

## Non-total body irradiation containing preparative regimen in alternative donor bone marrow transplantation for severe aplastic anemia

J-H Lee, S-J Choi, J-H Lee, Y-S Lee, M Seol, S-G Ryu, J-S Lee, W-K Kim and K-H Lee

Department of Internal Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

### Summary:

**Using non-total body irradiation (TBI) containing preparative regimens, 13 patients with severe aplastic anemia (SAA) were transplanted from an alternative donor in a single institute. In total, 12 donors were unrelated volunteers and one was an HLA one-locus mismatched sibling. Median time from diagnosis of SAA to bone marrow transplantation (BMT) was 10.1 months (range, 1.6–180.1). Nine patients had received immunosuppressive treatment with ATG before BMT, while four had not. Preparative regimens consisted of cyclophosphamide plus ATG in nine patients, cyclophosphamide plus fludarabine in two patients, and cyclophosphamide plus fludarabine plus ATG in two patients. All patients received non-T-cell depleted bone marrow from the donor. Cyclosporine plus methotrexate were given for GVHD prophylaxis. All patients engrafted on a median of day 21 (range, 15–27). Grade III–IV acute GVHD developed in three (23%) of 13 patients and extensive chronic GVHD in four (31%) of 12 evaluable patients. With a median follow-up duration of 1138 days (range, 118–1553), 10 patients are alive with durable engraftment showing 74.6% (95% confidence interval, 49.5–99.7%) of survival rate. Cause of the deaths was CNS bleeding in one and chronic GVHD in two. In conclusion, non-TBI containing preparative regimen could ensure durable engraftment in alternative donor BMT for SAA and showed promising results.**

*Bone Marrow Transplantation* (2005) 35, 755–761.

doi:10.1038/sj.bmt.1704880

Published online 28 February 2005

**Keywords:** non-TBI; preparative regimen; alternative donor; BMT; severe aplastic anemia

have an HLA-identical related donor and proceed to BMT as first-line therapy.<sup>1,2</sup> Aggressive immunosuppressive treatment with one or more cycles of antithymocyte globulin (ATG) with or without cyclosporine is the widely accepted primary therapeutic option for patients who do not have an HLA-identical related donor and this type of treatment has up to an 80% initial response rate with modest toxicity.<sup>3,4</sup> The results of BMT from an alternative donor other than an HLA-identical related donor have been less encouraging because of high rates of graft failure and severe graft-versus-host disease (GVHD).<sup>1,2,5</sup> To overcome graft failure, more intensive pretransplant preparative regimens, which included total body irradiation (TBI) in most studies, have been investigated for alternative donor transplants.<sup>5–8</sup> The rigorous preparative regimens, although effective in securing engraftment, resulted in increased toxicity without survival improvement.<sup>9,10</sup> Furthermore, use of TBI is likely to result in malignant diseases, especially in young patients.<sup>11</sup>

Graft failure after allogeneic BMT for SAA has been strongly associated with allo-sensitization to histocompatibility antigens through previous blood product transfusions.<sup>12–14</sup> Previously transfused patients have lower overall survival compared to untransfused patients, mainly because of complications of graft failure.<sup>15,16</sup> Most alternative donor transplants were performed late in the course of disease after multiple cycles of immunosuppressive treatment and the patients were extensively exposed to allo-antigens due to multiple blood product transfusions. High incidence of graft failure in alternative donor transplants might be ascribed to allo-sensitization through prior transfusions as well as higher degree of histocompatibility differences compared to HLA-matched sibling donor transplants. Canine studies demonstrated that gamma irradiation and leukocyte filtration of blood products before transfusion almost eliminated allo-sensitization to minor histocompatibility antigens and prevent graft rejection of dog leukocyte antigen identical marrow grafts.<sup>12,17–20</sup> Changes in transfusion policies, which use irradiated leukocyte-poor blood products, are considered to be one of important reasons that have led to significant decrease in graft failure after allogeneic BMT for SAA.<sup>21</sup> Reports of several large cooperative studies also showed that outcomes of alternative donor transplants have steadily improved.<sup>22–25</sup> Recently, efforts to reduce dose intensity of preparative regimen have been made for alternative donor transplants with promising results.<sup>23,26–29</sup>

Although allogeneic bone marrow transplantation (BMT) provides the best possibility of cure for patients with severe aplastic anemia (SAA), only about one-third of the patients

Correspondence: Dr J-H Lee, Department of Internal Medicine, Asan Medical Center, University of Ulsan College of Medicine, 388-1 Pungnap-2dong, Songpa-gu, Seoul 138-736, Korea;

E-mail: jhlee3@amc.seoul.kr

Received 27 October 2004; accepted 14 January 2005

Published online 28 February 2005

Using non-TBI containing preparative regimens, 13 patients with SAA were transplanted from an alternative donor in our center and we report on the results of BMT in these patients.

