Bone Marrow Transplantation (2006) 38, 665–669

www.nature.com/bmt

ORIGINAL ARTICLE

Nonmyeloablative stem cell transplantation for nonmalignant diseases in children with severe organ dysfunction

A Kikuta[1], M Ito[1], K Mochizuki[1], M Akaihata[1], K Nemoto[1], H Sano[1] and H Ohto[2]

[1]*Department of Pediatrics, Fukushima Medical University School of Medicine, Fukushima, Japan and* [2]*Division of Blood Transfusion and Transplantation Immunology, Fukushima Medical University School of Medicine, Fukushima, Japan*

Allogeneic stem cell transplantation (SCT) can cure several nonmalignant diseases in children. However, patients frequently have significant morbidity before transplantation and there is a high transplant-related mortality. Nonmyeloablative SCT might achieve the same goals but with less toxicity. Six pediatric patients with nonmalignant diseases underwent nonmyeloablative SCT from different stem cell sources. All patients were conditioned with fludarabine/melphalan with additional anti-thymocyte globulin for haploidentical grafts and prophylaxis for graft-versus-host disease (GVHD) consisting of tacrolimus and methotrexate with additional prednisolone for haploidentical grafts. Hematopoietic stem cells were neither T-cell depleted nor purged. All patients had severe organ dysfunction that precluded transplantation with conventional conditioning. Five of the six are alive and in complete disease resolution at a median of 19 months (range, 7–53 months) after SCT. One patient died of bacteremia before engraftment. Three patients achieved complete donor chimerism. Two patients remained stable mixed chimerism. Short-term toxicities were minimal. Acute and chronic GVHD were not seen. In summary, the fludarabine-based nonmyeloablative regimen followed by SCT provides a good approach for children with nonmalignant diseases. Even patients with severe organ dysfunctions had adequate engraftment with acceptable toxicities.

Bone Marrow Transplantation (2006) **38,** 665–669. doi:10.1038/sj.bmt.1705511; published online 2 October 2006

Keywords: nonmyeloablative stem cell transplantation; nonmalignant disease; child; organ dysfunction

Introduction

Allogeneic stem cell transplantation (SCT) has been proven as an effective means of treating various malignant and nonmalignant diseases.[1,2] The optimal approach to SCT in children with nonmalignant disease who have infection and organ dysfunction has yet to be determined. The beneficial effect of myeloablative regimens, however, is counterbalanced by increased short- and long-term toxicity. In particular, infection and organ dysfunction contribute to the high treatment-related mortality rates seen with conventionally conditioned SCT in older children who have acquired organ dysfunction.[3,4] Recently, some groups[5–7] have reported SCT for children with hematological malignancies and nonmalignancies who were on highly immunosuppressive but nonmyeloablative regimens. Such regimens are of interest for children with nonmalignant diseases, as there is no obvious need for intensive therapy or graft-versus-host disease (GVHD). In view of the experimental nature of such an approach, our initial studies were performed on patients in whom SCT with conventional conditioning therapy was contraindicated because of severe complications. We undertook a pilot study to determine engraftment rate, toxicity and GVHD associated with a fludarabine-based regimen[8] and tacrolimus-based GVHD prophylaxis[9–11] for children with nonmalignant diseases.

Patients and methods

Patients

Six consecutive patients with nonmalignant diseases at Fukushima Medical University underwent SCT (seven SCTs including one second SCT), all of whom were given a fludarabine-based nonmyeloablative conditioning regimen between April 2001 and October 2005. Patients' characteristics, including pre-SCT risk factors, are shown in Table 1. All of these patients would have been ineligible for a conventional myeloablative SCT, including two with severe combined immunodeficiency (SCID), two with severe chronic active Epstein–Barr virus infection in the active phase, one with chronic granulomatous disease (CGD) and one with hyper-IgM syndrome. The median

Correspondence: Dr A Kikuta, Department of Pediatrics, Fukushima Medical University School of Medicine, 1 Hikarigaoka, Fukushima City, Fukushima 960-1295, Japan.
E-mail: akikuta@fmu.ac.jp
Received 25 April 2006; revised 31 July 2006; accepted 2 September 2006; published online 2 October 2006

