Table 3. Univariate and multivariate analyses of treatment-related mortality

Variable	No.	Univariate analysis Hazard ratio (95% CI)	Univariate analysis P	Multivariate analysis Hazard ratio (95% CI)	Multivariate analysis P
Age at transplantation			.035		—
Younger than 40 y	158	1.00		—	
40 y or older	75	1.82 (1.04-3.17)		—	
Clinical subtype			.349		—
Indolent	38	1.00		—	
Lymphoblastic	84	1.47 (0.66-3.32)		—	
Clinical subtype			.103		—
Indolent	38	1.00		—	
Aggressive	111	1.91 (0.88-4.16)		—	
Aggressive lymphoma			.045		—
PTCL	22	1.00		—	
Non-PTCL	89	2.85 (1.02-7.94)		—	
Response to chemotherapy			< .001		< .001
Sensitive	128	1.00		1.00	
Resistant	105	3.41 (1.97-5.88)		2.95 (1.66-5.25)	
Prior autograft			< .001		< .001
No	193	1.00		1.00	
Yes	40	4.74 (2.23-10.07)		4.09 (1.85-9.04)	
Prior radiotherapy			.010		—
No	152	1.00		—	
Yes	81	2.05 (1.18-3.55)		—	
Years of transplantation			.225		—
1996-2001	187	1.00		—	
1990-1995	46	1.49 (0.78-2.86)		—	
Donor			.295		—
HLA-matched	197	1.00		—	
HLA-mismatched	36	1.46 (0.72-2.98)		—	
HLA-matched donor			.437		—
Related	154	1.00		—	
Unrelated	43	1.24 (0.72-2.15)		—	
Source of stem cells*			.544		—
BM	159	1.00		—	
PBSCs	70	1.09 (0.82-1.46)		—	
Conditioning regimen			.144		—
TBI-containing	193	1.00		—	
Others	40	1.67 (0.84-3.30)		—	
GVHD prophylaxis†			.169		—
Cyclosporin + methotrexate	204	1.00		—	
Tacrolimus + methotrexate	22	1.86 (0.77-4.51)		—	
Acute GVHD			.537		—
No	78	1.00		—	
Yes	155	1.19 (0.69-2.06)		—	
Chronic GVHD			< .001		.029
No	79	1.00		1.00	
Yes	154	2.76 (1.53-4.98)		2.02 (1.07-3.77)	

CI indicates confidence interval; PTCL, peripheral T-cell lymphoma; HLA, human leukocyte antigen; BM, bone marrow; GVHD, graft-versus-host disease; and —, not applicable.

*Those who received cord blood (n = 2) or BM + PBSC (n = 2) were excluded because of the small number of patients.

†Seven patients using other GVHD prophylaxis were excluded.

in Japan, focusing on the background problems of myeloablative therapy and the identification of risk factors for TRM and OS. We showed that long-term, lymphoma-free survival could be achieved in approximately 40% of patients. Patients with FL had a better prognosis, consistent with previous reports.[8,10] Even in patients with aggressive lymphoma or LBL, long-term survival of 35% was identified, consistent with previous reports.[8,9] However, there were no significant differences between clinical subtypes (eg, aggressive versus indolent or PTCL versus non-PTCL) in a multivariate analysis. Because rituximab became commercially available after 2001 in Japan, patients with B-cell NHL who received anti-CD20 antibody therapy were not included in this study. The clinical effect of the introduction of rituximab on outcome after allogeneic transplantation should be carefully evaluated in a future study.

Our study confirmed a high TRM rate (42%) after conventional allo-HSCT with a myeloablative regimen, consistent with previous reports.[4-8,25] One of the major causes of death was severe regimen-related toxicities, which included interstitial pneumonitis, venoocclusive disease, cardiac and renal toxicity, and organ hemorrhage. Although TBI-based regimens are frequently chosen because lymphoma cells are considered to be sensitive to irradiation, they have also been associated with long-term complications, including

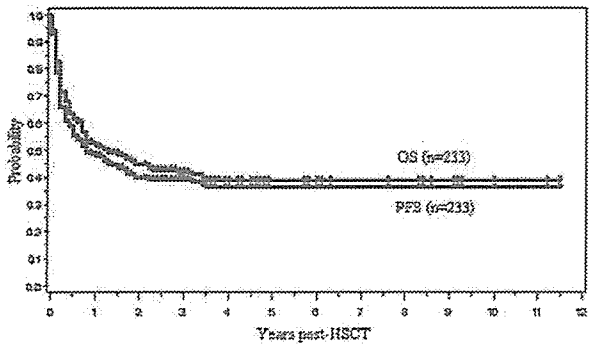

Figure 2. Overall survival (OS) and progression-free survival (PFS) for all 233 patients.

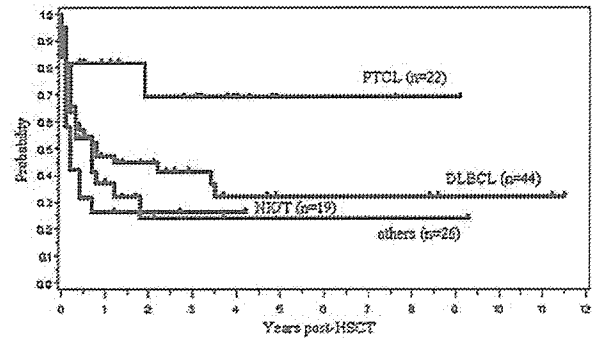

Figure 4. Overall survival for patients with 4 histologic subtypes of aggressive lymphoma. PTCL indicates peripheral T-cell lymphoma, unspecified; DLBCL, diffuse large B-cell lymphoma; NK/T, extranodal NK/T-cell lymphoma, nasal type.

interstitial pneumonitis.[26,27] Because most patients received TBI-based regimens as reported,[4,5,7] we failed to detect any significant differences in TRM between those who received or did not receive TBI.

Another major cause of death in our study was GVHD and/or infection. Of the 98 patients who died of treatment-related complications in our study, 29 (30%) died of infection. At least half of the patients (15 of 29) who died of infectious complications also had GVHD. In a prospective trial of allo-HSCT for patients with NHL, infection accounted for 63% of all TRM,[28] whereas other studies, including ours, have reported an incidence of 25% to 30%.[4,6] In practical transplantation procedures, complications are usually multifactorial, and it is always very difficult to define the exact cause of death, which may account for the wide variations in the incidence of infections among those who died of TRM (18%-63%) in previous reports.[4,5,28,29]

In this study, the incidence of chronic GVHD was high (48%), and chronic GVHD was a risk factor for TRM. The reason for the higher incidence of chronic GVHD in our study compared with the IBMTR report[9,30] was that the IBMTR study included data of patients who died within 100 days after allo-HSCT, whereas we excluded these patients. Unexpectedly, the incidence of chronic GVHD was higher in patients who had GVHD prophylaxis with tacrolimus plus methotrexate than in those with cyclosporin plus methotrexate. In Japan, there is a clear tendency to select tacrolimus rather than cyclosporine for GVHD prophylaxis in unrelated or HLA-mismatched transplantation.[31,32] In addition, PBSCT is not yet permitted for unrelated transplantation. Altogether, the higher incidence of GVHD observed in the tacrolimus group may simply reflect that patients with a higher risk of GVHD were selected to receive tacrolimus.

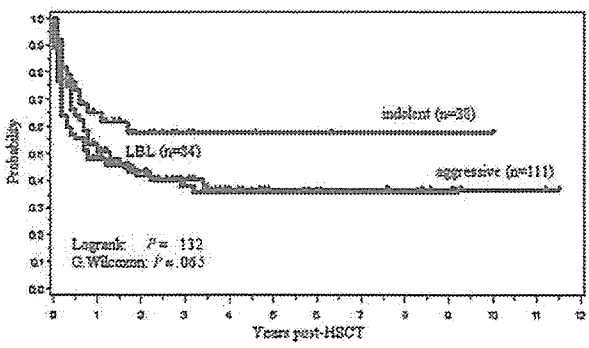

Figure 3. Overall survival stratified according to the clinical subtype. Indolent lymphoma included all grades of FL and extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue. Aggressive lymphoma included all lymphomas except for indolent and lymphoblastic lymphoma (LBL).

We found that the incidence of disease relapse/progression of NHL was low (21%). High TRM in the early phase of the transplantation course may mask later disease relapse/progression, and this made it difficult to estimate the relapse rate in this study. OS and PFS were not affected by the severity of acute GVHD. Our limited analysis failed to confirm a GVL effect after myeloablative allo-HSCT. Although the risk of relapse for patients with acute or chronic GVHD was not significantly different from that of patients without acute or chronic GVHD in previous studies with malignant lymphoma,[8,10,30] a study from the Japan Marrow Donor Program showed that the development of grade II to IV acute GVHD was associated with a lower incidence of disease progression after unrelated HSCT.[31] It has been reported that a low level of acute GVHD was associated with improved OS, and all levels of acute GVHD were associated with a decrease in the relapse rate for intermediate-grade NHL.[8] High levels of acute GVHD had a deleterious effect on OS but were associated with an improved relapse rate for LBL.[8] Thus, our study confirmed that greater effort is required to reduce GVHD-related complications after myeloablative allo-HSCT.

