Introduction

Hematopoietic stem cell transplantation (SCT) is a potentially curative treatment for a range of hematologic malignancies. Although the use of an HLA-matched unrelated donor is well accepted when an HLA-identical sibling donor is unavailable, the risk of transplantation- related complications may be increased.[1] Despite improvements in clinical and supportive care, transplant- related life-threatening complications, including graft-versus-host disease (GVHD), infections and disease relapse, remain an enormous obstacle to overcome.[2] Although HLA matching is the major genetic determinant of clinical outcome after allogeneic SCT, recent evidence suggests that non-HLA immune-associated genes are also implicated.[3] Previous investigations have revealed that several single nucleotide polymorphisms (SNP) which affect individual immune response to infections and inflammatory reactions are associated with the risk of GVHD and transplant outcomes.[4-15]

NKG2D is an activating and co-stimulatory receptor belonging to the C-type lectin-like family of transmembrane proteins and is expressed as a homodimer on natural killer (NK) cells, $CD8^+$ $\alpha\beta^+$ T cells, $\gamma\delta^+$ T cells and activated macrophages.[16-18] The ligands for NKG2D, such as MHC class I-chain related proteins (MICA and MICB), UL16 binding proteins are usually absent or expressed at very low levels in normal cells but are up-regulated by cellular stress including heat shock and microbial infections and are frequently expressed in epithelial tumor cells.[19] Ligand engagement of NKG2D triggers cell-mediated cytotoxicity and co-stimulates cytokine production through a DAP10-phosphoinositol 3-kinase dependent pathway and plays an important role in the elimination of tumors and infected cells.[16-18,20]

Recently, SNP were identified between *LNK1* and *HNK1* haplotypes of the *NKG2D* gene.[21] In Japanese individuals, the *HNK1* haplotype is associated with greater activity of NK cells in the peripheral blood[21,22] and a lower prevalence of cancers originating from epithelial cells.[21,23,24] The present study investigates the impact of donor and recipient polymorphisms in the *NKG2D* gene on the clinical outcomes of patients undergoing allogeneic myeloablative bone marrow transplantation using an HLA allele-matched unrelated donor.

Design and Methods

Patients

NKG2D genotyping was performed on a total 145 recipients with hematologic malignancies and their unrelated donors who were part of the Japan Marrow Donor Program (JMDP). The recipients underwent transplantation, following myeloablative conditioning, with T-cell-replete marrow from an HLA-A, -B, -C, -DRB1 allele-matched donor between November 1995 and March 2000. HLA genotypes of the HLA-A, -B, -C, and -DRB1 alleles of the patients and donors were determined by the Luminex microbead method described previously. (Luminex 100 System; Luminex, Austin, TX, USA).[25,26] No patient had a history of prior transplantation. The final clinical survey of these patients was completed by November 1, 2007. Diagnoses were acute myeloid leukemia (n=49; 34%), acute lymphoblastic leukemia (n=37; 26%), chronic myeloid leukemia (n=41; 28%), myelodysplastic syndrome (n=11; 8%) and malignant lymphoma (n=7; 5%), (Table 1). The recipients were defined as having standard risk disease if they had acute myeloid or lymphoblastic leukemia in first complete remission, malignant lymphoma in complete remission, chronic myeloid leukemia in any chronic phase or myelodysplastic syndrome. All other patients were designated as having high-risk disease. Myeloid malignancies included acute myeloid leukemia, chronic myeloid leukemia and myelodysplastic syndrome, whereas lymphoid malignancies included acute lymphoblastic leukemia and malignant lymphomas. Cyclosporine or tacrolimus- based regimens were used in all patients for GVHD prophylaxis whereas anti-T-cell therapy, such as anti-thymocyte globulin and *ex vivo* T-cell depletion, was not. All patients and donors gave their written informed consent to molecular studies, according to the declaration of Helsinki, at the time of transplantation. The project was approved by the Institutional Review Board of Kanazawa University Graduate School of Medicine and the JMDP.

NKG2D genotyping

NKG2D was genotyped using the TaqMan-Allelic discrimination method[27] with a 9700-HT real time polymerase chain reaction (PCR) system (Applied Biosystems, Foster City, CA, USA) and results were analyzed using allelic discrimination software (Applied Biosystems). The genotyping assay was conducted in 96-well PCR plates. The amplification reaction contained template DNA, TaqMan universal master mix and a specific probe (product No. C_9345347_10; Applied Biosystems) for rs1049174, a single locus featuring a G-C substitution to distinguish between the *HNK1* (G) and *LNK1* (C) haplotypes of the *NKG2D* gene.[21,23,24]

Data management and statistical analysis

Data were collected by the JMDP using a standardized report form. Follow-up reports were submitted at 100 days, 1 year and annually after transplantation. Pre-transplant cytomegalovirus serostatus was routinely tested only in patients but not in their donors. Engraftment was confirmed by an absolute neutrophil count of more than 0.5×10^9/L for at least 3 consecutive days. Acute and chronic GVHD were diagnosed and graded using established criteria.[28,29] Overall survival was defined as the number of days from transplantation to death from any cause. Disease relapse was defined as the number of days from transplantation to disease relapse. Transplant-related mortality was defined as death without relapse. Any patients who were alive at the last-follow-up date were censored. When collecting data, only the main cause of death was recorded if two or more causes were combined. Data on etiological agents of infections, post-mortem changes and supportive care (including prophylaxis of infections and therapy of GVHD, which were given on an institutional basis), were not available for this

cohort of patients. The analysis was performed using Excel 2007 (Microsoft Corp, Redmond, WA, USA), OriginPro version 8.0J (Lightstone Inc, Tokyo, Japan), and R (The R Foundation for Statistical Computing, Perugia, Italy).[30] The probability of overall survival was calculated using the Kaplan-Meier method and compared using the log-rank test. The probabilities of transplant-related mortality, disease relapse, acute GVHD, chronic GVHD, and each cause of death were compared using the Grey test[31] and analyzed using cumulative incidence analysis,[30] considering relapse, death without disease relapse, death without acute GVHD, death without chronic GVHD, and death without each cause as respective competing risks. The analysis was stratified for patients with standard-risk disease and high-risk disease to take into account the already recognized prognostic differences. The variables considered were recipient age at time of transplantation, sex, recipient cytomegalovirus serosta-