Table 1 Patient characteristics

UPN	*Disease*	*Age (years)/sex*	*Conditioning regimen*	*Donor*	*HLA match*	*TNC/kg ($\times 10^8$)*	*Risk factor*
1	CAEBV	8.1/M	FLU/MEL	MFD	6/6	12.7	Lung and liver damage
2	SCID	0.5/F	FLU/MEL	MFD	6/6	8.1	Severe VZV infection, encephalitis
3	HIGM	11.5/M	FLU/MEL	MUD	6/6	1.5	Bronchiectasis, chronic bronchitis
4-1	CGD	2.2/M	FLU/MEL/ATG	HIFD	3/6	5.7	Multiple abscesses in brain, lung, liver and skin
4-2	CGD	2.4/M	FLU/BU/TBI(2 Gy)	UCB	5/6	0.9	Second SCT after late graft failure, osteomyelitis
5	SCID	1.5/M	FLU/MEL	UCB	5/6	0.8	Disseminated BCG infection, bacteremia
6	CAEBV	16.4/F	FLU/MEL	MFD	6/6	9.4	Cardiac failure

Abbreviations: ATG = anti-thymocyte globulin; CAEBV = chronic active Epstein–Barr virus infection; CGD = chronic granulomatous disease; FLU = fludarabine; HIFD = haploidentical family donor; HIGM = hyper-IgM syndrome; MEL = melphalan; MFD = matched family donor; MUD = matched unrelated donor; SCID = severe combined immunodeficiency; SCT = stem cell transplantation; TNC = total nucleated cell dose; UCB = unrelated cord blood; VZV = varicella zoster virus.

age at transplantation was 5.2 years (range, 0.5–16.4 years). The HLA typing was confirmed by high-resolution typing in all cases except for unrelated cord blood (UCB), and UCB was confirmed by low-resolution typing. Hematopoietic stem cells were neither T-cell depleted nor purged. Approval was obtained from the Institutional Review Board of the Fukushima Medical University. Informed consent was obtained from patients or guardians before enrollment in a nonmyeloablative SCT protocol.

Conditioning regimen and transplantation

Five patients were conditioned with fludarabine (30 mg/m^2 once daily i.v. for 5 consecutive days) and melphalan (70 mg/m^2 once daily i.v. for 2 consecutive days) in the patients receiving matched family/unrelated donor transplantations. Patients receiving non-T-cell-depleted haploidentical family donor transplantations received rabbit anti-thymocyte globulin (ATG; 2.5 mg/kg once daily i.v. from days −4 to −1) together with fludarabine and melphalan as additional immunosuppression. One patient (UPN4) who showed late graft failure at the first transplantation received fludarabine (30 mg/m^2 once daily i.v. for 6 consecutive days), busulfan (4 mg/kg p.o. in divided doses daily for 2 days) and 2-Gy total body irradiation (TBI) in a single fraction at the second transplantation.

The median total nucleated cell dose (TNC) was 8.1×10^8/kg (range, 1.5–12.7 $\times 10^8$/kg) in bone marrow and peripheral blood stem cell and the median TNC was 0.85×10^8/kg (range, 0.8–0.9 $\times 10^8$/kg) in UCB. GVHD prophylaxis consisted of tacrolimus (0.03 mg/kg/day i.v. continuous infusion from day −1) and methotrexate (10 mg/m^2 i.v. on day 1, 7 mg/m^2 i.v. on days 3 and 6). When patients were able to tolerate oral intake, they were switched to oral tacrolimus at 0.3 mg/kg/day, with the dose divided into two and given every 12 h. The dose was adjusted to maintain trough levels of 7–15 ng/ml during 12 months after SCT and was tapered. Patients receiving non-T-cell-depleted haploidentical transplantations received 2 mg/kg prednisolone per day starting on day 0 together with tacrolimus and methotrexate. Prednisolone was tapered on day 14 in the absence of GVHD. The severity of GVHD was graded according to the consensus criteria.[12]

Supportive care

All patients received prophylaxis with trimethoprim–sulfamethoxazole against *Pneumocystis carinii* infection. They received broad-spectrum antibiotics, fluconazole and acyclovir for the prophylaxis of bacterial, fungal and herpesvirus infection, respectively. Additionally, one patient with SCID and disseminated Bacillus Calmette-Guerin (BCG) received antituberculosis therapy with rifampicin and isoniazid. Immunoglobulin (0.2 g/kg/dose, i.v.) was infused weekly until day 100, and then biweekly until 6 months after SCT. G-CSF (5 μg/kg/day) was started on day 1 following the infusion of stem cells. Cytomegalovirus (CMV) treatment with ganciclovir was started when CMV antigenemia was detected on routine weekly examination.