We confirmed that chemoresistance before allo-HSCT and prior autograft were significant risk factors for both OS and TRM. RIST or a less organ-toxic myeloablative allo-HSCT using a combination of fludarabine plus intravenous busulfan may be applied more safely in this population to reduce TRM.[19-21,33,34] However, further studies are needed to determine whether reduced-intensity conditioning could control activity of chemoresistant disease. In contrast to previous studies, we showed that prior radiotherapy was associated with a significantly worse OS, which may be related to the fact that 44 (54%) of the 81 patients who had a history of local radiotherapy had refractory disease at transplantation. Hence, it might be that prior radiotherapy was a marker of survival for more advanced and refractory disease.

In conclusion, we confirmed that myeloablative allo-HSCT is a curative therapeutic option in a subset of patients with NHL, but it carries a high risk of toxicities and TRM. Chemoresistant disease and a history of previous autograft are risk factors for both OS and TRM. Whether the introduction of a reduced-intensity transplantation procedure results in reduction of TRM should be evaluated, and more effective GVHD prophylaxis while maintaining a GVL effect should be developed.

Table 4. Univariate and multivariate analyses of overall survival

Variable	No.	Univariate analysis Hazard ratio (95% CI)	Univariate analysis P	Multivariate analysis Hazard ratio (95% CI)	Multivariate analysis P
Age at transplant			.134	—	—
Younger than 40 y	158	1.00		—	—
40 y or older	75	1.32 (0.92-1.90)		—	—
Clinical subtype			.126	—	—
Indolent	38	1.00		—	—
Lymphoblastic	84	1.57 (0.88-2.80)		—	—
Clinical subtype			.045	—	—
Indolent	38	1.00		—	—
Aggressive	111	1.77 (1.01-3.11)		—	—
Aggressive lymphoma			.004	—	—
PTCL	22	1.00		—	—
Non-PTCL	89	3.45 (1.47-7.69)		—	—
Response to chemotherapy			< .001	—	—
Sensitive	128	1.00		—	—
Resistant	105	3.31 (2.30-4.76)		3.12 (2.16-4.51)	< .001
Prior autograft			< .001	—	—
No	193	1.00		—	—
Yes	40	2.59 (1.73-3.87)		2.18 (1.43-3.30)	< .001
Prior radiotherapy			< .001	—	—
No	152	1.00		—	—
Yes	81	1.99 (1.41-2.83)		1.47 (1.02-2.11)	.037
Years of transplantation			.932	—	—
1996-2001	187	1.00		—	—
1990-1995	46	1.02 (0.67-1.54)		—	—
Donor			.076	—	—
HLA-matched	197	1.00		—	—
HLA-mismatched	36	1.50 (0.96-2.33)		—	—
HLA-matched donor			.769	—	—
Related	154	1.00		—	—
Unrelated	43	0.93 (0.58-1.50)		—	—
Source of stem cells*			.095	—	—
BM	159	1.00		—	—
PBSCs	70	1.37 (0.95-2.00)		—	—
Conditioning regimen			.107	—	—
TBI-containing	193	1.00		—	—
Others	40	1.42 (0.93-2.17)		—	—
GVHD prophylaxis†			.227	—	—
Cyclosporin + methotrexate	204	1.00		—	—
Tacrolimus + methotrexate	22	1.40 (0.81-2.40)		—	—
Acute GVHD-time‡	—	1.25 (0.85-1.84)	.264	1.28 (0.87-1.90)	.213

CI indicates confidence interval; PTCL, peripheral T-cell lymphoma; HLA, human leukocyte antigen; BM, bone marrow; GVHD, graft-versus-host disease; and —, not applicable.

*Those who received cord blood (n = 2) or BM + PBSCs (n = 2) were excluded because of the small number of patients.

†Seven patients using other GVHD prophylaxis were excluded.

‡Acute GVHD was treated as time-dependent variable.

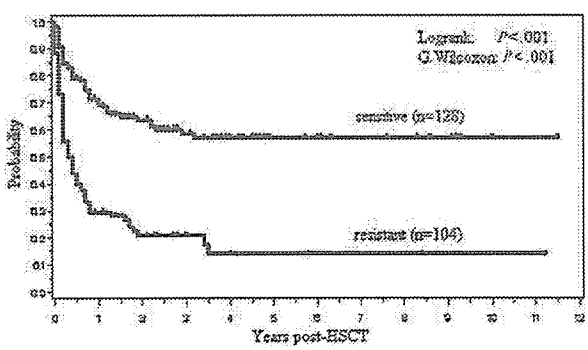

Figure 5. The relation between overall survival and response to chemotherapy.

Acknowledgments

We thank the many physicians in the participating teams who contributed to this survey.

Appendix

The following institutions contributed data to this study: Asahikawa Medical College Hospital, Hokkaido University Hospital, Sapporo Hokuyu Hospital, Akita University Hospital, Gunma Saiseikai Maebashi Hospital, Jichi Medical School Hospital, Suifu Hospital, Saitama Cancer Center Hospital, Jikei University Kashiwa Hospital, Chiba Aoba Municipal

Hospital, Tokyo Metropolitan Komagome Hospital, National Cancer Center Hospital, Keio University Hospital, Toranomon Hospital, Yokohama City University Hospital, Kanagawa Cancer Center, Tokai University Hospital, Kurobe City Hospital, Kanazawa University Hospital, Ishikawa Prefectural Central Hospital, Nagoya City University Hospital, Japanese Red Cross Nagoya First Hospital, Nagoya Daini Red Cross Hospital, Meitetsu Hospital, JA Aichi Showa Hospital, Kyoto University Hospital, Kyoto Prefectural University of Medicine Hospital, Osaka University Hospital, Osaka City University Hospital, Kansai Medical University Hospital, Kinki University Hospital, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka City General Hospital, Rinku General Medical Center Izumisano Hospital, Hyogo College of Medicine Hospital, Hyogo Medical Center for Adults, Okayama University Hospital, Okayama Medical Center, Shimane Prefectural Central Hospital, Takamatsu Red Cross Hospital, Ehime Prefectural Central Hospital, University of Occupational and Environmental Health Hospital, Kitakyushu Municipal Medical Center, Kyushu Cancer Center, Kokura Memorial Hospital, Fukuoka University Hospital, Hamanomachi Hospital, Harasanshin Hospital, Saga Prefectural Hospital Koseikan, Sasebo Municipal General Hospital, Miyazaki Prefectural Miyazaki Hospital, Imamura Bun-in Hospital, and Ryukyu University Hospital.

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ELSEVIER

EXPERIMENTAL HEMATOLOGY

Experimental Hematology 35 (2007) 13–20

Roles of DRB1*1501 and DRB1*1502 in the pathogenesis of aplastic anemia

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(Received 16 May 2006; revised 6 September 2006; accepted 7 September 2006)

Objective. **Although a number of reports have documented a significantly increased incidence of HLA-DR15 in aplastic anemia (AA), the exact role of HLA-DR15 in the immune mechanisms of AA remains unclear. We herein clarify the difference between DRB1*1501 and DRB1*1502, the two DRB1 alleles that determine the presentation of HLA-DR15, in the pathophysiology of AA.**

Materials and Methods. **We investigated the relationships of the patients* HLA-DRB1 allele with both the presence of a small population of $CD55^-CD59^-$ (PNH-type) blood cells and the response to antithymocyte globulin (ATG) plus cyclosporin (CsA) therapy in 140 Japanese AA patients.**

Results. **Of the 30 different DRB1 alleles, only DRB1*1501 (33.6% vs 12.8%, $p_c < 0.01$) and DRB1*1502 (43.6% vs 24.4%, $p_c < 0.01$) displayed significantly higher frequencies among the AA patients than among a control. AA patients possessing HLA-DR15 tended to be old, and especially, the frequency of DRB1*1502 in patients 40 years of age and older (52.4%) was markedly higher than that in those younger than 40 years old (16.2%, $p_c < 0.01$). Only DRB1*1501 was significantly associated with the presence of a small population of PNH-type cells and it also showed a good response to ATG plus CsA therapy in a univariate analysis. A multivariate analysis showed only the presence of a small population of PNH-type cells to be a significant factor associated with a good response to the immunosuppressive therapy ($p < 0.01$).**

Conclusions. **Although both DRB1*1501 and DRB1*1502 contribute to the development of AA, the methods of contribution differ between the two alleles.** © **2007 International Society for Experimental Hematology. Published by Elsevier Inc.**

Aplastic anemia (AA) is a syndrome characterized by pancytopenia and bone marrow hypoplasia. Although the etiology remains unclear, the immune destruction of hematopoietic stem cells has been considered the most important mechanism of bone marrow failure in AA [1]. One important finding supporting the role of such autoimmune mechanisms in AA is the high incidence of a certain HLA allele in AA patients. A number of reports have documented a significantly increased incidence of HLA-DR2 or the split antigen HLA-DR15 in AA [2–5]. We previously demonstrated a strong association between DRB1*1501 and a susceptibility to AA, in which the hematopoietic function improves with administration of cyclosporin A (CsA) [6]. Some reports have also demonstrated that HLA-DR15 or DRB1*1501 can predict the response to immunosuppressive therapy (IST) in patients with AA and myelodysplastic syndrome (MDS) [7–9], while others have failed to identify HLA-DR15 as a predictor for the response to antithymocyte globulin (ATG) therapy [3,10,11].

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0301-472X/06 $–see front matter.
doi: 10.1016/j.exphem.2006.09.002

In our previous study, AA patients carrying DRB1*1502, another major allele corresponding to HLA-DR15 in Japanese, did not show a better response to CsA than those without HLA-DR15 [6]. The exact role of HLA-DR15 in the immune mechanisms of AA thus remains unclear, probably because of the low number of patients that have been studied for DRB1 alleles and the general heterogeneity in the pathogenesis of AA.