## Patients and methods

### Patients

In total, 13 adult patients with SAA, six males and seven females, underwent allogeneic BMT using an alternative donor between May 1999 and February 2004 at the Asan Medical Center (Table 1). Six of these patients were reported previously.<sup>30</sup> Median age of the patients was 22 years (range, 15–34). One patient had SAA/PNH syndrome and all other patients had acquired idiopathic SAA. SAA was defined as having any two of absolute neutrophil count (ANC) less than 500/ $\mu$ l, platelet count less than 20 000/ $\mu$ l, and corrected reticulocyte count less than 1%. Bone marrow biopsy should reveal hypocellularity.<sup>31</sup> The median interval from diagnosis of SAA to BMT was 10.1 months (range, 1.6–180.1). Nine patients had received systemic immunosuppressive treatment with one cycle of ATG  $\pm$  cyclosporine before BMT, while four had not. Three patients had pretransplant neutrophil counts below 200/ $\mu$ l. All patients had been transfused before BMT with a median of 12 U (range, 6–41) of red blood cells and a median of 66 U (range, 6–353) of platelets. All patients had 80 or more of Karnofsky performance scale at the time of HCT.

The selection of donors was based on serological typing for HLA A, B, and DR according to standard techniques.<sup>32</sup> Allogeneic BMT was considered if HLA-identical or HLA one-locus mismatched related or unrelated donor was available. If a patient had two or more alternative donor candidates, an HLA C matched donor was preferred. Molecular typing by sequence-specific oligonucleotide probes was performed for the DRB1 loci in three patients (UPNs 312, 330, and 339) and for the A, B, and C loci in two patients (UPNs 330 and 339). One donor was an HLA

one-locus mismatched sibling and 12 donors were unrelated volunteers, among whom five were HLA ABCDR-matched, four were HLA ABDR-matched, and three were HLA ABDR-one-locus mismatched with respective recipients (Table 2). Three sex pairs were female-to-male and eight recipient-donor pairs were ABO-incompatible. Patients received a median of  $0.68 \times 10^8$  mononuclear cells/kg (range, 0.37–1.46) and a median of  $4.31 \times 10^6$  CD34+ cells/kg (range, 1.45–10.90).

### Preparative regimens

Preparative regimen was Cy-ATG (cyclophosphamide (50 mg/kg/day on days -5 to -2) plus ATG (Atgam<sup>®</sup> 30 mg/kg/day on days -4 to -2)) until June 2003, but two (UPNs 120 and 241) of 10 patients during the period had experienced an anaphylactic reaction to ATG prior to BMT and received fludarabine (30 mg/m<sup>2</sup>/day on days -4 to -2) in place of ATG. Since July 2003, three patients were included into a randomized trial, which was intended to reduce the cyclophosphamide dose of preparative regimen in allogeneic BMT for SAA. The patients received one of two preparative regimens, which were Cy-ATG (cyclophosphamide (50 mg/kg/day on days -5 to -2) plus ATG (Atgam<sup>®</sup> 30 mg/kg/day on days -4 to -2)) in one patient (UPN 312) and Cy-Flu-ATG (cyclophosphamide (50 mg/kg/day on days -3 to -2), fludarabine (30 mg/m<sup>2</sup>/day on days -6 to -2) plus ATG (Atgam<sup>®</sup> 30 mg/kg/day on days -4 to -2 or Thymoglobuline<sup>®</sup> 3 mg/kg/day on days -4 to -2)) in two patients (UPNs 330 and 339) (Table 2).

### Transplantation procedure

All patients were nursed in laminar air flow rooms. Ciprofloxacin and acyclovir were administered for gut decontamination and viral prophylaxis, respectively. Hyper-hydration and mesna were given for the prevention of cyclophosphamide-induced hemorrhagic cystitis. All cellular blood products were leukocyte-depleted and irradiated prior to transfusion. Immunoglobulin (500 mg/kg) was administered intravenously on day -7, every other week until day

**Table 1** Pre-transplant patient characteristics

UPN	Sex	Age (year)	Diagnosis	Time to BMT from diagnosis (month)	Previous therapy	Pre-BMT PB cell count			Previous transfusion		PS
						cReti	PLT	ANC	Red cells	Platelets	
101	M	15	SAA	3.8	Oxym	0.06	5	112	6 U	180 U	90
120	M	23	SAA	55.0	ATG, Oxym	1.27	17	434	17 U	53 U	80
130	M	28	SAA	1.6	Oxym	0.02	12	72	12 U	66 U	80
136	F	17	PNH/SAA	4.2	None	0.46	18	975	8 U	10 U	90
141	F	15	SAA	2.6	None	0.51	16	394	6 U	6 U	90
145	M	25	SAA	12.7	ATG, CSA	0.47	17	520	14 U	298 U	90
208	F	26	SAA	13.4	ATG, CSA, Fludara	0.06	14	216	13 U	81 U	90
241	F	34	SAA	10.1	ATG, CSA	0.27	5	324	6 U	46 U	90
248	F	19	SAA	6.8	ATG, CSA	0.05	16	64	41 U	353 U	90
272	M	20	SAA	7.2	ATG	0.58	3	570	28v	230 U	80
312	M	22	SAA	40.1	ATG, CSA, Oxym	0.33	14	972	7 U	20 U	80
330	F	33	SAA	180.1	ATG, CSA, Oxym	0.30	4	540	9 U	60 U	80
339	F	19	SAA	14.1	ATG, CSA, Oxym	0.24	17	561	28 U	134 U	80