Table 1. **Characteristics of the donors and recipients.**

	Standard-risk disease (n=93, 64%) Donor *NKG2D* haplotype					High-risk disease (n=52, 36%) Donor *NKG2D* haplotype				
	HNK1 negative n=55, 59%		*HNK1* negative n=38, 41%			*HNK1* positive n=28, 54%		*HNK1* negative n=24, 46%		
Variable	N.	Ratio	N.	Ratio	p	N.	Ratio	N.	Ratio	p
Age, years										
Recipient					0.08					0.39
Median	31		23			23		22		
Range	1-50		1-50			7-46		2-48		
Donor					0.54					
Median	33		28			34		29		0.02
Range	22-49		21-50			21-47		21-50		
Recipient *NKG2D* haplotype					0.17				0.48	
HNK1 positive	33	60%	28	74%		19	68%	14	58%	
HNK1 negative	22	40%	10	26%		9	32%	10	42%	
Sex, male					0.37					0.77
Recipient	30	55%	23	61%		19	68%	15	63%	
Donor	42	76%	23	61%		19	68%	13	54%	
Recipient/donor sex					0.23					0.86
Sex matched	31	56%	20	53%		18	64%	16	67%	
Male/female	6	11%	9	24%		5	18%	5	21%	
Female/male	18	33%	9	24%		5	18%	3	13%	
Disease					0.86					0.99
Acute myeloid leukemia	14	25%	9	24%		14	50%	12	50%	
Acute lymphoblastic leukemia	10	18%	8	21%		10	36%	9	38%	
Myelodysplastic syndrome	6	11%	5	13%		0	0%	0	0%	
Malignant lymphoma	2	4%	3	8%		1	4%	1	4%	
Chronic myeloid leukemia	23	42%	13	34%		3	11%	2	8%	
ABO matching					0.37					0.18
Matched	35	64%	19	50%		14	50%	17	71%	
Major mismatch	11	20%	10	26%		6	21%	5	21%	
Minor mismatch	9	16%	9	24%		8	29%	2	8%	
Bi-directional	0	0%	1	3%		0	0%	1	4%	
Conditioning regimen					0.93					0.51
With total body irradiation	43	78%	30	79%		26	93%	21	88%	
Without total body irradiation	12	22%	8	21%		2	7%	3	13%	
Pretransplant CMV serostatus				0.30					0.99	
CMV-negative recipient	14	25%	5	13%		6	27%	5	21%	
Missing data	4	7%	2	5%		5	18%	4	17%	
GVHD prophylaxis					0.58					0.11
With cyclosporine	51	93%	34	89%		27	96%	20	83%	
With tacrolimus	4	7%	4	11%		1	4%	4	17%	
TNC, $\times 10^8$/kg					0.40					0.04
Median	5.4		5.8			5.8		8.2		
Range	2.3-14.6		2.3-57.6			2.9-20.0		2.4-42.8		
Engraftment	53	96%	38	100%	0.23	28	100%	23	96%	0.28

CMV: cytomegalovirus; TNC: total nucleated cell count harvested.

tus before transplantation, disease characteristics (disease type and disease lineage), donor characteristics (age, sex, sex compatibility, and ABO compatibility), transplant characteristics (total body irradiation-containing regimen, tacrolimus versus cyclosporine, and total nucleated cell count harvested per recipient weight). The median was used as the cut-off point for continuous variables. The χ^2 test and Mann-Whitney test were used to compare results of two groups. The Hardy-Weinberg equilibrium for the *NKG2D* gene polymorphism was tested using the Haploview program.[32] Multivariate Cox models were used to evaluate the hazard ratio associated with the NKG2D polymorphism. Co-variates found to be statistically significant in univariate analyses ($p \leq 0.10$) were included in the models. For both the univariate and multivariate analyses, p values were two-sided and outcomes were considered to be statistically significant with $p \leq 0.05$.

Results

Frequencies of NKG2D haplotype

The *NKG2D* gene polymorphism was analyzed in 145 pairs of unrelated donors-recipients of bone marrow following myeloablative conditioning (Table 1). The haplotype frequencies of *LNK1/LNK1*, *HNK1/LNK1* and *HNK1/HNK1* were 43%, 42% and 15%, respectively in donors and 35%, 45% and 20%, respectively in recipients. These frequencies were similar to those reported in previous studies in Japanese populations[21,24] and were in accordance with the Hardy-Weinberg equilibrium (p=0.80).

Transplant outcomes according to NKG2D haplotype

With a median follow-up of 115 months among survivors (range, 74 to 140 months), 30 recipients (21%) had relapsed or progressed and 62 (47%) had died. Three patients (2%) died before engraftment. The analysis of the influence of the *NKG2D* genotype on clinical outcomes after transplantation was stratified according to whether the recipients had standard-risk disease or high-risk disease to account for the already recognized prognostic difference. The overall survival at 5 years in patients with standard-risk disease was 63% while that of patients with high-risk disease was 44% (p=0.06). The 5-year cumulative incidences of trasplant-related mortality were 32% and 27%, respectively (p=0.33) and those of disease relapse were 10% and 31%, respectively (p=0.0006).

The transplant outcomes according to *NKG2D* genotype are summarized in Table 2. Patients with standard-risk disease receiving transplants from donors with the *HNK1* haplotype had a significantly better 5-year overall survival (73% *vs.* 49%, p=0.01; Figure 1A) and lower transplant-related mortality rate (22% *vs.* 45%, p=0.02; Figure 1B) than those receiving transplants from donors without the *HNK1* haplotype. No difference was noted in disease relapse in relation to the donors' polymorphism (9% *vs.* 11%, p=0.81; Figure 1C) or in the development of grades II to IV acute GVHD (28% *vs.* 41%, p=0.25) or chronic GVHD (37% *vs.* 41%, p=0.83). When patients with acute myeloid leukemia or myelodysplastic syndrome were separately analyzed, there was still no difference in disease relapse in relation to NKG2D polymorphisms (*data not shown*). In patients with high-risk disease, the donor *HNK1* haplotype had no significant effects on transplant outcomes (Table 2).

Multivariate analysis

Any factors found to be significant in univariate analyses were included in the multivariate analysis. When patients with standard-risk disease were analyzed, the *HNK1* haplotype in donors remained statistically significant in multivariate analyses for both overall survival and transplant-related mortality (Table 3). The presence of the *HNK1* haplotype in the donor resulted in better overall survival (hazard ratio, 0.44; 95% confidence interval, 0.23 to 0.85; p=0.01) and transplant-related mortality (hazard ratio, 0.42; 95% confidence interval, 0.21 to 0.86; p=0.02).

Table 2. **Univariate analysis of the association of *NKG2D* polymorphisms with clinical outcomes after transplantation.**

	N	5-year OS	p	5-year TRM	p	5-year relapse	p	Grade II-IV acute GVHD	p	Chronic GVHD	p
Standard-risk disease											
Donor *NKG2D* haplotype			0.01		0.02		0.81		0.25		0.83
HNK1-positive	55	73%		22%		9%		28%		37%	
HNK1-negative	38	49%		45%		11%		41%		41%	
Recipient *NKG2D* haplotype			0.39		0.31		0.93		0.48		0.98
HNK1-positive	61	62%		33%		10%		37%		39%	
HNK1-negative	32	66%		28%		9%		25%		38%	
High-risk disease											
Donor *NKG2D* haplotype			0.91		0.77		0.93		0.08		0.47
HNK1-positive	28	43%		26%		33%		54%		44%	
HNK1-negative	24	46%		29%		29%		30%		35%	
Recipient *NKG2D* haplotype			0.41		0.43		0.10		0.40		0.68
HNK1-positive	33	42%		23%		39%		39%		37%	
HNK1-negative	19	47%		35%		18%		50%		47%	

OS: overall survival; TRM: transplant-related mortality.

The donor and recipient *HNK1* haplotype did not significantly influence the transplant outcomes in patients with high-risk disease.

Main causes of death

The main causes of death according to the *HNK1* haplotype of the donors and recipients are illustrated in Figure 2A for patients with standard-risk disease, and in Figure 2B for those with high-risk disease. In patients with standard-risk disease receiving transplants from *HNK1*-negative donors, the most frequent cause of death was acute GVHD, followed by interstitial pneumonia. Transplants from *HNK1*-positive donors resulted in a statistically significantly reduced incidence of death attributed to acute GVHD (Figure 3A; p=0.006) as well as a trend toward a lower incidence of death attributed to interstitial pneumonia (Figure 3B; p=0.09). Other causes of death did not differ according to the *HNK1* haplotype.