Another interesting aspect of HLA-DR15 is the association with the expansion of paroxysmal nocturnal hemoglobinuria (PNH) clones. Several studies have revealed the frequency of HLA-DR15 to be significantly higher in patients with AA and MDS possessing PNH-type blood cells and in florid PNH than in normal controls [10,12], however, the relationship between DRB1 alleles corresponding to DR15 and increased PNH-type cells in AA has not yet been studied in detail. The close relationship between HLA-DR15 and the expansion of PNH clones suggests that the T-cell responses against certain antigen presented by HLA-DR15 or other HLA-class II alleles in linkage disequilibrium with DR15 in hematopoietic stem cells may cause bone marrow failure, thus allowing PNH-type stem cells to survive.

We previous demonstrated the frequency of HLA-DR15 to markedly increase in patients with MDS-refractory anemia (RA) and a small population of PNH-type cells (>0.003% for granulocyte, >0.005% for red blood cells [RBCs]), as demonstrated by sensitive flow cytometry [13]. In that study, RA patients possessing a small population of PNH-type cells displayed favorable responses to CsA. An investigation of a large number of AA patients treated with IST using the same methods to detect small populations of PNH-type cells would thus clarify the role of DRB1 alleles corresponding to HLA-DR15 and PNH-type cells in the immune mechanisms of AA and their mutual relationships. To test this hypothesis, we investigated the relationship between the DRB1 allele in such patients and both the presence of a small population of PNH-type cells and the response to ATG plus CsA therapy in 140 Japanese AA patients.

Materials and methods

Patients

Table 1 summarizes the patient characteristics. The 140 Japanese AA patients were diagnosed at Kanazawa University Hospital, hospitals that participate in a cooperative study led by the Intractable Disease Study Group of Japan, and other referring institutions from April 1999 through November 2005. The study subject included 77 patients who were tested for any correlation between the presence of a minor population in PNH-type cells and the response to IST in our previous study [14]. The severity of AA was classified according to the criteria proposed by Camitta [15] and Marsh et al. [16]. All participants provided written, informed consent to all procedures associated with the study, which was approved by the Ethical Committee at our institution (study number 46). This study also conforms to the recently revised tenets of the Helsinki protocol.

Table 1. Patient characteristics

Characteristics	n	Range
Total (n)	140	NA
Age at diagnosis (y)	60	12–92
Gender: Male/female	65/75	NA
Severity: Severe/moderate	65/75	NA
Neutrophil count ($\times 10^9$/L)	720	0–2226
Platelet count ($\times 10^9$/L)	20	2–118
Reticulocyte count ($\times 10^9$/L)	28	2–106
No. of patients with clonal abnormality (n)	11	NA

NA = not applicable.

Detection of PNH-type cells

We performed two-color flow cytometry of the granulocytes and RBCs according to our previously described method [14,17,18]. First, 3–5 mL heparinized blood was drawn from each patient. To detect the PNH-type granulocytes, phycoerythrin (PE)-labeled anti-CD11b monoclonal antibodies (mAbs; Becton Dickinson, Mountain View, CA, USA), fluorescein-isothiocyanate (FITC)-labeled anti-CD55 mAbs (clone IA10, mouse IgG2a; Pharmingen, San Diego, CA, USA), and FITC-labeled anti-CD59 mAbs (clone p282, mouse IgG2a; Pharmingen) were used in combination with isotype-matched control mAbs, as described previously. To detect PNH-type RBCs, PE-labeled anti-glycophorin A mAbs (clone JC159, DAKO, Glostrup, Denmark) were used instead of anti-CD11b mAbs . Fresh blood was diluted to 3% using phosphate-buffered saline, and 50 mL diluted blood was incubated with 4 mL PE-labeled anti-glycophorin A mAbs , FITC-labeled anti-CD55 and anti-CD59 mAbs on ice for 25 minutes. A total of at least 1×10^5 $CD11b^+$ granulocytes and glycophorin A^+ RBCs within each corresponding gate were analyzed using FACScan flow cytometry (Becton Dickinson). In order to avoid any false-positive results, we excluded $CD11b^{dim}$ and glycophorin A^{dim} cells from the analyses using careful gating because these cells include damaged cells those are often mistakenly judged to be PNH-type cells because of their poor binding to anti-CD55 and anti-CD59 mAbs. This flow cytometry method failed to detect 0.003% or more $CD55^-CD59^-CD11b^+$ granulocytes or 0.005% or more $CD55^-CD59^-$glycophorin-A^+ RBCs in any of 183 healthy individuals. We, therefore, defined the presence of >0.003% $CD55^-CD59^-CD11b^+$ granulocytes $CD55^-CD59^-$glycophorin-A^+ RBCs to be abnormal [14,18].

Determination of DRB1 alleles

DRB1 alleles of 140 AA patients and 491 healthy Japanese randomly selected from general population [19] were determined using polymerase chain reactions with sequence-specific primers (PCR-SSP) (Micro SSP HLA DNA typing trays; One Lambda, Canoga Park, CA, USA). Genomic DNA was prepared from blood samples using a DNA extraction kit (Generation capture column kit; Gentra, Minneapolis, MN, USA).

ATG plus CsA therapy and response criteria

Seventy-seven of 140 patients (55.0%) were treated with ATG (15 mg/kg/day, 5 days; Lymphoglobuline, Aventis Behring, King of Prussia, PA, USA) and CsA (Novartis, Basel, Switzerland, 6 mg/kg/day) within 1 year of diagnosis. The dose of CsA was adjusted to maintain trough levels at between 150 and 250 ng/mL and the appropriate dose was administered for at least 6 months. Granulocyte colony-stimulating factor (filgrastim, 300 μg/m^2 or lenograstim, 5 μg/kg) was administered to some patients. The response to ATG plus CsA therapy was evaluated according to the response criteria described by Camitta [20]. A complete response was defined as hemoglobin normal for age, neutrophil count $>1.5 \times 10^9$/L, and platelet count more than 150×10^9/L. A partial response was defined as transfusion-independent and no longer meeting criteria for severe disease in patients with severe AA, and it was defined as transfusion independence (if previously dependent) or doubling of the normalization of at least one cell line or an increase in the baseline hemoglobin of more than 30 g/L (if initially <60 g/L), a neutrophil count of $>0.5 \times 10^9$/L (if initially $<0.5 \times 10^9$/L), and a platelet count of more than 10×10^9/L (if initially $<20 \times 10^9$/L) in patients with moderate AA.

Statistical analysis

The allele frequency defined as the proportion of patients with at least one copy of a specific gene was determined by direct counting. The χ^2 test compared the allele frequencies of HLA-DRB1 between the patient groups and a Japanese control population, composed of 491 healthy unrelated individuals selected at random from the general population [19]. The corrected value of p (p_c) was calculated by multiplying p with the number of alleles tested (n = 30). The χ^2 test, Fisher's exact test, and logistic procedures [21] analyzed associations between prevalence of increased PNH-type cells and genetic factors, and between individual pretreatment variables and the response to ATG plus CsA therapy. The Kaplan-Meier methods graphically compared the cumulative incidence of the response to ATG and CsA therapy and the time to event, while the log-rank test analyzed differences between the patients who possess HLA-DRB1*1501, DRB1*1502 and DRB1 alleles other than these two alleles. All statistical analyses were performed using the JMP version 5.0.1J software program (SAS Institute, Cary, NC, USA).

Table 2. Frequencies of HLA-DRB1 alleles in Japanese AA patients and controls

	AA patients (n = 140)		Controls (n = 491)		
HLA-DRB1 allele	n	%*	n	%*	p_c value**
0101	10	7.1	64	13.0	NS
0301	0	0.0	4	0.8	NS
0401	2	1.4	17	3.5	NS
0403	4	2.9	18	3.7	NS
0404	0	0.0	2	0.4	NS
0405	35	25.0	129	26.3	NS
0406	5	3.6	32	6.5	NS
0407	2	1.4	2	0.4	NS
0409	0	0.0	1	0.2	NS
0410	1	0.7	17	3.5	NS
0701	0	0.0	2	0.4	NS
0801	0	0.0	0	0.0	NS
0802	6	4.3	36	7.3	NS
0803	8	5.7	84	17.1	NS
0901	36	25.7	148	30.1	NS
1001	2	1.4	2	0.4	NS
1101	7	5.0	22	4.5	NS
1201	7	5.0	34	6.9	NS
1202	2	1.4	12	2.4	NS
1301	0	0.0	4	0.8	NS
1302	11	7.9	61	12.4	NS
1401	2	1.4	21	4.3	NS
1402	0	0.0	2	0.4	NS
1403	4	2.9	13	2.6	NS
1405	4	2.9	18	3.7	NS
1406	2	1.4	10	2.0	NS
1407	0	0.0	1	0.2	NS
1501	47	33.6	63	12.8	<0.01
1502	61	43.6	120	24.4	<0.01
1602	2	1.4	4	0.8	NS

AA = aplastic anemia; NS = not significant.
*Allele frequencies were determined by dividing the number of patients carrying one or two specific alleles by the total number of individuals.
**Corrected p value (p_c) was calculated by multiplying the p value with the number of alleles (n = 30) tested.