UPN = unique patient number; SAA = severe aplastic anemia; PNH = paroxysmal nocturnal hemoglobinuria; Oxym = oxymetholone; ATG = antithymocyte globulin; CSA = cyclosporine A; PB = peripheral blood; cReti = corrected reticulocyte count (%); PLT = platelet counts ( $\times 10^3/\mu$ l); ANC = absolute neutrophil counts ( $/\mu$ l); PS = Karnofsky performance status score.

**Table 2** Histocompatibility data, preparative regimen, and bone marrow cell dose

UPN	Donor		ABO blood type		Type of donor	Preparative regimen	Histocompatibility data			Bone marrow cell dose	
	Sex	Age	Patient	Donor			HLA locus mismatch	Patient unique alleles	Donor unique alleles	MNC ( $\times 10^8$ /kg)	CD34+ ( $\times 10^6$ /kg)
101	M	32	O+	O+	Unrelated	Cy-ATG	None	—	—	0.68	4.28
120	M	30	B+	B+	Unrelated	Cy-Flu	DR, (C)	DR9 (C8)	(C3)	1.46	9.14
130	F	26	B+	B+	Sibling	Cy-ATG	B	B35	B40	0.63	1.65
136	M	29	A+	B+	Unrelated	Cy-ATG	(C)	(C7)	(C8)	0.83	3.03
141	M	36	B+	A+	Unrelated	Cy-ATG	None	—	—	0.55	2.90
145	M	25	A+	AB+	Unrelated	Cy-ATG	B, (C)	B61 (C3)	B60 (C7)	0.38	2.15
208	M	27	O+	A+	Unrelated	Cy-ATG	(C)	(C7)	(C3)	0.37	1.40
241	M	26	B+	A+	Unrelated	Cy-Flu	None	—	—	1.40	10.00
248	M	27	O+	O+	Unrelated	Cy-ATG	None	—	—	1.10	4.70
272	F	31	A+	O+	Unrelated	Cy-ATG	None	—	—	0.40	5.70
312	M	42	A+	A+	Unrelated	Cy-ATG	(C)	(C7)	—	0.75	6.60
330	M	28	O+	B+	Unrelated	Cy-Flu-ATG <sup>a</sup>	DRB1	DRB1 1202	DRB1 1502	0.54	4.30
339	M	38	O+	B+	Unrelated	Cy-Flu-ATG <sup>b</sup>	(C)	(C 0304)	(C 0702)	0.91	9.50

UPN = unique patient number; Cy-ATG = cyclophosphamide (50 mg/kg/day on days -5 to -2) plus ATG (Atgam<sup>®</sup> 30 mg/kg/day on days -4 to -2); Cy-Flu = cyclophosphamide (50 mg/kg/day on days -5 to -2) plus fludarabine (30 mg/m<sup>2</sup>/day on days -4 to -2); Cy-Flu-ATG = cyclophosphamide (50 mg/kg/day on days -3 to -2), fludarabine (30 mg/m<sup>2</sup>/day on days -6 to -2) plus ATG (<sup>a</sup>Atgam<sup>®</sup> 30 mg/kg/day on days -4 to -2 or <sup>b</sup>Thymoglobuline<sup>®</sup> 3 mg/kg/day on days -4 to -2<sup>b</sup>); TNC = total nucleated cells; MNC = mononuclear cells; CD34+ = CD34+ cells.

120, and monthly until day 180. Patients were treated for prophylaxis of GVHD by administration of cyclosporine (1.5 mg/kg intravenously every 12 h starting on day -1) plus a short course of methotrexate (15 mg/m<sup>2</sup> intravenously on day 1, and 10 mg/m<sup>2</sup> intravenously on days 3, 6, and 11). No measure was performed for the prevention of hepatic veno-occlusive disease (VOD). On day 0, non-T-cell depleted marrow from the donor was infused over 3–4 h. Recombinant human granulocyte colony-stimulating factor (rhG-CSF) (450 µg) was given intravenously once daily starting on day 0 or day 5. All female patients received oral contraceptives until platelet counts increased over 100 × 10<sup>3</sup>/µl. Total parenteral nutrition was given if indicated.