Discussion

The current study showed an association between the *NKG2D-HNK1* haplotype in unrelated donors of HLA-matched myeloablative bone marrow transplants (haplotype frequency, 61%) and a significantly reduced transplant-related mortality and better overall survival for their recipients with standard-risk disease. The polymorphism of the donor *NKG2D* gene did not influence disease relapse or the development of grades II to IV acute GVHD or chronic GVHD in the patients. One possible explanation for the absence of the beneficial effects of the *HNK1* haplotype in patients with high-risk disease may be that the number of cases in the study was insufficient for a meaningful assessment of the effect. Alternatively, disease progression may precede the emergence of the potential advantageous effects of the *HNK1* donor haplotype that could protect the recipient from severe transplant-related complications. There was a larger difference in disease relapse between patients with standard-risk disease and those with high-risk disease: 10% and 31% at 3 years after transplantation, respectively.

NKG2D plays important roles in immunity to microbial infections and is especially prominent in controlling viral and bacterial infections.[16] Therefore, the reduced transplant-related mortality in patients with standard-risk disease receiving grafts from donors with the *HNK1* haplotype in this study might be a consequence of increased resistance to infections in the recipients. However, the hypothesis is too speculative because of the unavailability of data on causes of infections in this cohort. Further studies will be needed to clarify whether the *HNK1* haplotype in donors can effectively protect patients against infections.

Several studies have shown that NK cell activity has an important role in the outcomes of patients undergoing allogeneic transplantation.[33,34] Alloreactive NK cells reduced the risk of relapse of acute myeloid leukemia without increasing the incidence of GVHD, resulting in a marked improvement of event-free survival in a series of haploidentical transplant recipients.[35,36] In HLA-identical sibling transplants, the absence of HLA-C and HLA-B ligand for donor-inhibitory killer immunoglobulin-like receptors (KIR) provided benefits in terms of survival and relapse of patients with acute myeloid leukemia and myelodysplastic syndrome in recipients of T-cell-depleted SCT.[37] On the other hand, the JMDP found that KIR ligand mismatch was unfavorably correlated with relapse of leukemia and survival in patients undergoing T-cell-replete unrelated bone marrow transplants.[38] All patients in the present study received grafts from an HLA-A, -B, and -C allele-matched donor, implying KIR ligand match between each patient and donor. It is an open question whether the *NKG2D* polymorphism could affect the outcomes of patients undergoing transplantation with KIR-mismatched grafts.

In this study, major and minor ABO incompatibilities between the donor and recipient tended to be associated with poorer transplant outcomes, regardless of the risk

Table 3. **Multivariate analysis of the association of *NKG2D* polymorphisms with clinical outcomes after transplantation.**

Variable	Overall survival			Transplant-related mortality			Relapse			Grades II-IV acute GVHD			Chronic GVHD		
	HR	95% CI	p	HR	95% CI	p	HR	95% CI	p	HR	95% CI	p	HR	95% CI	p
Standard-risk disease															
HNK1-positive donor	0.44	0.23-0.85	0.01	0.42	0.21-0.86	0.02	0.71	0.19-2.67	0.61	0.83	0.39-1.75	0.63	0.83	0.39-1.75	0.62
HNK1-positive recipient	1.22	0.60-2.50	0.58	1.32	0.61-2.87	0.48	1.11	0.28-4.48	0.88	1.54	0.66-3.57	0.32	1.06	0.49-2.31	0.88
Donor age, >31 years	-	-	-	-	-	-	-	-	-	2.17	0.95-4.96	0.07	-	-	-
Major ABO incompatibility	-	-	-	-	-	-	-	-	-	3.12	1.49-6.56	0.003	0.50	0.17-1.45	0.20
Minor ABO incompatibility	2.42	1.17-5.03	0.02	-	-	-	-	-	-	-	-	-	0.29	0.07-1.24	0.10
High-risk disease															
HNK1-positive donor	0.68	0.30-1.51	0.34	0.62	0.20-1.91	0.40	1.25	0.41-3.80	0.69	1.87	0.69-5.07	0.22	1.55	0.60-4.01	0.37
HNK1-positive recipient	1.41	0.65-3.07	0.39	0.76	0.25-2.29	0.63	2.35	0.66-8.44	0.19	0.47	0.18-1.22	0.12	0.92	0.35-2.38	0.86
Age, >26 years	1.95	0.93-4.09	0.08	6.30	1.86-21.32	0.003	-	-	-	-	-	-	-	-	-
Donor age, >31 years	-	-	-	-	-	-	0.53	0.17-1.65	0.27	-	-	-	-	-	-
Minor ABO incompatibility	2.94	1.19-7.25	0.02	-	-	-	-	-	-	5.10	2.08-12.52	0.004	-	-	-

category of the disease. These findings are compatible with those of a previous study by the JMDP,[39] although the impact of ABO incompatibilities on SCT outcomes is controversial.

This study also identified age as a significant predictive factor for transplant-related mortality in the patients with standard-risk disease. This is consistent with the results of a previous study[40] showing that age over 35 years increased the risk of transplant-related mortality after allogeneic myeloablative SCT in high-risk patients.

A possible limitation of this study is the fact that no direct evidence is yet available regarding the ability of *NKG2D* polymorphisms to protect against microbial infections. The association observed between the *NKG2D* haplotype and transplant outcome might be due to another genetic polymorphism in linkage disequilibrium responsible for a better transplant outcome. One candidate gene is *NKG2F* (*KLRC4*), which is located in the NK complex region adjacent to the *NKG2D* gene, because an intrinsic SNP (rs2617171) in the gene has been reported to be in complete linkage with the *NKG2D* genotype.[24] Alternatively, polymorphisms may not be directly associated with controlling infection, but rather may be associated with other factors, such as sensitivity to treatment against GVHD or protection against organ toxicities related to transplants, which also influence the transplant outcome. These hypotheses have yet to be verified give the insufficient evidence.

Polymorphisms in genes encoding for nucleotide-binding oligomerization domain 2 (NOD2)/caspase recruitment domain 15 (CARD15),[9] heme oxygenase-1 (HO-1) promoter,[6] the Toll-like receptor 4,[4] CC chemokine ligand (CCL) 5 promoter,[32] transforming growth factor (TGF) β1,[11] interleukin (IL) 12, tumor necrosis factor (TNF) α,[15] IL-23,[5] mannose-binding lectin (MBL),[10] Fcγ receptor IIa (FcγRIIa), myeloperoxidase (MPO), FcγRIIIb, IL-1Ra, IL-10,[12] Fc receptor-like 3 (FCRL3), peptidylarginine deimi-