Results

Frequencies of DRB1 alleles in AA patients

Table 2 summarizes the frequencies for the 30 different DRB1 alleles identified in the 140 AA patients and 491 controls. Only the frequencies of DRB1*1501 (33.6% vs 12.8%, $p_c < 0.01$, odds ratio = 3.43) and DRB1*1502 (43.6% vs 24.4%, $p_c < 0.01$, odds ratio = 2.39) were significantly higher among the AA patients than among controls. Figure 1 illustrates the numbers of patients with DRB1*1501 and/or DRB1*1502 and the patients without either of the two alleles in the different age groups. Two peaks in the age distribution of the patients were noted, namely, at 20 to 29 years old and at 60 to 79 years old. After dividing the patients into young (younger than 40 years old, n = 37) and old (40 years or older, n = 103) groups, 82.5% of patients in the older group carried at least one of DRB1*1501 or DRB1*1502. Frequency of DRB1*1502 in the older group (54 of 103 patients, 52.4%) was significantly higher ($p_c = 0.03$) than that in the younger group (6 of 37 patients, 16.2%). No significant difference in the frequency of DRB1*1501 was identified between the two groups (36 of 103 patients, 35.0% vs 11 of 37 patients, 29.7%, $p = 0.56$).

Prevalence of patients possessing PNH-type cells

A wide range of PNH-type granulocytes (0.005–23.0%; median, 0.153%) and PNH-type RBCs (0.007–6.57%; median, 0.094%) were detected in 92 of 140 (65.7%) AA patients. When patients were divided into four groups according to presence of DRB1*1501 and DRB1*1502, the proportions of PNH$^+$ patients were 66.7% (4 of 6 patients) in the

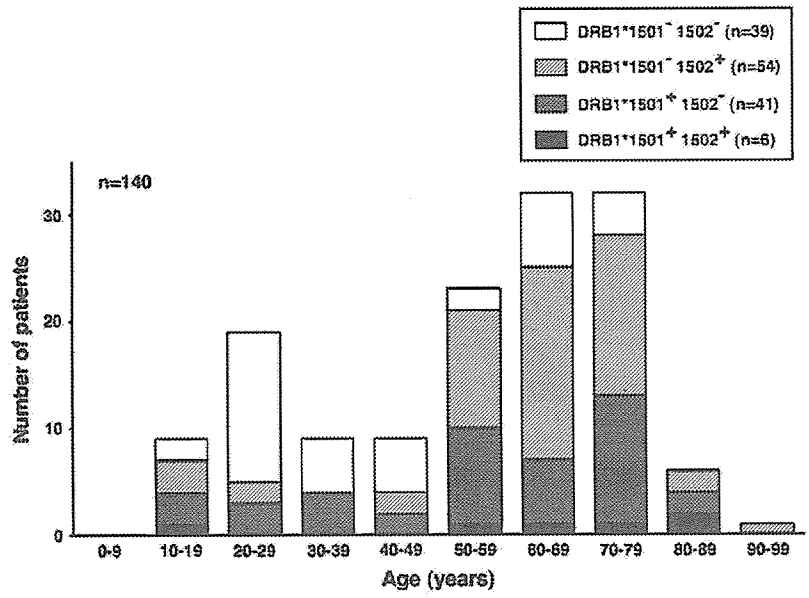

Figure 1. Age distribution of aplastic anemia (AA) patients with or without HLA-DR15. The number of AA patients with or without HLA-DR15 in different age groups is shown. DRB1*1501$^+$1502$^+$, patients with both DRB1*1501 and DRB1*1502; DRB1*1501$^+$1502$^-$, patients with DRB1*1501 but not DRB1*1502; DRB1*1501$^-$1502$^+$, patients with DRB1*1502 but not DRB1*1501; DRB1*1501$^-$1502$^-$, patients with neither DRB1*1501 nor DRB1*1502.

DRB1*1501$^+$1502$^+$ patients, 85.3% (35 of 41 patients) in DRB1*1501$^+$1502$^-$, 59.3% (32 of 54 patients) in DRB1*1501$^-$1502$^+$ and 53.8% (21 of 39 patients) in DRB1*1501$^-$1502$^-$.

Allele frequencies in the PNH$^+$ and PNH$^-$ AA patients

We next divided the 140 AA patients for whom both DRB1 alleles were determined into PNH$^+$ patients (n = 92) and patients without a small population of PNH-type cells (PNH$^-$ patients, n = 48), and then compared the frequency of each DRB1 allele among the three different groups including the PNH$^+$ patients, PNH$^-$ patients, and controls (Fig. 2). The frequency of DRB1*1501 compared to the controls was significantly higher in only the PNH$^+$ patients (39 of 92 patients, 42.4%, $p_c < 0.01$), not in PNH$^-$ patients (8 of 48 patients, 16.7%). On the other hand, the frequency of DRB1*1502 in comparison to the controls was higher in both the PNH$^+$ patients (37 of 92 patients, 40.2%, $p_c = 0.05$) and PNH$^-$ patients (24 of 48 patients, 50.0%). Frequencies of other DRB1 alleles, including DRB1*0405, were similar among PNH$^+$ patients, PNH$^-$ patients, and controls.

Correlation of HLA-DR15 alleles with the prevalence of increased PNH-type cells in AA patients

We analyzed the associations between the prevalence of PNH-type cells and genetic factors, such as age, sex, severity, chromosomal abnormality, and HLA-DRB1 allele to determine which factors might contribute to a slight increase in PNH-type cells in our AA patients. The presence of DRB1*1501 ($p < 0.01$, odds ratio = 3.68) was the only significant factor associated with an increase in the proportion of PNH-type cells based on a univariate analysis, and a multivariate analysis confirmed this result ($p < 0.01$). The presence of DRB1*1502 was not considered to be a contributing factor.

Favorable factors affecting response to ATG plus CsA therapy

Fifty-five of 77 patients (71.4%) improved with ATG plus CsA therapy. The factors favorably affecting the response to IST in the AA patients were examined under a univariate and multivariate analysis (Table 3). Only the presence of PNH-type cells was significantly associated with the response to IST based on a multivariate analysis. After taking into account the kinetics of the response to treatment, we made Kaplan-Meier curves to determine the probability of response to IST in three different groups of patients as defined by DRB1 alleles (Fig. 3). There were significant differences in the probability of the response to IST between the DRB1*1501$^+$1502$^-$ patients and either the DRB1*1501$^-$1502$^+$ patients ($p < 0.01$) or the DR15$^-$ patients ($p = 0.01$) (Fig. 3A). However, these differences in the probability of response IST were no longer observed when the probability of response was compared in either the PNH$^+$ patients or the PNH$^-$ patients (Fig. 3B, C).

Discussion

This study demonstrated for the first time that, in addition to DRB1*1501, which is a major DRB1 allele determining

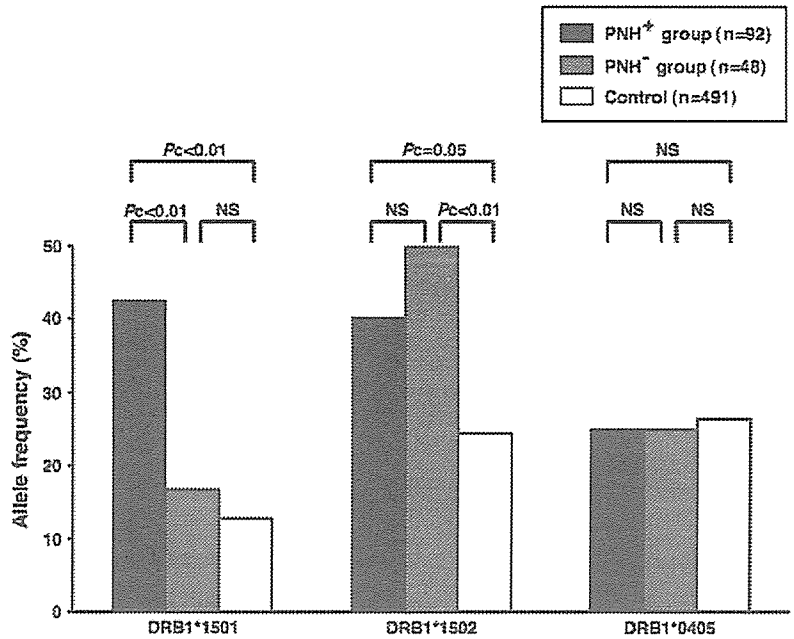

Figure 2. HLA-DRB1 allele frequencies in paroxysmal nocturnal hemoglobinuria $(PNH)^+$ and PNH^- aplastic anemia (AA) patients. Frequencies of the three alleles, DRB1*1501, DRB1*1502, and DRB1*0405 are compared in the PNH^+ AA patients, PNH^- AA patients, and controls.

the presentation of HLA-DR15 in Caucasian [2,3] and Chinese populations [4], DRB1*1502 is frequently present in Japanese AA patients. This finding, based on a large number of patients, suggests that the DR15 molecule plays a definite role in development of a subset of AA. Another novel finding in the present study was that the significantly increased frequency of HLA-DR15 was only observed in old AA patients. The frequency of HLA-DR15 reached up to 80% in AA patients 40 years of age or older. The apparent age-dependent differences in HLA-DR15 frequency suggest that the pathophysiology of AA in older patients may therefore differ from that in younger patients. Several studies of Japanese pediatric patients have revealed a relatively high incidence of MDS secondary to AA compared to adult patients [22–24]. Given the lower frequency of HLA-DR15, pediatric AA may thus display a higher proportion of bone marrow failure caused by nonimmune mechanisms than adult AA.