#### Monitoring of the patients

All patients were prospectively monitored for the occurrence of post transplant toxicities, including GVHD, hepatic VOD, infections, and other transplantation-related toxicities. Blood was drawn daily for complete blood counts, including reticulocyte counts. Blood chemistry and electrolytes, including magnesium level, were determined twice weekly, or more frequently if necessary, whereas prothrombin time (PT) and activated partial thromboplastin time (PTT) were measured weekly. Acute and chronic GVHD was diagnosed on the basis of clinical symptoms, laboratory tests, and whenever possible, histopathological findings of the skin, oral mucosa, liver, or gastrointestinal tract,<sup>33,34</sup> and was classified according to clinical criteria.<sup>35,36</sup> Hepatic VOD was diagnosed in patients having at least two of the following before day 30: (1) hyperbilirubinemia (bilirubin ≥ 2.0 mg/dl), (2) painful hepatomegaly, and (3) unexplained weight gain (> 2% from baseline), with no other explanation for these signs and symptoms present at the time of diagnosis.<sup>37</sup> Severity of VOD was classified as mild, moderate or severe.<sup>38</sup> Cytomegalovirus (CMV) infection was monitored weekly using shell vial culture<sup>39</sup>

until July 1997, thereafter, by both shell vial culture and CMV antigenemia assay;<sup>40,41</sup> ganciclovir 5 mg/kg every 12 h was initiated when CMV infection or disease was documented.

Toxicities within 100 days after allogeneic BMT were graded according to NCI Common Terminology Criteria for Adverse Events (CTCAE) v3.0, which classifies each toxicity as grades I through V.

#### Evaluation of bone marrow engraftment and hematopoietic chimerism

The first day of ANC of 500/µl or more for 2 consecutive days was recorded for bone marrow engraftment. The first day of unsupported platelet count of 20 000/µl or more for 7 consecutive days and that of reticulocyte count of 1% or more for 3 consecutive days were also recorded.

Hematopoietic chimerism was evaluated in all patients using peripheral blood samples from the donor and the recipient by PCR amplification of short tandem repeats (STRs) or amelogenin loci.<sup>42</sup> After BMT, recipient peripheral blood samples were drawn monthly for the first 3 months and then every 3 months for additional 1–2 years or until death. A panel of nine paired primers for STR loci including CSF1PO, F13A01, FESFPS, LPL, TPOX, TH01, HPRTB, vWA, and F13B was used in the initial screening process to identify the most informative STR locus in a given donor–recipient pair. Amelogenin displays a 212 base X-specific band and a 218 base Y-specific band, thus allowing discrimination between X and Y-chromosomes. Complete donor chimerism was defined as the presence of only donor type hematopoietic cells after allogeneic BMT. Mixed chimerism was defined as coexistence of both recipient and donor hematopoietic cells after allogeneic BMT. The degree of mixed chimerism was defined as the proportion of recipient cells in a given sample and determined by the proportion of the peak areas corre-

sponding to recipient signals as compared to the sum of peak areas of the donor and recipient signals.

**Results**

*Engraftment data and hematopoietic chimerism*

All patients were engrafted on a median of day 21 (range, 15–27). Except one patient (UPN 330) who died of CNS bleeding on day 53, all patients also achieved unsupported platelet count over 20 000/ $\mu$ l on a median of day 27 (range, 21–90) and reticulocyte count over 1% on a median of day 32 (range, 22–77) (Table 3). The patients required a median of 12 U of red blood cells (range, 4–24) and a median of 110 U of platelets (range, 70–379). All patients attained stable complete donor chimerism although three patients experienced transient mixed chimerism, which was con-

verted into a complete donor chimerism without any intervention.

*Post-transplant toxicities*

Eight patients (67%) developed acute GVHD: four had grade 1, one had grade 2, and 3 had grade 3 (Table 4). Six patients responded to methylprednisolone therapy and two patients progressed to chronic GVHD. Skin was involved in six patients, gastrointestinal in four, and liver in one. Chronic GVHD occurred in eight (67%) of 12 evaluable patients on a median of day 154 (range, 96–206); limited disease in four and extensive disease in four. Hepatic VOD was diagnosed only in one patient (8%) and severity was mild. CMV infection was documented in eight patients (62%) and preemptive ganciclovir treatment was given for 9–105 days (median, 35.5). No patient developed CMV disease. Hemorrhagic cystitis occurred in three patients

**Table 3** Engraftment data and hematopoietic chimerism

UPN	Engraftment day			Transfusion (units) <sup>a</sup>		Hematopoietic chimerism (%)					
	ANC $\geq$ 500/ $\mu$ l	PLT $\geq$ 20K/ $\mu$ l	Reti $\geq$ 1%	Red cells	Platelets <sup>b</sup>	1 mo	3 mo	6 mo	12 mo	24 mo	36 mo
101	18	27	26	4	96	CDC	CDC	CDC	8%	CDC	CDC
120	25	32	32	12	100	CDC	CDC	CDC	CDC	CDC	CDC
130	15	21	22	12	70	CDC	CDC	CDC	CDC	CDC	CDC
136	20	23	40	24	322	10 <sup>c</sup>	13 <sup>c</sup>	8 <sup>c</sup>	CDC	CDC	CDC
141	21	26	30	16	74	10 <sup>c</sup>	<5 <sup>c</sup>	—	CDC	CDC	—
145	21	29	29	14	200	CDC	CDC	CDC	CDC	—	—
208	20	25	36	11	72	CDC	CDC	CDC	CDC	CDC	—
241	20	26	49	10	84	CDC	CDC	CDC	CDC	—	—
248	27	35	77	8	190	CDC	CDC	CDC	CDC	—	—
272	26	36	44	24	158	CDC	CDC	CDC	—	—	—
312	25	90	31	16	212	CDC	CDC	CDC	—	—	—
330	23	—	49	12	379	CDC	—	—	—	—	—
339	22	27	28	10	110	CDC	CDC	—	—	—	—

<sup>a</sup>Transfusion requirements within 100 days after BMT.