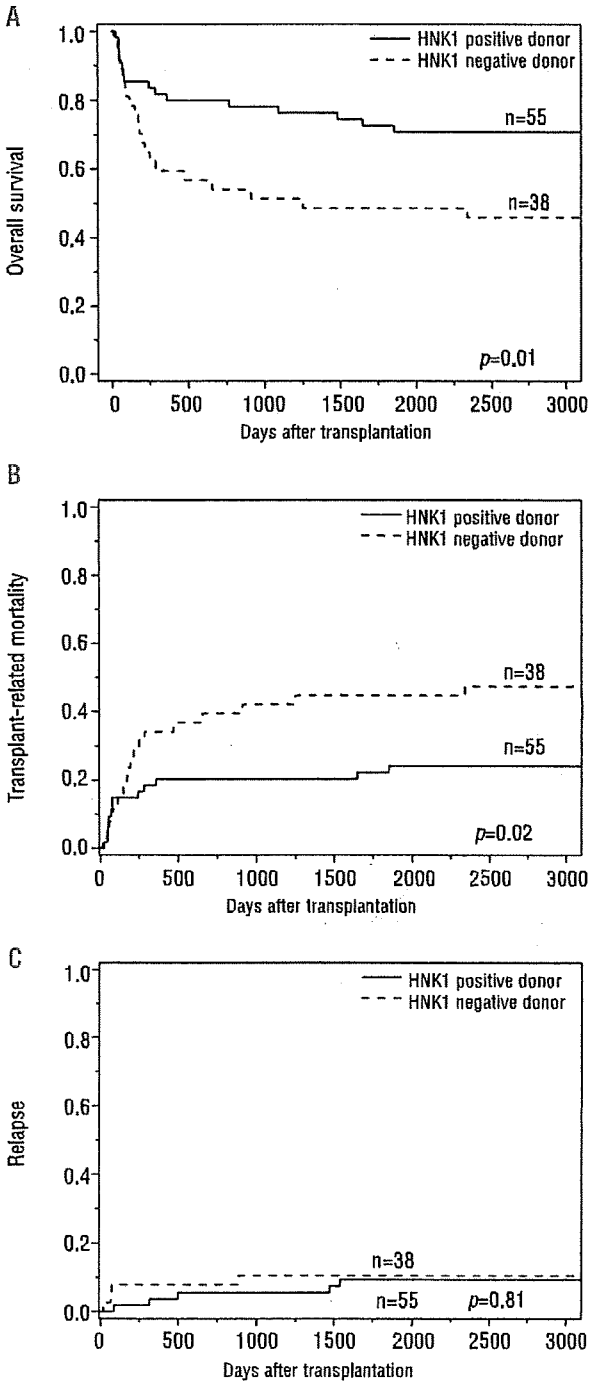

Figure 1. Kaplan-Meier analysis of (A) overall survival, (B) cumulative incidence of transplant-related mortality and (C) disease relapse after transplantation according to the donor *NKG2D* polymorphism in patients with standard-risk disease. Patients with donors with the *HNK1* haplotype had better overall survival and lower transplant-related mortality. Donor haplotype had no significant impact on disease relapse.

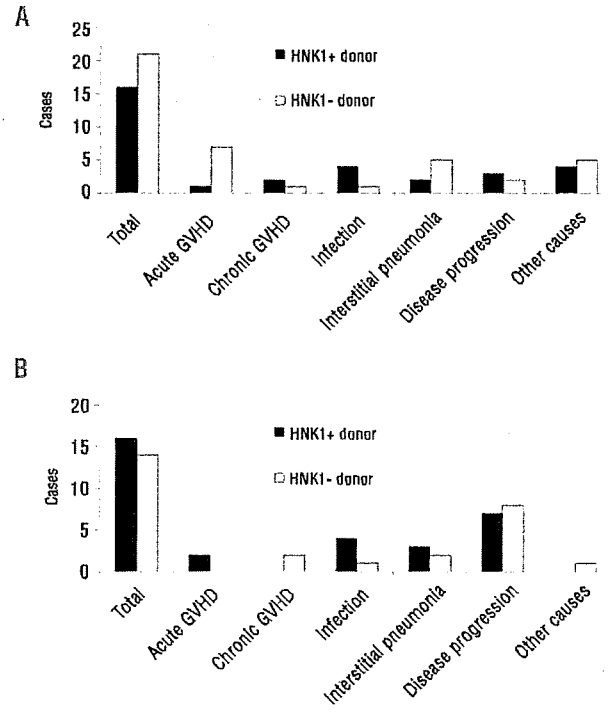

Figure 2. Main causes of death after transplantation according to the *NKG2D* polymorphism in patients with (A) standard-risk disease (B) high-risk disease.

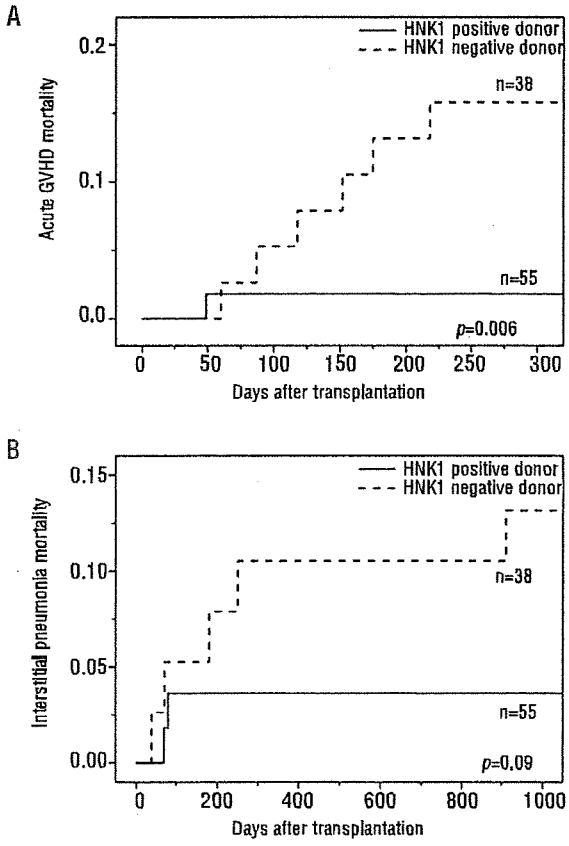

Figure 3. **Cumulative incidence of deaths due to (A) acute GVHD and (B) interstitial pneumonia after transplantation in patients with standard-risk disease. The *HNK1* haplotype in donors was associated with a significantly lower incidence of deaths due to acute GVHD (p=0.006) as well as a trend toward a lower incidence of deaths due to interstitial pneumonia (p=0.09).**

nase citullinating enzymes 4 (PADI4)[13] and methylenetetrahydrofolate reductase (MTHFR)[14] have been shown to influence the outcome after allogeneic SCT. Most of them are associated with the development of GVHD. Only the *NOD2/CARD15* and *HO-1* promoter polymorphisms have a significant impact on overall survival after SCT. Furthermore, the impact of the *HO-1* promoter polymorphisms depends on donor cells but not on recipient cells, as observed with the *NKG2D* polymorphism which, in the donor, was shown to be significantly associated with overall survival in the present study. This may prompt the determination of the donor *NKG2D* polymorphism prior to SCT in order to choose the best donor, expected to minimize transplant-related mortality after SCT, when multiple donors for a patient are available. Otherwise, prior information on the donor *NKG2D* polymorphism may be helpful in selecting risk-specific appropriate precautions following transplantation.

In conclusion, the present data suggest that the *NKG2D* polymorphism, in addition to HLA disparity between recipients and donors, affects prognosis after a bone marrow transplant from an unrelated donor. However, care should be made in drawing conclusions because the number of patients in the present study was small. The finding of a gene polymorphism may not be equivalent to differences in gene expression, which may be influenced by multiple factors because the NKG2D receptor is found on many tissues and cells.[41] Experimental evidence is required to substantiate the effect of the *NKG2D* polymorphism on immune function. We next plan to conduct a prospective study to confirm these results and to extend this investigation to other transplantation settings, such as related donor SCT, reduced-intensity SCT, HLA-mismatched SCT and SCT for patients with non-hematologic malignancies.