Analysis of chimerism

Chimerism was assessed by standard cytogenetic analysis in male/female donor–recipient combination and the various numbers of tandem repeats analysis with multiple informative alleles in sex-matched donor–recipient combinations.

Results

Toxicity and survival

Five of the six patients are alive and in complete disease resolution (CR) at a median of 19 months (range, 7–53 months). One patient (UPN5) had *Pseudomonas aeruginosa* bacteremia and disseminated BCG before transplantation and died on day 28 secondary to multi-organ failure. Of the five surviving patients, one patient (UPN4-2) had CMV and human herpesvirus 6 reactivation. One patient (UPN3) developed bacteremia and had CMV and varicella zoster virus reactivation. Three patients in total suffered from bacteremia. Lung bleeding in one and mild mucositis in one were observed and controlled. Veno-occlusive disease and Epstein–Barr virus reactivation were not seen.

Engraftment and chimerism

Primary engraftment occurred in five of the six patients. The median times to neutrophil ($>500/\mu$l) and platelet ($>2 \times 10^4/\mu$l) recovery were 15 days (range, 11–15 days) and 17 days (range, 14–24 days), respectively. Two patients achieved full donor chimerism on days 20 and 53. Two

patients remained stable mixed donor chimerism (50–84% donor type and 43–59% donor type) at 19 and 28 months, respectively. One patient (UPN4-1) with CGD who had multiple abscesses in the brain, lungs, liver and skin received granulocyte transfusion together with antibiotics at first SCT and these abscesses resolved. He achieved a maximum of 42% donor chimerism on day 30, but had late graft failure on day 77. He (UPN4-2) received the second transplantation from UCB and achieved full donor chimerism on day 28 after second SCT.

GVHD

Acute and chronic GVHD were not observed even in the second SCT (Table 2).

Immune reconstitution

Immune reconstitution was assessed by recovery of $CD3^+$, $CD4^+$ and $CD8^+$ T cells, phytohemagglutinin A (PHA) stimulation index, and $CD19^+$ B cells at a median of 19 months (range, 7–53 months) after SCT. All five evaluable patients have had good recovery of T-cell numbers, and have achieved normal age-adjusted CD3 counts. In all patients, CD8 recovery preceded CD4 recovery. Two of the five patients now have normal CD4 counts, and the remaining three patients still have low CD4 counts. With regard to T-cell function, all five patients have had normal PHA stimulation index at 6 months after SCT. In the current study, four of the five patients achieved normal CD19 counts, but none of the patients continued to undergo prophylactic i.v. immunoglobulin replacement therapy.

Quality of life

At the time of writing this report, five of the six patients are alive and well, and have Lansky scores of 90–100% at a median of 19 months after SCT.

Discussion

We have shown that reliable donor stem cell engraftment is possible by using nonmyeloablative conditioning for the patients with severe organ dysfunctions who could not be candidates for SCT with conventional conditioning. Most patients were cured and there was improvement with respect to their severe infections and organ dysfunctions. Although too small for statistical analysis, the overall survival data compare favorably with historical data,[13] particularly for the patients receiving HLA-mismatched transplantations. In our study, two patients receiving cells from an HLA-mismatched donor survive and are in CR. Viral reactivations were frequent, as previously reported for immunosuppressive conditioning,[14] but only one patient died of direct infectious causes.

Engraftment was one of the main end points of this study. Although the patient with SCID did not achieve primary engraftment, he had disseminated BCG infection and Gram-negative bacteremia at the time of transplantation, which was done as a heroic measure. The patient with CGD achieved primary engraftment but subsequently showed late graft failure. In this patient, late graft failure

Table 2 Hematological reconstitution and transplant outcome

UPN	*Disease*	*ANC > 500 days*	*PLT > 2 × 10⁴ days*	*Chimerism*			*GVHD*		*Complications*	*Outcome*
				Maximum	*Day of 100%*	*At last follow-up*	*Acute*	*Chronic*		
1	CAEBV	14	16	100%	53	100%	0	0	Hemolysis, bacteremia	Alive – CR, 53 months
2	SCID	11	14	59%	NE	57%	0	0	Lung bleeding	Alive – CR, 28 months
3	HIGM	15	24	84%	NE	50%	0	0	CMV, VZV, bacteremia	Alive – CR, 19 months
4-1	CGD	15	17	42%	NE	NE	0	NE	Late graft failure	day 77
4-2	CGD	17	59	100%	28	100%	0	0	CMV, HHV6	Alive – CR, 15 months
5	SCID	NE	NE	NE	NE	NE	NE	NE	Bacteremia, MOF, disseminated BCG	Dead, day 28
6	CAEBV	14	14	100%	20	100%	0	0	—	Alive – CR, 7 months

Abbreviations: BCG = Bacillus Calmette-Guerin; CAEBV = chronic active Epstein–Barr virus infection; CGD = chronic granulomatous disease; CMV = cytomegalovirus; CR = complete disease resolution; GVHD = graft-versus-host disease; HIGM = hyper-IgM syndrome; HHV = human herpesvirus; MOF = multi-organ failure; NE = non-evaluable; SCID = severe combined immunodeficiency; VZV = varicella zoster virus.

occurred after the first non-T-cell-depleted HLA haploidentical SCT, despite the sufficient number of infused TNC. The possible risk factors for graft failure in our case seemed to be donor–recipient HLA mismatch, and the first conditioning regimen (fludarabine/melphalan/ATG) may have been insufficient to achieve stable engraftment.[6] The second conditioning including 2-Gy TBI might account for the high rates of engraftment.[15–17]

Although most groups[7,18] using nonmyeloablative conditioning for SCT have reported an incidence of acute GVHD comparable to that seen with conventional SCT, we did not observe acute GVHD. This likely reflects, at least in patients, the younger age of our patients, but it may also be related to our use of tacrolimus. Furthermore, the use of ATG in our regimen for non-T-cell-depleted haploidentical BMT from his mother, so close to the time of stem cell infusion, might have effectively induced partial T-cell depletion *in vivo*.[19,20] It is difficult to comment on the frequency of chronic GVHD, but the fact that this complication did not occur is encouraging. Tacrolimus-based prophylaxis regimens are of interest for nonmalignant diseases as there is no obvious need for GVHD.

Finally, we have demonstrated that nonmyeloablative SCT permits rapid engraftment from family and unrelated, matched and mismatched donors with minimal toxicity, even in the presence of severe organ dysfunctions. Clearly, one of the difficulties in drawing conclusion is that we describe heterogeneous diseases with different stem cell sources used. Moreover, the follow-up period is short. Because we avoided conventional-dose TBI and high-dose chemotherapy, we expect that long-term side effects of myeloablative SCT, such as growth retardation and learning disabilities, will be absent in the future of the children of this investigation.

Acknowledgements

This study was supported partially by grants from the Fukushima Society for the Promotion of Medicine.