<sup>b</sup>In total, 1 U of single donor pheresis was calculated as 6 U of random donor platelet concentrates.

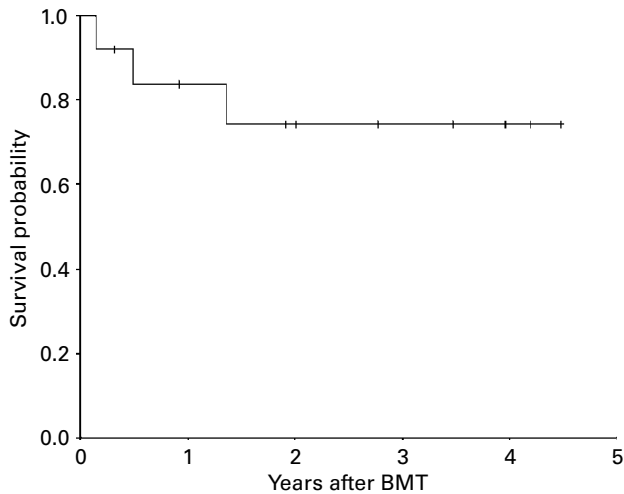
<sup>c</sup>Numerical values denote the proportions of recipient cell population.

UPN = unique patient number; ANC = absolute neutrophil count; PLT = platelet count; Reti = reticulocyte count; mo = post transplant month; CDC = complete donor chimerism.

**Table 4** Post transplant complications and outcomes

UPN	Acute GVHD	Chronic GVHD	CMV Ag	VOD	Hemocystitis	NCI grade of toxicities within 100 days after BMT								Follow-up	
						Pulm	Hep	Renal	Card	GI	Infec	Bleed	Coag		Metab
101	Gr 1	Limited	pos	None	None	0	4	1	0	2	3	0	1	3	Alive (day 1533)
120	None	Limited	pos	None	None	0	3	1	0	2	3	0	1	3	Alive (day 1447)
130	None	Limited	pos	Mild	None	2	3	1	0	2	3	0	1	4	Alive (day 1636)
136	Gr 3	Extensive	pos	None	Gr 1	2	3	2	0	4	3	0	1	4	Alive (day 1534)
141	None	None	neg	None	None	1	3	0	0	2	0	0	0	3	Alive (day 1266)
145	Gr 3	Extensive	pos	None	Gr 3	1	4	3	0	4	3	0	2	4	Died (day 497)
208	Gr 1	None	pos	None	None	0	3	1	0	2	3	0	1	3	Alive (day 1009)
241	None	Limited	neg	None	None	0	2	0	0	2	3	0	0	4	Alive (day 734)
248	None	None	neg	None	None	0	1	1	0	2	3	0	1	3	Alive (day 701)
272	Gr 3	Extensive	pos	None	None	1	3	1	2	4	3	0	3	4	Died (day 180)
312	Gr 1	Extensive	pos	None	None	0	3	1	1	2	3	0	1	4	Alive (day 335)
330	Gr 2	NA	neg	None	Gr 1	0	4	0	2	3	0	4	0	3	Died (day 53)
339	Gr 1	None	neg	None	None	0	2	0	1	3	0	0	1	3	Alive (day 118)

Gr = grade; NA = not applicable; CMV Ag = CMV antigenemia; pos = positive; neg = negative; Hemo cystitis = hemorrhagic cystitis; Pulm = pulmonary; Hep = hepatic; Card = cardiac; GI = gastrointestinal; Infec = infection; Bleed = bleeding; Coag = coagulation; Metab = metabolic.



**Figure 1** Overall survival curve.

(23%) on days 25, 58, and 474, respectively. When toxicities within 100 days after BMT were graded by CTCAE v3.0, toxicities of grade III or more developed as follows: pulmonary toxicities in 0 (0%), hepatic in 10 (77%), renal in 1 (8%), coagulation in 1 (8%), cardiac in 0 (0%), gastrointestinal in 5 (39%), infection in 10 (77%), bleeding in 1 (8%), and metabolic in 13 (100%).