In contrast to the findings of previous reports, DRB1*1501 appeared to confer a better chance of response to regimens including ATG than other DRB1 alleles, including DRB1*1502. We previously demonstrated that DRB1*1501 predicts the response to CsA, but not to ATG [11]. In the previous study, only 6 of 59 ATG-treated patients received CsA. The combined use of CsA and the larger number of ATG-treated patients in the present study probably accounts for the different findings regarding the role of DRB1*1501 in predicting the response to ATG therapy. DRB1*1501 may affect the response of AA to ATG therapy only when CsA is administered in combination with ATG.

Several previous studies failed to confirm the role of HLA-DR15 in predicting the response to ATG [3,10]. Most previous studies analyzed DRB1 alleles using low-resolution methods that are unable to sufficiently distinguish DRB1*1502 from DRB1*1501. DRB1*1502 accounts for 3% to 7% of the DRB1 alleles corresponding to DR15 even in Caucasians [25], and this frequency may even be higher in AA patients, particularly among AA patients 40 years of age or older. As a result, some patients with DR15 who did not respond to ATG in previous studies may have been $DRB1*1502^+$, rather than $DRB1*1501^+$. The results of this study indicate the importance of

Table 3. Pretreatment variables associated with a response to antithymocyte globulin plus cyclosporin A therapy

	p Value	
Favorable factors	univariate*	multivariate**
Gender (male vs female)	0.32	0.47
Age (at least 40 y vs younger)	0.79	0.37
Severity (severe vs moderate)	0.61	0.86
HLA-DRB1*1501 (positive vs negative)	0.03	0.19
HLA-DRB1*1502 (positive vs negative)	0.61	0.46
PNH-type cells (positive vs negative)	<0.01	<0.01

*Fisher's exact probability test.
**Wald c^2 test for a logistic regression model.
PNH = paroxysmal nocturnal hemoglobinuria.

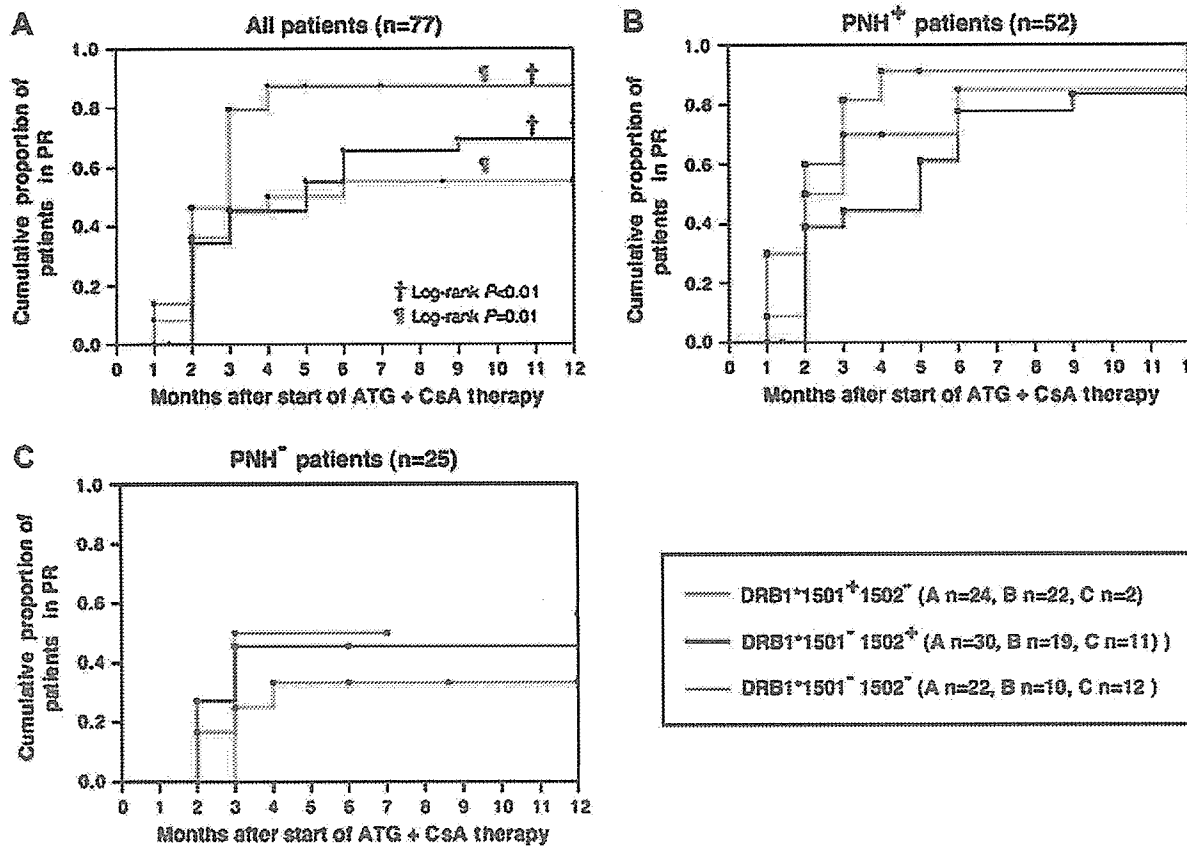

Figure 3. Kinetics of response to antithymocyte globulin (ATG) plus cyclosporin A (CsA) therapy. Kaplan-Meier curves for the response in the different groups of patients based on the DRB1 alleles are shown. DRB1*1501^{+}1502^{+} patients were not showed in this figure because only one patient (he was paroxysmal nocturnal hemoglobinuria [PNH]$^{+}$) was available for the analysis. (A) all patients; (B), PNH^{+} patients; (C), PNH^{-} patients.

accurately determining the DRB1 alleles using high-resolution methods to clarify the role of HLA-DR15 in predicting a response to IST.

A higher frequency of HLA-DR15 among PNH^{+} patients in comparison to PNH^{-} patients has been reported by Maciejewsky et al. [26]. The present study confirmed this finding using a different flow cytometry assay that distinguished PNH^{+} patients from PNH^{-} patients using lower levels of glycosylphosphatidyl inositol-anchored protein-deficient cells than the assay used in the previous study. Our methods also identified a significant difference between DRB1*1501 and DRB1*1502 in the minimal expansion of PNH clones. The frequencies of both alleles increased in the PNH^{+} patients in comparison to normal controls, thus supporting the preliminary results of our study of 23 patients with refractory anemia [13]. However, only DRB1*1501 represented a genetic factor significantly associated with an increase in the proportion of PNH-type cells in AA patients in the present study because the frequency of DRB1*1502 was high in both PNH^{+} and PNH^{-} AA patients, thus indicating that the minimal expansion of PNH clones is not affected by DRB1*1502. Together with the difference in the response rate to IST between DRB1*1501^{+} and DRB1*1502^{+} AA patients, all these findings suggest that DRB1*1501 and DRB1*1502, therefore, play a different role in the pathogenesis of AA.

In AA patients carrying DRB1*1501, the presentation of autoantigen by this molecule may readily induce a cell-mediated attack against hematopoietic stem cells that may be associated with minimal expansion of a PNH clones. Previous studies have demonstrated that the presence of a CD4^{+} T-cell attack against hematopoietic stem cells allows the survival of PNH-type stem cells [27,28]. On the other hand, polymorphic gene alleles of myelosuppressive cytokines, in linkage disequilibrium with DRB1*1502 may predispose individuals with HLA-DRB1*1502 toward development of AA. In keeping with this hypothesis, a recent study on diabetes mellitus patients revealed that a haplotype of TNFa12-DRB1*1502 was, therefore, more frequent in patients likely to develop insulin-dependency than in those who do not develop insulin-dependency [29]. Several reports have demonstrated TNFa12 to be associated with a higher secretion of tumor necrosis factor-α [30].

HLA-DR15 molecules derived from DRB1*1502 differ from those derived from DRB1*1501 in only one amino acid at position 86 (valine for DRB1*1502 and glycine for DRB1*1501) of the β-chain [31]. This structural similarity indicates that antigenic epitopes presented by these molecules are common [32,33]. For most autoimmune diseases where DRB1*1501 is associated with susceptibility in patients from Western countries, DRB1*1502 is expected to play the same role as DRB1*1501 in Japanese patients. However, in Japanese patients with multiple sclerosis, the frequency of DRB1*1502 is not increased in comparison to that in the controls [34,35]. As a result, DRB1*1502 appears to contribute to development of some autoimmune diseases via different mechanisms to DRB1*1501. In AA patients carrying DRB1*1501, certain antigens of which presentation requires position 86 of the β-chain to be glycine may likely induce an immune system attack to hematopoietic progenitor cells. It is also possible that DRB5*0101 and DRB5*0102, which are in complete linkage disequilibrium with DRB1*1501 and DRB1*1502, respectively, in the Japanese population [19] may be responsible for the difference because DRB5*0101 differs from DRB5*0102 by three amino acids in the antigen-peptide binding domain.

Our data may be relevant to the management of AA. Although the incidence of HLA-DR15 is significantly higher in AA patients than in the normal controls, only DRB1*1501 was found to be a predictive marker for a good response to ATG plus CsA therapy. AA patients with DRB1*1502 who do not show an increased proportion of PNH-type cells may not benefit from IST. HLA-DR typing has been considered to be useful for predicting a good response to IST in AA patients [7,8], but this costly test may not be necessary in the circumstance where the highly sensitive flow cytometry is available because the presence of a small population of PNH-type cells is the only significant factor that affects the response to ATG plus CsA therapy based on the findings of our multivariate analysis. Prospective studies are called for to confirm these findings.