References

1 Giralt S, Estey E, Albitar M, van Besien K, Rondon G, Anderlini P *et al*. Engraftment of allogeneic hematopoietic progenitor cells with purine analog-containing chemotherapy: harnessing graft-versus-leukemia without myeloablative therapy. *Blood* 1997; **89**: 4531–4536.
2 Niederwieser D, Maris M, Shizuru JA, Petersdorf E, Hegenbart U, Sandmaier BM *et al*. Low-dose total body irradiation (TBI) and fludarabine followed by hematopoietic cell transplantation (HCT) from HLA-matched or mismatched unrelated donors and postgrafting immunosuppression with cyclosporine mycophenolate mofetil (MMF) can induce durable complete chimerism and sustained remissions in patients with hematological diseases. *Blood* 2003; **101**: 1620–1629.
3 Fischer A, Friedrich W, Fasth A, Blanche S, Deist FL, Girault D *et al*. Reduction of graft failure by monoclonal antibody (anti-LFA-1 CD11a) after HLA nonidentical bone marrow transplantation in children with immunodeficiencies, osteopetrosis, and Fanconi's anemia: a European group for immunodeficiency/European Group for Bone Marrow Transplantation Report. *Blood* 1991; **77**: 249–256.
4 Antoine C, Müller S, Cant A, Cavazzana-Calvo M, Veys P, Vossen J *et al*. Long-term survival and transplantation of haematopoietic stem cells for immunodeficiencies: report of European experience 1968–99. *Lancet* 2003; **361**: 553–560.
5 Jacobsohn DA, Emerick KM, Scholl P, Melin-Aldana H, O'Gorman M, Duerst R *et al*. Nonmyeloablative hematopoietic stem cell transplant for X-linked hyper-immunoglobulin M syndrome with cholangiopathy. *Pediatrics* 2004; **113**: 122–127.
6 Horwitz ME, Barret AJ, Brown MR, Carter CS, Childs R, Gallin JI *et al*. Treatment of chronic granulomatous disease with nonmyeloablative conditioning and a T-cell-depleted hematopoietic allograft. *N Engl J Med* 2001; **344**: 881–888.
7 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.
8 Amrolia P, Gaspar HB, Hassan A, Webb D, Jones A, Sturt N *et al*. Nonmyeloablative stem cell transplantation for congenital immunodeficiencies. *Blood* 2000; **96**: 1239–1246.
9 Ratanatharathorn V, Nash RA, Przepiorka D, Devine SM, Klein JL, Weisdolf D *et al*. Phase III study comparing methotrexate and tacrolimus (prograf, FK506) with methotrexate and cyclosporine for graft-versus-host disease prophylaxis after HLA-identical sibling bone marrow transplantation. *Blood* 1998; **92**: 2303–2314.
10 Nash RA, Antin JH, Karanes C, Fay JW, Avalos BR, Yeager AM *et al*. Phase 3 study comparing methotrexate and tacrolimus with methotrexate and cyclosporine for prophylaxis of acute graft-versus-host disease after marrow transplantation from unrelated donors. *Blood* 2000; **96**: 2062–2068.
11 Uberti JP, Ayash L, Braun T, Reynolds C, Silver S, Ratanatharathorn V. Tacrolimus as monotherapy or combined with minidose methotrexate for graft-versus-host disease prophylaxis after allogeneic peripheral blood stem cell transplantation: long-term outcomes. *Bone Marrow Transplant* 2004; **34**: 425–431.
12 Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J *et al*. Report of the 1994 consensus conference on acute GVHD grading. *Bone Marrow Transplant* 1995; **15**: 825–828.
13 Peters C, Shapiro EG, Anderson J, Anderson J, Henslee-Downey PJ, Klemperer MR *et al*. Hurler syndrome: II. Outcome of HLA-genotypically identical sibling and HLA-haploidentical related donor bone marrow transplantation in fifty-four children. *Blood* 1998; **91**: 2601–2608.
14 Rao K, Amrolia PJ, Jones A, Jones A, Cale CM, Naik P *et al*. Improved survival after unrelated donor bone marrow transplantation in children with primary immunodeficiency using a reduced-intensity conditioning regimen. *Blood* 2005; **105**: 879–885.
15 Baker KS, Wagner JE. Novel conditioning regimens and nonmyeloablative stem cell transplants. *Curr Opin Pediatr* 2002; **14**: 17–22.
16 Baker 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.
17 Lowsky R, Takahashi T, Liu YP, Dejbakhsh-Jones S, Grumet FC, Shizuru JA *et al*. Prospective conditioning for acute graft-versus-host disease. *N Engl J Med* 2005; **353**: 1321–1331.

18 Mielcarek M, Martin PJ, Leisenring W, Flowers MED, Maloney DG, Sandmaier BM *et al*. Graft-versus-host disease after nonmyeloablative versus conventional hematopoietic stem cell transplantation. *Blood* 2003; **102**: 756–762.
19 Crawley C, Szydlo R, Lalancette M, Bacigalupo A, Lange A, Brune M *et al*. Outcomes of reduced-intensity transplantation for chronic myeloid leukemia: an analysis of prognostic factors from the Chronic Leukemia Working Party of the EBMT. *Blood* 2005; **106**: 2969–2976.
20 Bacigalupo A, Lamparelli T, Bruzzi P, Guidi S, Alessandrino PE, di Bartolomeo P *et al*. Antithymocyte globulin for graft-versus-host disease prophylaxis in transplants from unrelated donors: 2 randomized studies from Gruppo Italiano Trapianti Midollo Osseo (GITMO). *Blood* 2001; **98**: 2942–2947.