### Survival

The median follow up duration of surviving patients was 1138 days (range, 118–1553). Overall survival rate was 74.6% (95% confidence interval, 49.5–99.7%) (Figure 1). Three patients died and cause of the deaths was CNS bleeding in one (UPN 330) and chronic GVHD in two (UPNs 272 and 145). In these three patients, there was no evidence of secondary graft failure. Other 10 patients are alive with stable engraftment. They do not require transfusion and have 90 or more of Karnofsky performance scale on last follow-up. Three patients are under treatment for chronic GVHD.

### Discussion

Our results suggest that non-TBI preparative regimen may be sufficient to ensure durable engraftment in alternative donor BMT for SAA. This finding is in contrast to earlier reports, which showed that nonirradiation-containing regimens provided insufficient immunosuppression to prevent rejection among patients receiving unrelated or HLA-mismatched related marrow graft.<sup>8,43</sup> Although graft rejection was prevented with TBI doses of 10–14 Gy, GVHD and infections are still frequent barriers to successful BMT.<sup>6,9,44</sup> Promising results have been reported from recent studies to achieve engraftment with less intensive conditioning regimens.<sup>23,26–29</sup> An NMDP-sponsored study showed that a TBI dose of 2 Gy in combination with cyclophosphamide and ATG was sufficient to allow for engraftment.<sup>23</sup> In two small studies, Campath-1H or fludarabine based conditioning regimens enabled alterna-

tive donor hematopoietic cells to engraft in pediatric SAA patients.<sup>27,29</sup> Successful engraftment and better outcomes with less intensive conditioning regimen in recent studies including ours may be explained in several ways. Advances in molecular HLA-typing method have led to better donor selection. Two large registry data demonstrated that HLA genotypic matching resulted in significantly favorable effects on survival and GVHD.<sup>22,45</sup> Recently, most patients received irradiated leukocyte-poor blood products before BMT. As with the HLA-identical sibling BMT, this change in transfusion practice might be critical to decrease the risk of graft rejection in alternative donor BMT for SAA. Interval from diagnosis to BMT is an important prognostic factor for survival after alternative donor BMT for SAA.<sup>22,23</sup> In earlier studies, most patients had long duration of disease and failed one or more courses of systemic immunosuppressive therapy before BMT. In contrast, the patients in our study had received no or only one cycle of systemic immunosuppressive therapy. Durable engraftment is likely with alternative donor BMT carried out earlier in the course of SAA. In addition, genetic differences between ethnic groups may explain, at least in part, the durable engraftment of our patients. Lower incidence of GVHD in the Japanese patients was assumed to reflect a lower degree of diversity of HLA and minor histocompatibility antigens among the Japanese compared to the patients of the Western countries.<sup>46</sup> The frequency of IL-10 592A allele, which is associated with lower incidence of GVHD, is reported to be higher among Asians than the whites.<sup>47</sup>

In our study, incidence of GVHD was relatively high and chronic GVHD was a major factor contributing to two deaths. Major attributable factor to high incidence of GVHD might be higher degree of HLA disparity. The donor selection was based on serological typing of HLA antigens and four patient–donor pairs were phenotypically HLA-one-locus mismatched. With improved donor selection through molecular HLA-typing, patients may have superior outcome after unrelated donor BMT.<sup>22,23</sup> The occurrence of GVHD is related to the intensity of preparative regimen.<sup>48</sup> Although the preparative regimens in our study were less intensive and nonirradiation containing, it is necessary to investigate further refined preparative regimen, such as addition of Campath-1H and omission of cyclophosphamide.

In conclusion, our study suggests that non-TBI containing preparative regimen may be able to ensure durable engraftment in alternative donor BMT for adult patients with SAA.

### References

- 1 Marsh JC, Gordon-Smith EC. Treatment options in severe aplastic anaemia. *Lancet* 1998; **351**: 1830–1831.
- 2 Young NS, Barrett AJ. The treatment of severe acquired aplastic anemia. *Blood* 1995; **85**: 3367–3377.
- 3 Frickhofen N, Kaltwasser JP, Schrezenmeier H *et al*. Treatment of aplastic anemia with antilymphocyte globulin and methylprednisolone with or without cyclosporine. The German Aplastic Anemia Study Group. *N Engl J Med* 1991; **324**: 1297–1304.