Authorship and Disclosures

JLE and AT designed and performed the research, and contributed to the same aspects of the work; AT, JLE and SN wrote the paper; AT, YKa, and SOh performed the statistical analyses; MO, HS, HA, KM, SOk, MI, TF, YM, and YKo contributed to data collection.

The authors reported no potential conflicts of interest.

References

1. Weisdorf DJ, Anasetti C, Antin JH, Kernan NA, Kollman C, Snyder D, et al. Allogeneic bone marrow transplantation for chronic myelogenous leukemia: comparative analysis of unrelated versus matched sibling donor transplantation. Blood 2002; 99:1971-7.
2. Gratwohl A, Brand R, Frassoni F, Rocha V, Niederwieser D, Reusser P, et al. Cause of death after allogeneic haematopoietic stem cell transplantation (HSCT) in early leukaemias: an EBMT analysis of lethal infectious complications and changes over calendar time. Bone Marrow Transplant 2005;36:757-69.
3. Dickinson AM, Middleton PG, Rocha V, Gluckman E, Holler E. Genetic polymorphisms predicting the outcome of bone marrow transplants. Br J Haematol 2004; 127:479-90.
4. Bochud PY, Chien JW, Marr KA, Leisenring WM, Upton A, Janer M, et al. Toll-like receptor 4 polymorphisms and aspergillosis in stem-cell transplantation. N Engl J Med 2008; 359:1766-77.
5. Elmaagacli AH, Koldehoff M, Landt O, Beelen DW. Relation of an interleukin-23 receptor gene polymorphism to graft-versus-host disease after hematopoietic-cell transplantation. Bone Marrow Transplant 2008; 41:821-6.
6. Gerbitz A, Hillemanns P, Schmid C, Wilke A, Jayaraman R, Kolb HJ, et al. Influence of polymorphism within the heme oxygenase-I promoter on overall survival and transplantation-related mortality after allogeneic stem cell transplantation. Biol Blood Marrow Transplant 2008;14:1180-9.
7. Holler E, Rogler G, Brenmoehl J, Hahn J, Herfarth H, Greinix H, et al. Prognostic significance of NOD2/CARD15 variants in HLA-identical sibling hematopoietic stem cell transplantation: effect on long-term outcome is confirmed in 2 independent cohorts and may be modulated by the type of gastrointestinal decontamination. Blood 2006;107: 4189-93.
8. Holler E, Rogler G, Herfarth H, Brenmoehl J, Wild PJ, Hahn J, et al. Both donor and recipient

NOD2/CARD15 mutations associate with transplant-related mortality and GvHD following allogeneic stem cell transplantation. Blood 2004;104:889-94.
9. Mayor NP, Shaw BE, Hughes DA, Maldonado-Torres H, Madrigal JA, Keshav S, et al. Single nucleotide polymorphisms in the NOD2/CARD15 gene are associated with an increased risk of relapse and death for patients with acute leukemia after hematopoietic stem-cell transplantation with unrelated donors. J Clin Oncol 2007;25:4262-9.
10. Mullighan CG, Heatley S, Doherty K, Szabo F, Grigg A, Hughes TP, et al. Mannose-binding lectin gene polymorphisms are associated with major infection following allogeneic hemopoietic stem cell transplantation. Blood 2002;99:3524-9.
11. Noori-Daloii MR, Rashidi-Nezhad A, Izadi P, Hossein-Nezhad A, Sobhani M, Derakhshandeh-Peykar P, et al. Transforming growth factor-β1 codon 10 polymorphism is associated with acute GVHD after allogenic BMT in Iranian population. Ann Transplant 2007;12:5-10.
12. Rocha V, Franco RF, Porcher R, Bittencourt H, Silva WA Jr, Latouche A, et al. Host defense and inflammatory gene polymorphisms are associated with outcomes after HLA-identical sibling bone marrow transplantation. Blood 2002;100:3908-18.
13. Shimada M, Onizuka M, Machida S, Suzuki R, Kojima M, Miyamura K, et al. Association of autoimmune disease-related gene polymorphisms with chronic graft-versus-host disease. Br J Haematol 2007;139:458-63.
14. Soydan E, Topcuoglu P, Dalva K, Arat M. The impact of methylenetetrahydrofolate reductase (MTHFR) C677T gene polymorphism on transplant-related variables after allogeneic hematopoietic cell transplantation in patients receiving MTX as GVHD prophylaxis. Bone Marrow Transplant 2008;42:429-30.
15. Viel DO, Tsuneto LT, Sossai CR, Lieber SR, Marques SB, Vigorito AC, et al. IL2 and TNFA gene polymorphisms and the risk of graft-versus-host disease after allogeneic haematopoietic stem cell transplantation. Scand J Immunol 2007;66: 703-10.
16. Burgess SJ, Maasho K, Masilamani M, Narayanan S, Borrego F, Coligan JE. The NKG2D receptor: immunobiology and clinical implications. Immunol Res 2008;40:18-34.
17. Hyka-Nouspikel N, Phillips JH. Physiological roles of murine DAP10 adapter protein in tumor immunity and autoimmunity. Immunol Rev 2006;214:106-17.
18. Jamieson AM, Diefenbach A, McMahon CW, Xiong N, Carlyle JR, Raulet DH. The role of the NKG2D immunoreceptor in immune cell activation and natural killing. Immunity 2002;17:19-29.
19. Raulet DH. Roles of the NKG2D immunoreceptor and its ligands. Nat Rev Immunol 2003;3:781-90.
20. Groh V, Wu J, Yee C, Spies T. Tumour-derived soluble MIC ligands impair expression of NKG2D and T-cell activation. Nature 2002;419: 734-8.
21. Hayashi T, Imai K, Morishita Y, Hayashi I, Kusunoki Y, Nakachi K. Identification of the NKG2D haplotypes associated with natural cytotoxic activity of peripheral blood lymphocytes and cancer immunosurveillance. Cancer Res 2006;66: 563-70.
22. Imai K, Matsuyama S, Miyake S, Suga K, Nakachi K. Natural cytotoxic activity of peripheral-blood lymphocytes and cancer incidence: an 11-year follow-up study of a general population. Lancet 2000;356:1795-9.
23. Furue H, Kumimoto H, Matsuo K, Suzuki T, Hasegawa Y, Shinoda M, et al. Opposite impact of NKG2D genotype by lifestyle exposure to risk of aerodigestive tract cancer among Japanese. Int J Cancer 2008; 123:181-6.
24. Furue H, Matsuo K, Kumimoto H, Hiraki A, Suzuki T, Yatabe Y, et al. Decreased risk of colorectal cancer with the high natural killer cell activity NKG2D genotype in Japanese. Carcinogenesis 2008; 29:316-20.
25. Kawase T, Morishima Y, Matsuo K, Kashiwase K, Inoko H, Saji H, et al. High-risk HLA allele mismatch combinations responsible for severe acute graft-versus-host disease and implication for its molecular mechanism. Blood 2007;110:2235-41.
26. Sasazuki T, Juji T, Morishima Y, Kinukawa N, Kashiwabara H, Inoko H, et al. Effect of matching of class I HLA alleles on clinical outcome after transplantation of hematopoietic stem cells from an unrelated donor. Japan Marrow Donor Program. N Engl J Med 1998;339:1177-85.
27. Livak KJ. Allelic discrimination using fluorogenic probes and the 5' nuclease assay. Genet Anal 1999;14:143-9.
28. Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J, et al. 1994 Consensus Conference on Acute GVHD Grading. Bone Marrow Transplant 1995;15:825-8.
29. Shulman HM, Sullivan KM, Weiden PL, McDonald GB, Striker GE, Sale GE, et al. Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. Am J Med 1980;69:204-17.
30. Scrucca L, Santucci A, Aversa F. Competing risk analysis using R: an easy guide for clinicians. Bone Marrow Transplant 2007;40:381-7.
31. Gooley TA, Leisenring W, Crowley J, Storer BE. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. Stat Med 1999;18: 695-706.
32. Kim DH, Jung HD, Lee NY, Sohn SK. Single nucleotide polymorphism of CC chemokine ligand 5 promoter gene in recipients may predict the risk of chronic graft-versus-host disease and its severity after allogeneic transplantation. Transplantation 2007;84:917-25.
33. Dulphy N, Haas P, Busson M, Belhadj S, Peffault de Latour R, Robin M, et al. An unusual CD56(bright) CD16(low) NK cell subset dominates the early posttransplant period following HLA-matched hematopoietic stem cell transplantation. J Immunol 2008; 181:2227-37.
34. Hamby K, Trexler A, Pearson T, Larsen C, Rigby M, Kean L. NK cells rapidly reject allogeneic bone marrow in the spleen through a perforin- and Ly49D-dependent, but NKG2D-independent mechanism. Am J Transplant 2007;7:1884-96.
35. Ruggeri L, Capanni M, Urbani E, Perruccio K, Shlomchik W, Tosti A, et al. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Science 2002;295:2097-100.
36. Ruggeri L, Mancusi A, Burchielli E, Capanni M, Carotti A, Aloisi T, et al. NK cell alloreactivity and allogeneic hematopoietic stem cell transplantation. Blood Cells Mol Dis 2008;40: 84-90.
37. Hsu K, Keever-Taylor C, Wilton A, Pinto C, Heller G, Arkun K, et al. Improved outcome in HLA-identical sibling hematopoietic stem-cell transplantation for acute myelogenous leukemia predicted by KIR and HLA genotypes. Blood 2005;105: 4878-84.
38. Morishima Y, Yabe T, Matsuo K, Kashiwase K, Inoko H, Saji H, et al. Effects of HLA allele and killer immunoglobulin-like receptor ligand matching on clinical outcome in leukemia patients undergoing transplantation with T-cell-replete marrow from an unrelated donor. Biol Blood Marrow Transplant 2007;13: 315-28.
39. Kimura F, Sato K, Kobayashi S, Ikeda T, Sao H, Okamoto S, et al. Impact of ABO-blood group incompatibility on the outcome of recipients of bone marrow transplants from unrelated donors in the Japan Marrow Donor Program. Haematologica 2008;93: 1686-93.
40. Goldstone AH, Richards SM, Lazarus HM, Tallman MS, Buck G, Fielding AK, et al. In adults with standard-risk acute lymphoblastic leukemia, the greatest benefit is achieved from a matched sibling allogeneic transplantation in first complete remission, and an autologous transplantation is less effective than conventional consolidation/maintenance chemotherapy in all patients: final results of the International ALL Trial (MRC UKALL XII/ECOG E2993). Blood 2008;111: 1827-33.
41. Collins RW. Human MHC class I chain related (MIC) genes: their biological function and relevance to disease and transplantation. Eur J Immunogenet 2004;31:105-14.

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Infectious complications in patients receiving autologous CD34-selected hematopoietic stem cell transplantation for severe autoimmune diseases

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Abstract: Long-term analysis of infectious complication after high-dose immunosuppressive therapy with CD34-selected autologous hematopoietic stem cell transplantation for patients with severe autoimmune diseases (AD) was performed. Theoretically, CD34 selection can reduce the risk of reinfusion of autoreactive lymphocytes. However, it is also associated with a significant reduction in T cells, natural killer cells, and monocytes, which in turn may compromise immune reconstitution, thereby increasing the risk of infection. Moreover, AD compromises host immunity and causes organ damage resulting in dysfunction of the cutaneous or mucosal barrier. In this study, the incidence rate of infections is reported in 14 patients who underwent high-dose (200 mg/kg) cyclophosphamide therapy followed by reinfusion of CD34-selected autologous peripheral blood stem cells. Bacterial complication occurred in 3 of 14 (21%) patients. Cytomegalovirus reactivation and adenovirus hemorrhagic cystitis were observed in 9 (64%) and 2 (14%) patients, respectively. As for late infectious complications, 7 patients (50%) developed dermatomal varicella zoster virus infection. No infection-related mortality was seen in this case series. Because the risk for infections approaches that seen in allogeneic transplant recipients, infection surveillance, diagnostic workup, and prophylactic strategies similar to those applicable to allogeneic recipients are warranted.

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Pilot studies comprising high-dose immunosuppressive therapy followed by transplantation of autologous hematopoietic stem cells (HSC) were conducted to obtain safety and preliminary efficacy data in patients with severe autoimmune diseases (AD) (1–5).

Both unselected and CD34-selected peripheral blood stem cells (PBSC) have been used as sources of HSC (1–5). Theoretically, CD34 + cell selection of PBSC can reduce the possibility of reinfusion of autoreactive lymphocytes. However, the superiority of CD34-selected PBSC over unmanipulated PBSC has not been established. The safety and efficacy of CD34-selected autologous PBSC transplantation (PBSCT) for refractory AD have been investigated at our institute (6).

PBSCT-related complications include regimen-related toxicities and various infections. For the treatment of AD, PBSCT is a more toxic treatment modality than the conventional immunosuppressive therapies. Organ damage due to AD puts the patients at risk of regimen-related toxicities. Thus, careful selection of refractory AD patients for PBSCT is essential to minimize transplant-related mortality.

Infections are major contributors to morbidity and mortality in PBSCT. In hematological malignancies, CD34-selected autologous PBSCT has been reported to increase incidences of opportunistic infections compared with non-CD34-selected autologous PBSCT (7–11). Most AD patients had undergone immunosuppressive therapy, including cyclosporine and corticosteroids, before transplantation. AD

itself compromises host immunity to various infections. Furthermore, AD causes organ damage such as skin ulcers, esophageal dysmotility, and interstitial pneumonia (IP) resulting in dysfunction of the cutaneous or mucosal barrier. Thus, the understanding of infectious complications is important in increasing the safety of CD34-selected autologous PBSCT for AD.

Here, we retrospectively analyze infectious complications during the course of CD34-selected autologous PBSCT for severe AD.

Materials and methods

Protocol

The protocol of this phase I/II clinical trial (6) was approved by the ethics committee of Kyushu University Hospital. Written informed consent was obtained from all patients.

Patients and eligibility

Patients between 16 and 65 years of age were eligible at the time of pre-transplant evaluation. Patient eligibility depended on the diagnosis of AD, as previously described (6).

PBSC mobilization and CD34 + cell selection

PBSC were mobilized during hematological recovery after administration of cyclophosphamide (CY) (2 g/m^2/day) for 2 days, followed by a recombinant human granulocyte-colony stimulating factor (G-CSF, filgrastim; Kirin Brewery, Tokyo, Japan) at a dosage of 2 μg/kg/day. After collecting PBSC to obtain 2 × 10^6 CD34 + cells/kg or more by apheresis, CD34 + cells were positively selected using immunomagnetic beads with an anti-CD34 monoclonal antibody (CliniMACS, Miltenyi Biotec, Cologne, Germany).

Autologous PBSCT and supportive care

Patients were kept in HEPA-filtered rooms until engraftment. For pre-transplant conditioning, high-dose CY (50 mg/kg/day) was administered for 4 days, from days −5 to −2. Frozen-thawed CD34-selected PBSC were infused on day 0. All immunosuppressive and disease-modifying agents were discontinued upon HSC procurement, except systemic corticosteroids, which were tapered to a relatively low dose (5–15 mg of prednisolone/day) over 2–6 months after PBSCT. Acyclovir (intravenous 250 mg/day, from days 1 to 18), ciprofloxacin (by mouth [PO] 600 mg/day, from days −7 to 14), fluconazole (PO 200 mg/day, from days −7 to 30), and trimethoprim-sulfamethoxazole (TMP-SMX) (each 1920 mg/day; from days −14 to −2, and twice a week from days 30 to 180, respectively) were prophylactically administered, as previously described (6). Neutropenic fever was treated with intravenous administration of broad-spectrum cephalosporins according to the guidelines for the use of antimicrobial agents in neutropenic patients (12). After engraftment, weekly monitoring of cytomegalovirus (CMV) pp65 antigenemia was conducted until day 100 after transplant (13). If CMV antigenemia was detected, preemptive therapy was initiated with ganciclovir.

Diagnosis and definition of infections

The day of onset of infection was defined as the day the diagnostic test was performed.

Bacterial infections were categorized as bacteremias and site-specific infections (14). Varicella zoster virus (VZV) infections were defined as typical cutaneous vesicular lesions. CMV infection and disease were defined as previously described (13, 15). In brief, CMV infection was defined as isolation of the CMV virus or detection of the viral proteins or nucleic acids in body fluid or tissue specimen. CMV disease is defined by the presence of organ-specific signs and/or symptoms with the detection of CMV in test specimens (e.g., bronchoalveolar lavage in the lungs or biopsy samples in other organs). CMV infection with unexplained fever for at least 2 days within a 4-day period and the presence of neutropenia or thrombocytopenia is considered CMV syndrome. Hemorrhagic cystitis (HC) due to adenoviruses (AdV) was diagnosed when AdV were detected by either viral culture or polymerase chain reaction in macroscopic hematuria with clinical signs of cystitis. To exclude regimen-related HC, patients with *de novo* hematuria at least 10 days after HSC transplantation (HSCT) and no tendency toward generalized bleeding or bacteriuria were considered to have AdV HC (16). Fungal infection was defined by proven or probable invasive fungal infection (17) and clinical or radiological manifestation along with positive microbiological tests.

Results

Patients

Fourteen patients (4 males, 10 females) with a median age of 54 years (range 21–63 years) were examined (Table 1). Patients No. 1–11 were diagnosed as diffuse systemic sclerosis (SSc). Patient No. 1 was suffering from systemic lupus er-

Clinical characteristics of the autoimmune patients receiving CD34-selected transplant

Patient number	Disease	Age (years)	Sex	Complication	Previous therapies	Follow up (months)[1]
1	SSc/SLE	54	F	IP, digital ulcer	DEX, IVCY	72
2	SSc	55	M	IP, digital ulcer	PSL, IVCY	65
3	SSc	58	M	IP	PSL, IVCY	61
4	SSc	54	F	IP	PSL, IVCY	58
5	SSc	53	F	IP	PSL	56
6	SSc	49	F	IP	m-PSL, CsA, IVCY	52
7	SSc	33	F	IP	–	21
8	SSc	63	F	IP	PSL	36
9	SSc	61	F	IP	PSL, CsA	31
10	SSc	44	F	IP	PSL, IVCY	27
11	SSc	52	M	IP, digital ulcer	PSL, CsA, IVCY	23
12	DM	54	F	IP	PSL pulse, CsA, IVCY	70
13	DM	44	F	IP, skin ulcer	PSL pulse, CsA	12
14	WG	21	M	Exophthalmos	PSL pulse, IVCY	55

[1]After transplantation.
SSc, systemic sclerosis; SLE, systemic lupus erythematosus; IP, interstitial pneumonia; DEX, dexamethasone; IVCY, intravenous cyclophosphamide; PSL, prednisolone; m-PSL, methyl prednisolone; CsA, cyclosporine; DM, dermatomyositis; WG, Wegener's granulomatosis.

Table 1

ythematosus (SLE) for 22 years and SSc for 2 years. Although SLE was inactive, she had progressive IP and severe digital ulcers due to SSc. Patients No. 2–11 (SSc) and 12 and 13 (dermatomyositis) developed IP, which did not respond to immunosuppressive agents. Patients No. 3–6, 8, 9, and 11 showed severe skin sclerosis. Patient No. 3 had been in complete remission from non-Hodgkin's lymphoma for 1 year and was considered eligible. Patient No. 14 (Wegener's granulomatosis) presented with severe exophthalmos due to granuloma formation (18 mm in diameter) in the upper lateral region of the left orbit affecting the superior rectus muscle. He needed monthly steroid pulse therapy to prevent further growth of the granuloma. The Eastern Cooperative Oncology Group performance status (18) was <3 in all patients. CY and cyclosporine were administered to 9 patients and 5 patients, respectively. All patients, except Patient No. 7, were treated with corticosteroids. The median follow-up duration was 53.5 months after transplant (range 8–72 months).

Results are reported as of March 2008.

Infections

Bacterial

Nine of 14 patients developed febrile neutropenia at a median of 6 (0–9) days after PBSCT (Table 2). Among these, Patients No. 6 and 11 revealed *Streptococcus mitis* bacteremia on days 8 and 9 after PBSCT, respectively. Both were empirically treated with broad-spectrum cephalosporins. Vancomycin was added when the blood culture was reported positive. Patient No. 12 developed high-grade fever without signs of local infection on day 119 post PBSCT, and a blood culture turned out to be positive for *Listeria monocytogenes*. Empirical therapy was initiated with broad-spectrum cephalosporin but switched to penicillin/β-lactamase inhibitor after detection of the microbe. All patients responded to the therapy, and no fatal complications occurred.

Patient No. 14 developed *Mycobacterium gordonae* pneumonia 1343 days after PBSCT. However, he was on anti-tumor necrosis factor (TNF) antibody therapy because of the relapse at that time; thus the case is omitted from this study.

Viral

CMV. Nine of 14 patients developed CMV antigenemia at a median of 28 days (range 10–60 days) after PBSCT. All patients who developed CMV infection were seropositive for CMV antibody before PBSCT, and this was considered as reactivation. Patients were preemptively treated with ganciclovir (5 mg/kg twice a day) and none developed CMV disease. High levels of antigenemia were detected in Patients No. 1 and 6. With their clinical symptoms, these patients were considered to have CMV syndrome. Foscarnet

was administered to Patient No. 12 because CMV antigenemia persisted despite ganciclovir therapy.

VZV. Seven of 14 patients developed VZV infection at a median of 409 days (range 351–1263 days) after PBSCT. All patients were treated with either oral valacyclovir or intravenous acyclovir promptly after diagnosis. All patients had dermatomal disease, and no dissemination was observed.

AdV. Patients No. 1 and 6 developed AdV HC on days 64 and 33, respectively. Cidofovir was administered in both patients.

Fungal

No fungal infection was observed in this case series.

Discussion

We present a long-term analysis of infectious complications in AD patients who had undergone CD34-selected autologous PBSCT.

Bacterial infections were observed in 3 patients. Despite the increased frequency of isolation of viridans streptococci from the blood of neutropenic patients (19, 20), bacteremia from *S. mitis* was observed in only 2 patients in the present study. The incidence of streptococcal bacteremia (2/14; 14%) was the same as that reported in other studies (16–31%) (21, 22). Although antibacterial prophylaxis was undertaken with ciprofloxacin (23), the susceptibility of the pathogen to penicillin was intermediate in both cases; hence, vancomycin was added to the treatment regimen. *L. monocytogenes* infection in HSCT is rare (24) because TMP-SMX, traditionally used for prophylaxis, is active against the microbe. However, the patient did not take TMP-SMX when she developed *L. monocytogenes* bacteremia. As TMP-SMX is effective not only in prophylaxis for *Pneumocystis jirovecii* pneumonia but also against Listeria, Nocardia, and Toxoplasma, its prophylactic administration is mandatory.

CMV reactivation has been reported to be uncommon in unselected autologous PBSCT (25–27). However, 64% of our patients (9/14) became positive for CMV antigenemia after PBSCT and were treated with ganciclovir. Although no patient developed CMV disease, the level of antigenemia in 2 patients was so high that they might have developed CMV disease without monitoring and preemptive therapy. Although it is still not clear whether CD34 selection of autograft itself is a risk for CMV infection (7, 28), this proportion of CMV reactivation is comparable to that of CD34-selected autologous HSCT for hematological malignancies, or allogeneic HSCT (13). Thus, we consider CMV monitoring necessary in AD patients undergoing CD34-selected autologous PBSCT.

Infectious complications in autoimmune patients receiving CD34-selected transplant

Patient number	FN (days)	Bacterial infection (days)	CMV Ab	CMV (days)	VZV (days)	Others (days)
1	5	–	+	38	1263	AdV cystitis (64)
2	–	–	+	–	–	–
3	–	–	+	10	351	–
4	–	–	+	24	418	–
5	–	–	+	–	409	–
6	7	*Streptococcus mitis* (8)	+	33	–	AdV cystitis (31)
7	0	–	–	–	–	–
8	1	–	+	–	–	–
9	5	–	+	22	374	–
10	–	–	+	60	427	–
11	8	*Streptococcus mitis* (9)	+	28	–	–
12	5	*Lysteria monocytogenes* (119)	+	22	358	–
13	9	–	+	–	–	–
14	9	–	+	–	–	–

FN, febrile neutropenia; days, days after hematopoietic stem cell transplantation; CMV, cytomegalovirus infection; Ab, antibody VZV, varicella zoster virus infection; AdV, adenovirus.

Table 2

AdV HC developed in 2 of 14 patients (14%). The incidence of AdV infection in the autologous HSCT is reported to be 1% (29), and HC is a rare development. T-cell depletion and lymphopenia are the risk factors (30, 31) for AdV disease in adults, and severe lymphopenia was seen in both the patients (139 and 318/μL, respectively). They were successfully treated with cidofovir (16).

Regarding late infectious complications, 50% of the patients (7/14) developed VZV infection at a median of 409 days (range 351–1263 days) after PBSCT. Delayed recovery of CD4 + T cell (32, 33) and increased incidences of the VZV infections (8) have been reported in CD34-selected transplantation. In addition to CD34 selection of auto-graft, low-dose steroids were continued after PBSCT in our study. Delayed CD4 + T-cell recovery along with high incidence of late infection were both observed. We used prophylactic acyclovir from days 1 to 35 and did not use long-term low-dose acyclovir prophylaxis (25, 34, 35).

Data are conflicting whether CD34-selected PBSCT for hematologic malignancies and breast cancer cause increased incidence of infections compared with non-CD34-selected PBSCT. In this study, underlying AD itself, prolonged immunosuppressive therapy before PBSCT, CD34 selection of the auto-graft, and low-dose systemic steroid administration after the PBSCT might contribute to the high incidence of infectious complications.

In conclusion, our findings confirm a very high incidence of infectious complications after CD34-selected autologous PBSCT for AD. Because the risk for infections approaches that seen in allogeneic transplant recipients, infection surveillance, diagnostic workup, and prevention strategies similar to those applicable to allogeneic recipients are recommended.

References

1. Burt RK, Traynor A, Ramsey-Goldman R. Hematopoietic stem-cell transplantation for systemic lupus erythematosus. N Engl J Med 1997; 337 (24): 1777–1778.
2. Traynor AE, Schroeder J, Rosa RM, et al. Treatment of severe systemic lupus erythematosus with high-dose chemotherapy and haemopoietic stem-cell transplantation: a phase I study. Lancet 2000; 356 (9231): 701–707.
3. van Laar JM, Farge D, Tyndall A. Autologous Stem cell Transplantation International Scleroderma (ASTIS) trial: hope on the horizon for patients with severe systemic sclerosis. Ann Rheum Dis 2005; 64: 1515.
4. Nash RA, McSweeney PA, Crofford LJ, et al. High-dose immunosuppressive therapy and autologous hematopoietic cell transplantation for severe systemic sclerosis: long-term follow-up of the US multicenter pilot study. Blood 2007; 110 (4): 1388–1396.
5. Oyama Y, Barr WG, Statkute L, et al. Autologous non-myeloablative hematopoietic stem cell transplantation in patients with systemic sclerosis. Bone Marrow Transplant 2007; 40: 549–555.
6. Tsukamoto H, Nagafuji K, Horiuchi T, et al. A phase I–II trial of autologous peripheral blood stem cell transplantation in the treatment of refractory autoimmune disease. Ann Rheum Dis 2006; 65 (4): 508–514.
7. Holmberg LA, Boeckh M, Hooper H, et al. Increased incidence of cytomegalovirus disease after autologous CD34-selected peripheral blood stem cell transplantation. Blood 1999; 94 (12): 4029–4035.
8. Crippa F, Holmberg L, Carter RA, et al. Infectious complications after autologous CD34-selected peripheral blood stem cell transplantation. Biol Blood Marrow Transplant 2002; 8 (5): 281–289.
9. Miyamoto T, Gondo H, Miyoshi Y, et al. Early viral complications following CD34-selected autologous peripheral blood stem cell transplantation for non-Hodgkin's lymphoma. Br J Haematol 1998; 100 (2): 348–350.
10. Witzens-Harig M, Heilmann C, Hensel M, et al. Long-term follow-up of patients with non-Hodgkin lymphoma following myeloablative therapy and autologous transplantation of CD34 + -selected peripheral blood progenitor cells. Stem Cells 2007; 25 (1): 228–235.
11. Lin PC, Lee MY, Lin JT, Hsiao LT, Chen PM, Chiou TJ. Virus reactivation in high-risk non-Hodgkin's lymphoma patients after autologous CD34(+)-selected peripheral blood progenitor cell transplantation. Int J Hematol 2008; 87 (4): 434–439.
12. Hughes WT, Armstrong D, Bodey GP, et al. 2002 guidelines for the use of antimicrobial agents in neutropenic patients with cancer. Clin Infect Dis 2002; 34: 730–751.
13. Takenaka K, Gondo H, Tanimoto K, et al. Increased incidence of cytomegalovirus (CMV) infection and CMV-associated disease after allogeneic bone marrow transplantation from unrelated donors. The Fukuoka Bone Marrow Transplantation Group. Bone Marrow Transplant 1997; 19 (3): 241–248.
14. Narimatsu H, