy of total body irradiation. GVHD prophylaxis included tacrolimus and mycophenolate mofetil for 8 patients, tacrolimus, or cyclosporine A alone in 6. Neutrophil and platelet engraftment was achieved in 13 and 6 patients, respectively, of the 14 patients. The median time to engraftment was 23 days (range, 14-43 days) and 53 days (range, 44-102 days) for neutrophils and platelets, respectively. The cumulative incidence of neutrophil engraftment at day 60 and platelet engraftment at day 100 was 92.9% and 42.9%, respectively (Figure 1A-B). Chimerism analysis of the peripheral blood of 8 patients and the BM of 6 showed that donor chimerism was complete (donor > 90%) in all of them. The median length of time required to achieve complete donor chimerism was 14 days (range, 7-33 days; Figure 1A). Of the 14 patients, 9 (64%) developed pre-engraftment immune reactions. Five (36%) developed acute GVHD grades 2 to 4. No extensive chronic GVHD was observed in 6 evaluable patients (Table 3). Five patients remained alive at last contact, representing an estimated probability of

overall survival of 28.6% at 4 years (Figure 1C). All the patients who could not achieve platelet engraftment died, whereas 4 of 7 patients (57%) who achieved platelet engraftment survived. In 9 patients who died after RI-CBT, 5 patients died of relapsed leukemia. Non-relapse-related causes of death composed infection (n = 2), GVHD (n = 1), and multiple organ failure (n = 1). Marrow fibrosis disappeared in 2 evaluated patients who survived beyond 100 days.

This study demonstrated that umbilical cord blood results in successful engraftment, even for patients with severe marrow fibrosis in the setting of the RIC regimen, which was similar to that of other stem cell sources, such as BM and PBSCs.^{2-8,22} Although marrow fibrosis has historically been considered as a relative contraindication to transplantation because of concerns over an insufficient and/or dysfunctional niche in which allogeneic hematopoietic stem cell engraftment may proceed, recent outcomes of allo-HSCT for MF support the concept that marrow fibrosis is not an absolute barrier to allogeneic hematopoietic stem cell engraftment.¹ However, data from these reports are limited to transplantations with BM and PBSCs, and no information is available about umbilical cord blood. Delayed hematopoietic recovery and low engraftment rate, perhaps because of limited infused cell doses and

Table 2. Transplantation characteristics

Patient no.	TNC, ×10 ⁷ /kg	CD34 ⁺ , ×10 ⁵ /kg	Sex match	HLA match	Blood type match	Conditioning regimen	GVHD prophylaxis
1	2.52	0.823	MM	4/6	MM	F125/M80/TBI4	CsA
2	2.62	0.678	MM	4/6	MM	F125/M80/TBI4 + SRT	TAC
3	3.17	1.60	Match	4/6	Match	F125/M80/TBI4	TAC
4	2.43	NA	MM	4/6	Match	F125/M80/TBI4	TAC
5	3.94	2.26	MM	5/6	Match	F180/M140	TAC/MMF
6	2.31	0.887	MM	4/6	MM	F125/M80/TBI4	TAC
7	2.72	1.03	Match	4/6	MM	F125/Me140/TBI4	TAC/MMF
8	2.46	0.773	MM	4/6	Match	F180/M140	TAC
9	1.99	1.24	MM	4/6	MM	F125/M80/TBI4 + SRT	TAC/MMF
10	3.25	0.547	MM	4/6	Match	F125/M140/TBI4	TAC/MMF
11	3.31	1.31	MM	4/6	Match	F125/M80/TBI8	TAC/MMF
12	2.37	0.873	MM	4/6	MM	F125/M80/TBI8	TAC/MMF
13	2.51	0.993	MM	4/6	Match	Flu180/B12.8/TBI2	TAC/MMF
14	2.50	0.554	MM	5/6	Match	F125/M120	TAC/MMF

TNC indicates total nucleated cell count; MM, mismatch; F, fludarabine (mg/m²); M, melphalan (mg/m²); TBI, total body irradiation; CsA, cyclosporine; SRT, splenic radiation; TAC, tacrolimus; MMF, mycophenolate mofetil; and B, intravenous busulfan (mg/kg).

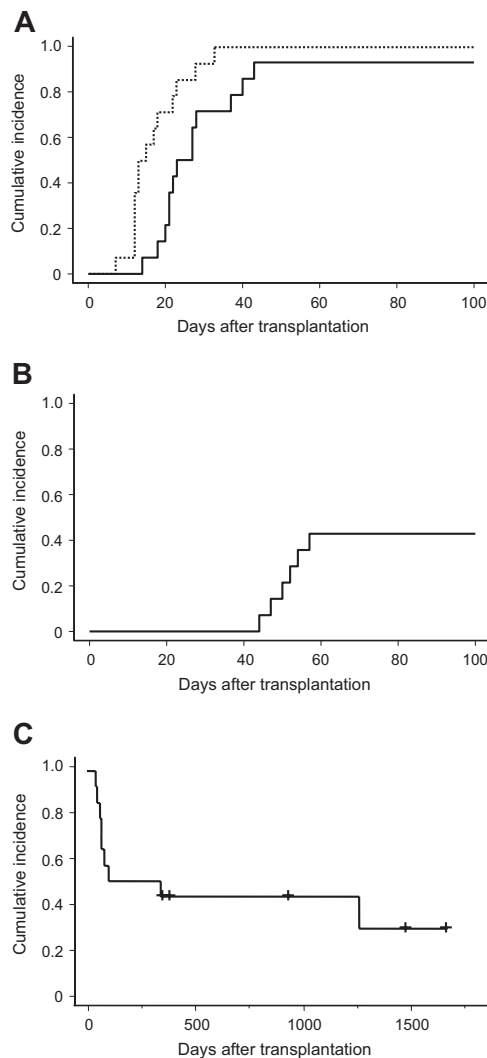


Figure 1. Cumulative incidence of engraftment. (A) Solid and broken lines indicate cumulative incidence of neutrophil engraftment and complete donor chimerism, respectively. (B) Cumulative incidence of platelet engraftment. (C) Overall survival.

HLA disparities, might limit the use of umbilical cord blood in these cases.^{13-15,19} However, the present study demonstrated an equivalent or superior engraftment rate after CBT compared with allo-HSCT using other stem cell sources.¹⁻⁸ We also confirmed an early chimerism switching in the present study. All 14 patients achieved complete donor chimerism at a median of 14 days, which was much earlier than that with neutrophil engraftment. Moreover, we histologically confirmed that RI-CBT had the potential to cure marrow fibrosis in 2 evaluated patients. These data suggest that RI-CBT is an encouraging strategy for treating MF.

Despite successful engraftment, overall survival was poor in the present study compared with previous reports. However, this result does not eliminate the feasibility of RI-CBT for MF patients. Our patient series included only one primary MF. In 13 of 14 patients, MF coexisted with AML simultaneously. High prevalence of concurrent AML with MF in the present study probably made overall survival poorer. However, MF with AML is also challenging issues in real clinical settings. Physicians occasionally face rapidly growing AML cases with concurrent marrow fibrosis, especially in the elderly, for whom urgent allo-HSCT is the only curative therapy. For those patients, CBT is attractive because of its accessibility. In this viewpoint, we think that the feasibility of RI-CBT suggested in the present study is encouraging.

In conclusion, our data suggest that RI-CBT is feasible, even for patients with severe marrow fibrosis, from the viewpoint of donor cell engraftment. Especially for MF with AML, further improvements are required in the next place to overcome poor survival resulting from relapse.

Acknowledgments

The authors thank all physicians (Dr Akiko Yoneyama, Dr Shigeyoshi Makino, Dr Hideki Araoka), nurses, pharmacists (Mr Tadaaki Ito, Ms Yumiko Uchida), transplantation coordinator (Ms Madoka Narita), data managers (Ms Naomi Yamada, Ms Kaoru Kobayashi, and Ms Rumiko Tsuchihashi), and support personnel for their care of the patients involved in this study.

This work was supported in part by the Japanese Ministry of Health, Labor, and Welfare (Research Grant for Tissue Engineering H17-014 and Research Grant for Allergic Disease and Immunology H20-015).

Table 3. Outcome of RI-CBT

Patient no.	Neutrophil engraftment, d	Platelet engraftment, d	Pre-engraftment immune reactions*	aGVHD 2-4	aGVHD 3-4	cGVHD	Survival	Survival from transplantation, d	Cause of death
1	27	52	No	Yes	No	No	Dead	1264	Relapse
2	22	54	Yes	No	No	NE	Alive	1672	NA
3	23	Not engrafted	Yes	Yes	Yes	NE	Dead	68	Infection
4	40	102	Yes	Yes	No	Limited	Alive	1481	NA
5	18	44	Yes	No	No	No	Dead	344	Relapse
6	14	Not engrafted	Yes	No	No	NE	Dead	78	Relapse
7	21	57	Yes	Yes	Yes	Limited	Alive	937	NA
8	Not engrafted	Not engrafted	No	No	No	NE	Dead	42	Infection
9	37	Not engrafted	Yes	No	No	NE	Dead	45	MOF
10	28	Not engrafted	Yes	Yes	Yes	NE	Dead	64	GVHD
11	27	Not engrafted	Yes	No	No	NE	Dead	61	Relapse
12	43	NA	No	No	No	Limited	Alive	392	NA
13	21	47	No	No	No	Limited	Alive	355	NA
14	20	50	No	No	No	NE	Dead	100	Relapse

aGVHD indicates acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; NE, not evaluable; NA, not applicable; and MOF, multiple organ failure.

*Pre-engraftment immune reactions were diagnosed when febrile patients developed skin eruption, diarrhea, jaundice, or body weight gain of more than 10% of baseline, with no direct evidence of infection or adverse effects of medication, developing more than 6 days before engraftment.¹⁸

Authorship

Contribution: S. Takagi performed transplantation, analyzed extracted data, and contributed to writing the paper; Y.O. analyzed histologic sections; N.U., K.T., K.I., M.T., H.Y., Y.A.-M., K.M., A.W., and S.M. performed transplantation and contributed to writing the paper; N.M. performed transplantation and supported

statistical analysis; K.O. reviewed histologic sections and contributed to writing the paper; and S. Taniguchi reviewed the study method and organized this study.

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

Correspondence: Shinsuke Takagi, Department of Hematology, Toranomon Hospital, 2-2-2 Toranomon, Minato-Ku, Tokyo 105-0001, Japan; e-mail: shinsuke-takagi@umin.net.

References

- Zang DY, Deeg HJ. Allogeneic hematopoietic cell transplantation for patients with myelofibrosis. *Curr Opin Hematol*. 2009;16(2):140-146.
- Guardiola P, Anderson JE, Bandini G, et al. Allogeneic stem cell transplantation for agnogenic myeloid metaplasia: a European Group for Blood and Marrow Transplantation, Societe Francaise de Greffe de Moelle, Gruppo Italiano per il Trapianto del Midollo Osseo, and Fred Hutchinson Cancer Research Center Collaborative Study. *Blood*. 1999;93(9):2831-2838.
- Deeg HJ, Gooley TA, Flowers ME, et al. Allogeneic hematopoietic stem cell transplantation for myelofibrosis. *Blood*. 2003;102(12):3912-3918.
- Daly A, Song K, Nevill T, et al. Stem cell transplantation for myelofibrosis: a report from two Canadian centers. *Bone Marrow Transplant*. 2003;32(1):35-40.
- Rondelli D, Barosi G, Bacigalupo A, et al. Allogeneic hematopoietic stem-cell transplantation with reduced-intensity conditioning in intermediate- or high-risk patients with myelofibrosis with myeloid metaplasia. *Blood*. 2005;105(10):4115-4119.
- Snyder DS, Palmer J, Stein AS, et al. Allogeneic hematopoietic cell transplantation following reduced intensity conditioning for treatment of myelofibrosis. *Biol Blood Marrow Transplant*. 2006;12(11):1161-1168.
- Kroger N, Zabelina T, Schieder H, et al. Pilot study of reduced-intensity conditioning followed by allogeneic stem cell transplantation from related and unrelated donors in patients with myelofibrosis. *Br J Haematol*. 2005;128(5):690-697.
- Merup M, Lazarevic V, Nahi H, et al. Different outcome of allogeneic transplantation in myelofibrosis using conventional or reduced-intensity conditioning regimens. *Br J Haematol*. 2006;135(3):367-373.
- Barker JN, Davies SM, DeFor T, Ramsay NK, Weisdorf DJ, Wagner JE. 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(10):2957-2961.
- 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(22):2265-2275.
- Rocha V, Labopin M, Sanz G, et al. Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia. *N Engl J Med*. 2004;351(22):2276-2285.
- Takahashi S, Iseki T, Ooi J, et al. Single-institute comparative analysis of unrelated bone marrow transplantation and cord blood transplantation for adult patients with hematologic malignancies. *Blood*. 2004;104(12):3813-3820.
- 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(5):1611-1618.
- Gluckman E, Rocha V, Arcese W, et al. Factors associated with outcomes of unrelated cord blood transplant: guidelines for donor choice. *Exp Hematol*. 2004;32(4):397-407.
- Terakura S, Azuma E, Murata M, et al. Hematopoietic engraftment in recipients of unrelated donor umbilical cord blood is affected by the CD34+ and CD8+ cell doses. *Biol Blood Marrow Transplant*. 2007;13(7):822-830.
- Rajantie J, Sale GE, Deeg HJ, et al. Adverse effect of severe marrow fibrosis on hematologic recovery after chemoradiotherapy and allogeneic bone marrow transplantation. *Blood*. 1986;67(6):1693-1697.
- Thiele J, Kvasnicka HM, Tefferi A, Barosi G, Orazi A, Vardiman JW. Primary myelofibrosis. In: *World Health Organization Classification of Tumours of Haematopoietic and Lymphoid Tissues*, 4th edition. Lyon, France: IARC Press; 2008:44-47.
- Kishi Y, Kami M, Miyakoshi S, et al. Early immune reaction after reduced-intensity cord-blood transplantation for adult patients. *Transplantation*. 2005;80(1):34-40.
- 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(8):1689-1695.
- 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(4):543-553.
- Kaplan E, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc*. 1958;53:457-481.
- Rondelli D. Allogeneic hematopoietic stem cell transplantation for myelofibrosis. *Haematologica*. 2008;93(10):1449-1450.