- 4 Bacigalupo A, Broccia G, Corda G *et al*. Antilymphocyte globulin, cyclosporin, and granulocyte colony-stimulating factor in patients with acquired severe aplastic anemia (SAA): a pilot study of the EBMT SAA Working Party. *Blood* 1995; **85**: 1348–1353.
- 5 Margolis DA, Casper JT. Alternative-donor hematopoietic stem-cell transplantation for severe aplastic anemia. *Semin Hematol* 2000; **37**: 43–55.
- 6 Margolis D, Camitta B, Pietryga D *et al*. Unrelated donor bone marrow transplantation to treat severe aplastic anaemia in children and young adults. *Br J Haematol* 1996; **94**: 65–72.
- 7 Kojima S, Inaba J, Kondo M *et al*. Unrelated donor marrow transplantation for severe acquired aplastic anemia using cyclophosphamide, antithymocyte globulin, and total body irradiation. *Blood* 1995; **85**: 291–292.
- 8 Wagner JL, Deeg HJ, Seidel K *et al*. Bone marrow transplantation for severe aplastic anemia from genotypically HLA-nonidentical relatives. An update of the Seattle experience. *Transplantation* 1996; **61**: 54–61.
- 9 Hows JM, Yin JL, Marsh J *et al*. Histocompatible unrelated volunteer donors compared with HLA nonidentical family donors in marrow transplantation for aplastic anemia and leukemia. *Blood* 1986; **68**: 1322–1328.
- 10 Gluckman E, Horowitz MM, Champlin RE *et al*. Bone marrow transplantation for severe aplastic anemia: influence of conditioning and graft-versus-host disease prophylaxis regimens on outcome. *Blood* 1992; **79**: 269–275.
- 11 Curtis RE, Rowlings PA, Deeg HJ *et al*. Solid cancers after bone marrow transplantation. *N Engl J Med* 1997; **336**: 897–904.
- 12 Storb R, Deeg HJ. Failure of allogeneic canine marrow grafts after total-body irradiation. Allogeneic ‘resistance’ versus transfusion-induced sensitization. *Transplantation* 1986; **42**: 571–580.
- 13 Storb R, Thomas ED, Buckner CD *et al*. Marrow transplantation in thirty ‘untransfused’ patients with severe aplastic anemia. *Ann Intern Med* 1980; **92**: 30–36.
- 14 Anasetti C, Doney KC, Storb R *et al*. Marrow transplantation for severe aplastic anemia. Long-term outcome in fifty ‘untransfused’ patients. *Ann Intern Med* 1986; **104**: 461–466.
- 15 Doney K, Leisenring W, Storb R, Appelbaum FR. Primary treatment of acquired aplastic anemia: outcomes with bone marrow transplantation and immunosuppressive therapy. Seattle Bone Marrow Transplant Team. *Ann Intern Med* 1997; **126**: 107–115.
- 16 Champlin RE, Horowitz MM, van Bekkum DW *et al*. Graft failure following bone marrow transplantation for severe aplastic anemia: risk factors and treatment results. *Blood* 1989; **73**: 606–613.
- 17 Bean MA, Storb R, Graham T *et al*. Prevention of transfusion-induced sensitization to minor histocompatibility antigens on DLA-identical canine marrow grafts by gamma irradiation of marrow donor blood. *Transplantation* 1991; **52**: 956–960.
- 18 Bean MA, Graham T, Appelbaum FR *et al*. Gamma-irradiation of pretransplant blood transfusions from unrelated donors prevents sensitization to minor histocompatibility antigens on dog leukocyte antigen-identical canine marrow grafts. *Transplantation* 1994; **57**: 423–426.
- 19 Bean MA, Graham T, Appelbaum FR *et al*. Gamma radiation of blood products prevents rejection of subsequent DLA-identical marrow grafts. Tolerance versus abrogation of sensitization to non-DLA antigens. *Transplantation* 1996; **61**: 334–335.
- 20 Storb R, Bean MA, Appelbaum FR *et al*. Treatment of marrow donor blood products with gamma-irradiation prevents transfusion-induced sensitization to DLA-identical marrow grafts. *Transplant Proc* 1991; **23**: 1697–1698.
- 21 Georges GE, Storb R. Allogeneic hematopoietic cell transplantation for aplastic anemia. In: Blume KG, Forman SJ, Appelbaum FR (eds) *Thomas’ Hematopoietic Cell Transplantation*. Blackwell Publishing, Inc.: Malden, 2004, pp 981–1001.
- 22 Kojima S, Matsuyama T, Kato S *et al*. Outcome of 154 patients with severe aplastic anemia who received transplants from unrelated donors: the Japan Marrow Donor Program. *Blood* 2002; **100**: 799–803.
- 23 Deeg HJ, Amlon ID, Harris RE *et al*. Marrow transplants from unrelated donors for patients with aplastic anemia: minimum effective dose of total body irradiation. *Biol Blood Marrow Transplant* 2001; **7**: 208–215.
- 24 Deeg HJ, Seidel K, Casper J *et al*. Marrow transplantation from unrelated donors for patients with severe aplastic anemia who have failed immunosuppressive therapy. *Biol Blood Marrow Transplant* 1999; **5**: 243–252.
- 25 Horowitz MM. Current status of allogeneic bone marrow transplantation in acquired aplastic anemia. *Semin Hematol* 2000; **37**: 30–42.
- 26 Kojima S, Inaba J, Yoshimi A *et al*. Unrelated donor marrow transplantation in children with severe aplastic anaemia using cyclophosphamide, anti-thymocyte globulin and total body irradiation. *Br J Haematol* 2001; **114**: 706–711.
- 27 Chan KW, Li CK, Worth LL *et al*. A fludarabine-based conditioning regimen for severe aplastic anemia. *Bone Marrow Transplant* 2001; **27**: 125–128.
- 28 Elebute MO, Ball SE, Gordon-Smith EC *et al*. Autologous recovery following non-myceloablative unrelated donor bone marrow transplantation for severe aplastic anaemia. *Ann Hematol* 2002; **81**: 378–381.
- 29 Vassiliou GS, Webb DK, Pamphilon D *et al*. Improved outcome of alternative donor bone marrow transplantation in children with severe aplastic anaemia using a conditioning regimen containing low-dose total body irradiation, cyclophosphamide and Campath. *Br J Haematol* 2001; **114**: 701–705.
- 30 Lee JH, Lee JH, Lee JS *et al*. Cyclophosphamide and antithymocyte globulin conditioning may be sufficient for Korean patients with early stage severe aplastic anemia transplanted with marrow from donors other than HLA-identical siblings. *Haematologica* 2001; **86**: 434–435.
- 31 Camitta BM, Rapoport JM, Parkman R, Nathan DG. Selection of patients for bone marrow transplantation in severe aplastic anemia. *Blood* 1975; **45**: 355–363.
- 32 Hopkins KA. Basic microlymphocytotoxicity test. In: Zachary AA, Teresi GA (ed.). *Laboratory Manual*. American Society of Histocompatibility and Immunogenetics: Lenexa, 1990, pp 195–201.
- 33 Shulman HM, Sullivan KM, Weiden PL *et al*. Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. *Am J Med* 1980; **69**: 204–217.
- 34 Tutschka PJ, Beschorner WE, Hess AD, Santos GW. Cyclosporin-A to prevent graft-versus-host disease: a pilot study in 22 patients receiving allogeneic marrow transplants. *Blood* 1983; **61**: 318–325.
- 35 Przepiorka D, Anderlini P, Saliba R *et al*. Chronic graft-versus-host disease after allogeneic blood stem cell transplantation. *Blood* 2001; **98**: 1695–1700.
- 36 Przepiorka D, Khouri I, Ippoliti C *et al*. Tacrolimus and minidose methotrexate for prevention of acute graft-versus-host disease after HLA-mismatched marrow or blood stem cell transplantation. *Bone Marrow Transplant* 1999; **24**: 763–768.
- 37 McDonald GB, Hinds MS, Fisher LD *et al*. Venous-occlusive disease of the liver and multiorgan failure after bone marrow transplantation: a cohort study of 355 patients. *Ann Intern Med* 1993; **118**: 255–267.
- 38 Bearman SI. The syndrome of hepatic venous-occlusive disease after marrow transplantation. *Blood* 1995; **85**: 3005–3020.