Acknowledgments

We wish to express our sincere thanks to Ms. M. Yoshii for technical assistance. In addition, we would also like to thank the following physicians for providing us with patient samples and clinical information: M. Ueda and M. Yamaguchi of Ishikawa Prefectural Central Hospital, H. Yamauchi of Kurobe City Hospital, H. Kobayashi of Nagano Red Cross Hospital, A. Urabe of NTT Kanto Hospital, A. Matsuda of Saitama Medical University Hospital, T. Matsunaga of Sapporo Medical University Hospital, K. Ohyashiki of Tokyo Medical University, M. Teramura of Tokyo Women's Medical University, Y. Terasaki of Toyama City Hospital, and T. Yoshida of Toyama Prefectural Central Hospital.

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Technology, Sports and Culture of Japan (KAKENHI 15390298) and grants from the Research Committee for the Idiopathic Hematopoietic Disorders, The Ministry of Health, Labour and Welfare, Japan.

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Case Report

Successful Treatment of Minimal Residual Disease–Positive Philadelphia Chromosome–Positive Acute Lymphoblastic Leukemia with Imatinib Followed by Reduced-Intensity Unrelated Cord Blood Transplantation after Allogeneic Peripheral Blood Stem Cell Transplantation

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Received March 22, 2006; received in revised form April 24, 2006; accepted May 2, 2006

Abstract

We describe a 35-year-old woman with Philadelphia chromosome–positive acute lymphoblastic leukemia (Ph^+ ALL) who received allogeneic sibling donor peripheral blood stem cell transplantation (PBSCT) and entered a second complete remission. Upon detection of BCR-ABL transcripts after PBSCT, the patient received imatinib, leading to molecular remission. Following the failure of donor leukocyte infusions, she underwent reduced-intensity unrelated cord blood transplantation (RI-UCBT), and has continued durable molecular remission for more than 30 months without substantial graft-versus-host disease. Because of a lack of adverse effects of imatinib on transplantation outcome, a treatment strategy consisting of molecular monitoring–guided initiation of imatinib followed by RI-UCBT may be promising in the management of Ph^+ ALL after allogeneic SCT.

Int J Hematol. 2006;84:170-173. doi: 10.1532/IJH97.06066

Key words: Philadelphia chromosome–positive acute lymphoblastic leukemia; Unrelated cord blood transplantation; Imatinib; Minimal residual disease

1. Introduction

The prognosis for adult patients with Philadelphia chromosome–positive acute lymphoblastic leukemia (Ph^+ ALL) is poor. Although allogeneic stem cell transplantation (SCT) is considered the only potentially curative therapy, a substantial proportion of patients undergoing allogeneic SCT develop hematologic relapse or experience disease progression. In such cases, further treatment is rarely successful [1-5]. Consequently, identification of patients at the highest risk prior to overt hematologic relapse is of great importance. The reverse transcription polymerase chain reaction (RT-PCR) is a sensitive method for detecting low-level transcripts of the breakpoint cluster region–Abelson oncogene locus (BCR-ABL) to assess minimal residual disease (MRD) in Ph^+ ALL [6]. Detection of BCR-ABL transcripts after allogeneic SCT is associated with a probability of hematologic relapse exceeding 90% [6,7].

Another important goal is to prevent MRD after allogeneic SCT from developing hematologic relapse. Imatinib (Glivec, STI571; Novartis Pharmaceuticals, East Hanover, NJ, USA), a selective protein tyrosine kinase inhibitor of BCR-ABL, has pronounced but brief antileukemic activity in patients with advanced Ph^+ ALL, including those with failing SCT [4,5]. Patients with Ph^+ ALL receiving imatinib after SCT on the basis of BCR-ABL transcript positivity have been shown to have a decreased rate of hematologic relapse and to experience prolonged disease-free survival (DFS) [8]. However, sustained molecular remissions are almost never expected with single-agent imatinib [4,5,8]. Despite the need

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for additional treatment options, no effective therapies are available at present.

Here, we report a Ph⁺ ALL patient with molecular evidence of recurrent leukemia after allogeneic peripheral blood stem cell transplantation (PBSCT) who was successfully treated with an unrelated cord blood transplantation (UCBT) following the induction of molecular remission by imatinib.

2. Case Report

In June 2002, a 35-year-old woman received a diagnosis of Ph⁺ ALL with additional karyotype abnormalities of –7 and der(9;22)(q10;q10). Major BCR-ABL chimeric messenger RNA was detected by RT-PCR. Although the patient achieved complete remission (CR) with an induction therapy consisting of cyclophosphamide, daunorubicin, vincristine, prednisolone, and L-asparaginase, based on the Japan Adult Leukemia Study Group ALL-97 protocol, she underwent hematologic relapse during the first course of consolidation therapy. The patient achieved a second CR, a complete cytogenetic remission but not molecular remission, in October 2002 after receiving imatinib at a daily oral dose of 600 mg for 27 days combined with vincristine and prednisolone. Immediately following conditioning therapy with cyclophosphamide at 120 mg/kg, cytarabine at 8 g/m^2, and total body irradiation with 12 Gy, the patient underwent an HLA-identical PBSCT (17.7 × 10^6/kg body weight CD34⁺ cells) with a brother as the stem cell donor. Cyclosporine A (CSA) and short-term methotrexate were used for prophylaxis against graft-versus-host disease (GVHD). The patient developed acute GVHD with stage 1 liver damage on day 50 but responded well to treatment with prednisolone in addition to CSA. Discontinuation of prednisolone and CSA did not induce GVHD recurrence. As previously described [9], MRD analyses using real-time quantitative RT-PCR (RQ-PCR) analysis of bone marrow samples were performed monthly from the start of the conditioning regimen. The patient gave written informed consent to participate in this study to assess the utility of MRD analysis after allogeneic SCT, which was reviewed and approved by an institutional review board at Kanazawa University Medical Center. The detection threshold of RQ-PCR in this study is 50 copies/1 μg RNA. The predictive value of the BCR-ABL transcript number for hematological relapse of Philadelphia-ALL in this setting is ≥50 copies/1 μg RNA (unpublished data). The MRD study showed the patient attaining molecular remission on day 28, as defined by a decrease in BCR-ABL transcripts below the detection threshold of RQ-PCR (Figure 1). Molecular evidence of recurrent leukemia on day 95 resulted in the re-initiation of treatment with 600 mg imatinib on day 103. Despite the subsequent detection of bone marrow BCR-ABL fusion-positive cells by fluorescence in situ hybridization (FISH), the patient regained molecular remission with imatinib monotherapy on day 120. The patient discontinued imatinib on day 146 due to the development of imatinib-induced pericardial effusion. Donor leukocyte infusions (DLI) on days 168 and 196 containing 1.2 × 10^8/kg and 1.3 × 10^8/kg CD3⁺ cells, respectively, from the same donor resulted in the reappearance of molecular relapse on day 248.

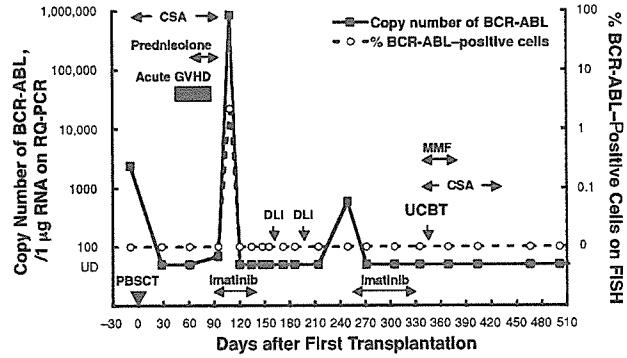

Figure 1. Results of longitudinal RQ-PCR and FISH analyses in bone marrow begun at the start of the preparative regimen for the first transplantation. The detection threshold of BCR-ABL transcripts was 50 copies/μg RNA. UD indicates undetectable levels of transcripts.

Resumption of imatinib on day 257 at a daily oral dose of 400 mg led to molecular remission again with minimal toxicity. In view of the low probability of sustained molecular remission with imatinib [4,5], the urgent need for stem cell transplantation, and the high incidence of regimen-related mortality after conventional second allogeneic SCT in patients with early relapse after first allogeneic SCT [10], reduced-intensity UCBT (RI-UCBT) was planned. The preparative regimen, based on a previous report [11], consisted of cyclophosphamide at 50 mg/kg on day –6, fludarabine at 40 mg/m^2 daily on days –6 to –2, and a single dose of 2 Gy of total body irradiation on day –1. Unrelated cord blood (UCB) that was phenotypically matched and genotypically mismatched at only the DRB1 locus was obtained through the Tohoku Cord Blood Bank. The patient received a UCB graft at a dose of 2.0 × 10^7 nucleated cells/kg of the recipient's body weight in October 2002, 343 days post–first transplantation. To prevent rejection of the graft and GVHD, CSA and mycophenolate mofetil (MMF) were started 3 days before transplantation. Granulocyte colony-stimulating factor was administered from day 1. The patient tolerated the conditioning regimen well, with neutrophil recovery (>5 × 10^8/L) occurring by day 13. Lineage-specific chimerism analysis 25 days posttransplantation showed 100% donor chimerism in both myeloid and T-lymphoid lineages. MMF was discontinued within 3 weeks of UCBT. A gradual tapering of CSA commenced on day 30, and CSA was withdrawn on day 80. On day 140, the patient developed chronic GVHD of the mouth that resolved without treatment. The patient continues to show good performance 30 months after the second transplantation and maintains molecular remission.

3. Discussion

In our patient, the early administration of imatinib, initiated upon molecular evidence of Ph⁺ ALL recurrence after allogeneic SCT, induced molecular remission that has continued after subsequent UCBT with early tapering of CSA. However, the relative contributions of MRD-oriented ima-

tinib treatment, CBT, and early tapering of CSA on her long duration of remission are uncertain.

Concerning imatinib for leukemia relapse after SCT, Wassmann et al [8] reported that in 14 (52%) of 27 Ph+ ALL patients receiving imatinib upon detection of MRD after SCT, BCR-ABL transcripts became undetectable after a median of 1.5 months. They emphasized that their 48% DFS at 18 months since MRD-triggered imatinib commencement surpassed the 5% DFS in a previous report [5] of imatinib treatment for any Ph+ ALL relapse after SCT, suggesting a superior response of imatinib in the setting of MRD. However, even with MRD-triggered imatinib after SCT, there is no plateau in survival curves [8]. These findings indicate that imatinib monotherapy is unable to maintain molecular remission in patients with Ph+ ALL, despite the benefit that treatment with imatinib may provide patients in relapse with a good platform, molecular remission, for subsequent treatment strategies such as SCT.

Takahashi et al [12] showed better outcomes in acute GVHD, treatment-related mortality, and DFS after UCBT than after BMT from unrelated donors. This report may suggest that UCBT could provide the best explanation for the good clinical course in our patient. Thus far, 2 patients with Ph+ ALL receiving cord blood grafts have been reported [13,14]. Wang et al [14] reported an 11-year-old male patient who received HLA-identical sibling donor CBT during hematologic relapse after chemotherapy. The patient relapsed on day 117 and died of leukemia on day 146. The second was a 3-year-old girl who received HLA 1-antigen-mismatched UCBT during the first hematologic CR [13]. She had maintained long-term remission, but died of leukemia 29 months after UCBT (personal communications). The present case is the first reported case of CBT used to treat a patient with a prior history of SCT. These observations are insufficient to determine the effectiveness of CBT for Ph+ ALL. However, given that no curative treatment has been established for patients with Ph+ ALL relapsing after allogeneic SCT [1-4,8], we suggest that CBT could become a promising therapeutic option for the management of such patients.

Our patient was tapered off CSA early after UCBT in an attempt to reduce the chance of relapse due to an enhanced GVL effect. This resulted in successful durable remission without the development of GVHD severe enough to require immunosuppressive therapy. Despite the induction of the GVL effect in some patients with advanced disease, the rapid tapering of CSA could place patients at a risk of developing fatal GVHD [15]. However, the immunological naivety [16] of cord blood lymphocytes may decrease the probability of intractable GVHD after UCBT, allowing the safe reduction of posttransplantation immunosuppression, while the shortened duration of immunosuppression may permit lymphocytes to exert a more potent antileukemic effect. This hypothesis is supported by clinical observations showing similar rates of disease relapse and lower rates of acute and chronic GVHD in adult patients receiving UCBT compared to those receiving allogeneic bone marrow transplantation or PBSCT [11,12,17-19]. In further support, a case report of a child with blast crisis CML was successfully treated with related cord blood transplantation and early withdrawal of CSA [20]. However, it is still unclear whether early tapering of immunosuppression therapy was instrumental in the maintenance of molecular remission in our patient. The correlation of the early tapering of immunosuppression therapy with the sustained molecular remission is only speculative.

Patients undergoing a second allogeneic SCT due to the recurrence of Ph+ ALL have a very poor prognosis because of increased regimen-related toxicity and a high rate of relapse. With the intention of avoiding severe toxicity, we used a reduced-intensity conditioning regimen that was well-tolerated and achieved durable donor engraftment with minimal GVHD in accordance with results in previous reports of RI-UCBT [11,19].

DLI has a very limited success rate in Ph+ ALL relapsing after allogeneic SCT [21], likely due in part to a leukemia burden too high at relapse to be eradicated by DLI. Accordingly, the monitoring of MRD after allogeneic SCT is useful for the maximizing antileukemic effects of DLI as well as those of a second transplant. Evidence to this effect can be seen in the reports of 2 patients with MRD levels of leukemia relapse after allogeneic SCT who obtained molecular remission following DLI [22,23]. Recently, Shimori et al [24] reported 2 patients with CML relapsing into lymphoid blast crisis and with Ph+ ALL, in which the initiation of imatinib led to the elimination of BCR-ABL fusions that was maintained after DLI. However, taking into consideration the persistence of BCR-ABL transcripts after DLI in both patients, the continuation of imatinib treatment, and the short-term follow-up within 5 months of imatinib treatment, it cannot be determined whether these effects are due to imatinib alone rather than the combination of imatinib and DLI. In conjunction with our observation that DLI was ineffective in a patient with molecular remission induced by imatinib prior to DLI, the efficacy of DLI in combination with imatinib remains unclear at present.

The advantages of UCBT are the immediate availability of cells, the absence of a risk to the donor, and a reduced need for HLA compatibility between the donor and recipient [11,2,17-19,25,26]. Because of the establishment of many cord banks, nearly every patient can find a potential cord blood graft, suggesting that a therapeutic approach using imatinib and UCBT guided by molecular monitoring for MRD after SCT could be applied in the majority of patients with Ph+ ALL. A subsequent study of a large group of patients is required to assess whether imatinib in combination with UCBT is a safe and effective therapy for patients with molecular evidence of recurrent Ph+ ALL after allogeneic SCT.

Acknowledgments

This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Technology, Sports and Culture (KAKENHI 18591049) and from the Ministry of Health, Labor and Welfare, Japan. We are indebted to the patient and her family, the cord blood donor, the Japan Cord Blood Network, the staff at the Division of Hematology and Oncology at Kanazawa University Medical Center, and Mami Yoshimura, Arisa Hamano, and Yumi Waridashi for their excellent technical assistance.

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Bone Marrow Transplantation (2006) 00, 1–4

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ORIGINAL ARTICLE

Reduced-intensity unrelated cord blood transplantation for treatment of metastatic renal cell carcinoma: first evidence of cord-blood-versus-solid-tumor effect

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We report a 69-year-old man with cytokine-resistant metastatic renal cell carcinoma treated with reduced-intensity unrelated cord blood transplantation. The patient achieved durable donor engraftment with minimal graft-versus-host disease. The patient showed regression of metastatic disease, providing the first evidence of a graft-versus-tumor effect on a solid tumor resulting from cord blood graft.
Bone Marrow Transplantation (2006) **0,** 000–000. doi:10.1038/sj.bmt.1705519
Keywords: reduced-intensity unrelated cord blood transplantation; metastatic renal cell carcinoma; graft-versus-tumor effect

Introduction

Metastatic renal cell carcinoma (RCC) is resistant to standard radiotherapy or chemotherapy, and patients with this disease have a poor outlook.[1] Although immunotherapy with cytokines such as interleukin 2 and interferon alpha can lead to regression of RCC in some patients, the response rate for these treatments remains around 10–20%, and response is usually temporary.[2] Recently, allogeneic stem cell transplantation utilizing mobilized peripheral blood from a matched donor has been investigated as an alternative immunotherapeutic strategy for the treatment of advanced RCC. The results of pilot reduced-intensity transplant trials for metastatic RCC are encouraging and show that responses can occur in patients with advanced metastatic disease that has failed to respond to conventional cytokine-based therapy.[1,3 15]

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Received 12 August 2005; revised 1 August 2006; accepted 14 August 2006

Unrelated cord blood (UCB) is considered an alternative hematopoietic stem cell source for transplantation, and its use in adult patients with hematologic disorders is increasing.[16 21] Thus far, UCB transfer has not been attempted in patients with a solid-organ malignancy such as RCC. Here, we report a patient with metastatic RCC treated with reduced-intensity unrelated cord blood transplantation (RI-UCBT).

A 56-year-old man with clear cell RCC of his right kidney underwent a right nephrectomy in March 1991. Six years later, metastatic diseases were found in the right upper jaw and pancreas and were partially removed. The remaining metastases grew and new metastases developed in the left lung, left kidney, retroperitoneal space and subcutaneous space. The patient was treated with a 12-week course of combination therapy of subcutaneous interferon alpha 2 MU/m^2 and interferon gamma 2 MU/m^2 five times per week. However, these metastases showed a progressive increase in the size. Because of the low probability of response to further conventional treatment for metastatic RCC, the patient was referred to our institute in February 2004 at the age of 69 years. Then, serum LDH level was 286 IU/l (normal range, 0–250), hemoglobin level 10.6 g/dl, serum calcium level 9.3 mg/dl and erythrocyte sedimentation rate 38 mm/h. Reduced-intensity allogeneic stem cell transplantation was considered in order to decrease regimen-related toxicity, but because of the lack of a suitable donor candidate among his family members, unrelated RI-UCBT was planned. The patient gave written informed consent to participate in an institutional review board-approved investigational protocol designed to evaluate graft-versus-tumor (GVT) effects in metastatic RCC after nonmyeloablative allogeneic transplantation. The preparative regimen, which was based on a previous report,[22] consisted of cyclophosphamide, 50 mg/kg, on day −6, fludarabine, 40 mg/m^2, daily on days −6 to −2, and a single dose of 200 cGy of total body irradiation on day −1. UCB, phenotypically mismatched at one HLA-B antigen and one DRB1 antigen, was obtained through the Japanese Cord Blood Bank Network (J-CBBN). The patient received a UCB graft at a dose of 2.0×10^7 nucleated cells/kg of recipient body weight in

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March 2004. To prevent rejection of the graft and graft-versus-host disease (GVHD), intravenous cyclosporine A (1.5 mg/kg b.i.d.) and oral mycophenolate mofetil (15 mg/kg b.i.d. until neutrophil engraftment) were started 3 days before transplantation. Granulocyte colony-stimulating factor (G-CSF) was initiated on day 1. The patient developed poor engraftment with at most 50% of peripheral blood granulocytes of donor origin. This resulted in graft rejection, with complete autologous recovery on day 41. One hundred and six days after first transplant, the patient received a second UCB graft from J-CBBN containing 2.2×10^7 nucleated cells/kg of recipient body weight, which was phenotypically mismatched at one HLA-B antigen and one DRB1 antigen. Conditioning therapy consisted of fludarabine, 25 mg/m^2, daily on days -7 to -3, melphalan, 80 mg/m^2, on day -2 and a single dose of 400 cGy of total body irradiation on day -1, as previously reported.[18] A continuous infusion of tacrolimus, 0.03 mg/kg, was started from 3 days before transplant for prophylaxis of GVHD and graft rejection. G-CSF was started on day 1. The patient tolerated the conditioning regimen well and exhibited rapid engraftment, with neutrophil rising above 5×10^8/l by day 15. Chimerism analysis of blood on day 20 after second transplant revealed 100% donor origin in both myeloid and T-lymphoid lineages. On day 47, grade II acute GVHD of the skin and gut developed. Acute GVHD improved rapidly after increasing doses of tacrolimus without corticosteroid therapy, but it became dependent on the treatment of tacrolimus. The tacrolimus was finally tapered off at 11 months, and thereafter no GVHD developed. Treatment response was evaluated monthly after transplantation according to the Response Evaluation Criteria in Solid Tumors (RECIST).[23] A computed tomography (CT) scan at 2 months showed substantial regression of metastasis in the left kidney and retroperitoneal space (Figure 1), and the patient was determined as partial remission (PR). The PR had lasted for 3 months until new metastatic lesions in the liver and pancreas appeared at 5 months after second transplantation, defined as progressive disease (PD). At the onset of PD, he had active GVHD of the gut, which was treated with oral tacrolimus alone. Metastatic lesions progressed in size very slowly until 18 months after second transplantation, but since then, they have been unchanged until the time of this writing. The association of the onset of GVHD with the development of PR as well as that of discontinuation of the immunosuppression with no further progression of disease is suggestive of a GVT effect in this patient. The patient continues to show a good performance 26 months after second transplantation without active GVHD.

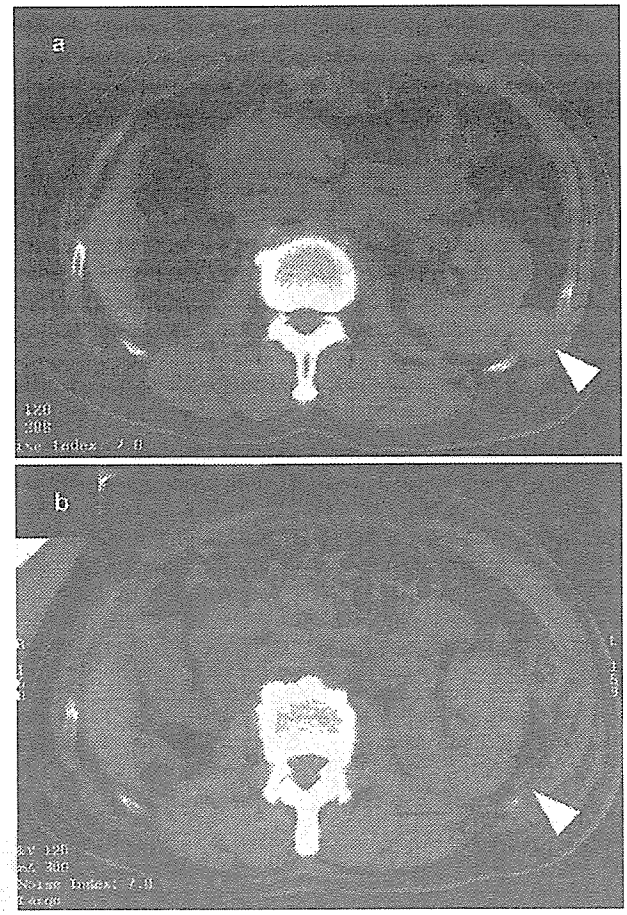

Figure 1 CT images of retroperitoneal metastasis (arrowheads) in a patient before second transplantation (**a**) and 2 months after second transplantation (**b**). Regression in the patient was concordant with the onset of acute GVHD of the skin and gut.

Discussion

Metastatic RCC is the solid tumor in which a GVT effect has been most expected. Childs *et al.*[3] and Childs and Otterud[24] have reported that of 50 patients with metastatic RCC who underwent allogeneic peripheral blood stem cell transplantation (PBSCT), 22 (44%) showed a disease response including four complete responses and 18 PRs, and five (10%) patients had a mixed response. However, the worldwide clinical experience of allogeneic SCT for metastatic RCC is limited, with approximately 200 cases reported in the literature.[3,15,24] One of the major restrictions of this approach is the requirement that transplant candidates have an HLA-identical related donor. This requirement may limit the use of allogeneic stem cell transplantation to a minority of patients with metastatic RCC. Our patient achieved long-term survival following RI-UCBT, despite the lack of a suitable donor candidate among his family members.

Cord blood, which is collected from the umbilical cord and placenta of healthy newborns, is an alternative source of hematopoietic stem cells.[25] Compared to adult peripheral blood or bone marrow, cord blood contains a greater proportion of highly proliferative hematopoietic progenitor cells,[26] which may account for myeloid and lymphoid reconstitution after cord blood transplantation (CBT) despite the presence of fewer cells (by 1–2 logs) in cord blood than in bone marrow or mobilized peripheral blood.

It was originally thought in CBT that the immunological naivety of cord blood lymphocytes[26] might produce a

lowered GVT effect at the expense of a lower GVHD incidence. However, clinical studies revealed similar rates of disease relapse and lower rates of acute and chronic GVHD in adult patients with hematologic malignancies receiving CBT compared to those receiving allogeneic bone marrow transplantation or PBSCT.[17,19,21] Although it remains unclear whether such favorable effects also occur in patients with metastatic RCC who undergo CBT, several observations support the hypothesis that similar alloimmune effects mediated by donor T cells could work in these patients.[24,27 30] Although the target antigens in GVT effects after allogeneic transplantation against metastatic RCC have not been determined, clinical and laboratory observations suggested that minor histocompatibility antigens (mHAs) could be mainly involved as target antigens in GVT effects for metastatic RCC after PBSCT, and donor T cells responding to mHAs could be generated.[24,27,28] The fact that cord blood can generate cytotoxic T cells specific for the mHA in the same way as peripheral blood and bone marrow[29,30] might imply that mHA-specific donor T cells contributive to a GVT effect against metastatic RCC are inducible in a patient receiving a cord blood graft as well.

As allogeneic stem cell transplantation is associated with many and sometimes severe toxic effects, we used a reduced-intensity conditioning regimen as described in previous reports,[18,22] which included low-dose total body irradiation in combination with cyclophosphamide and fludarabine or with melphalan and fludarabine. This RI-UCBT regimen proved to be well tolerated and achieved durable donor engraftment with minimal GVHD. Although our patient required a second RI-UCBT because of graft refection after first RI-UCBT, the demonstrated feasibility of secondary transplantation may be of benefit in the treatment of older cancer patients with RI-UCBT. Of note, the observation in the patient that retroperitoneal and renal metastasis regressed, despite a mixed response, provides the first evidence of a GVT effect by a cord blood graft on RCC.

The advantages of CBT are the immediate availability of cells, the absence of risk to the donor and a lower need for HLA compatibility between the donor and the recipient.[16 22] Because of the establishment of many cord blood banks, nearly every patient can find a potential cord blood graft, suggesting that CBT could substantially expand the use of allogeneic transplantation in patients with metastatic RCC. Despite these potential advantages, there are several disadvantages such as susceptibility to graft rejection, prolonged recovery of hematopoiesis and unavailability of donor lymphocyte infusions. A clinical study focusing on minimizing toxicities and controlling infectious complications as well as enhancing GVT effects is needed to optimize the success of CBT for treatment of advanced RCC.

Acknowledgements

This work was supported in part by Grant-in-Aids for Scientific Research from the Ministry of Education, Science, Technology, Sports and Culture (KAKENHI 15790490) and from the Ministry of Health, Labor and Welfare, Japan. We are indebted to the patient and his family for participating in this study, to the staff at the Division of Hematology and Oncology at Kanazawa University Hospital, to the Japan Cord Blood Bank Network for processing the cord blood units, to Dr Richard Childs for valuable suggestions for the current study and to Megumi Yoshii and Arisa Hamano for their excellent technical assistance.

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