- 39 Gleaves CA, Smith TF, Shuster EA, Pearson GR. Comparison of standard tube and shell vial cell culture techniques for the detection of cytomegalovirus in clinical specimens. *J Clin Microbiol* 1985; **21**: 217–221.
- 40 Pancholi P, Wu F, Della-Latta P. Rapid detection of cytomegalovirus infection in transplant patients. *Expert Rev Mol Diagn* 2004; **4**: 231–242.
- 41 St George K, Rinaldo Jr CR. Comparison of cytomegalovirus antigenemia and culture assays in patients on and off antiviral therapy. *J Med Virol* 1999; **59**: 91–97.
- 42 Choi SJ, Lee KH, Lee JH *et al*. Prognostic value of hematopoietic chimerism in patients with acute leukemia after allogeneic bone marrow transplantation: a prospective study. *Bone Marrow Transplant* 2000; **26**: 327–332.
- 43 Deeg HJ, Anasetti C, Petersdorf E *et al*. Cyclophosphamide plus ATG conditioning is insufficient for sustained hematopoietic reconstitution in patients with severe aplastic anemia transplanted with marrow from HLA-A, B, DRB matched unrelated donors. *Blood* 1994; **83**: 3417–3418.
- 44 Hows J, Szydlo R, Anasetti C *et al*. Unrelated donor marrow transplants for severe acquired aplastic anemia. *Bone Marrow Transplant* 1992; **10** (Suppl 1): 102–106.
- 45 Petersdorf EW, Gooley TA, Anasetti C *et al*. Optimizing outcome after unrelated marrow transplantation by comprehensive matching of HLA class I and II alleles in the donor and recipient. *Blood* 1998; **92**: 3515–3520.
- 46 Morishima Y, Kodera Y, Hirabayashi N *et al*. Low incidence of acute GVHD in patients transplanted with marrow from HLA-A,B,DR-compatible unrelated donors among Japanese. *Bone Marrow Transplant* 1995; **15**: 235–239.
- 47 Lin MT, Storer B, Martin PJ *et al*. Relation of an interleukin-10 promoter polymorphism to graft-versus-host disease and survival after hematopoietic-cell transplantation. *N Engl J Med* 2003; **349**: 2201–2210.
- 48 Deeg HJ, Spitzer TR, Cottler-Fox M *et al*. Conditioning-related toxicity and acute graft-versus-host disease in patients given methotrexate/cyclosporine prophylaxis. *Bone Marrow Transplant* 1991; **7**: 193–198.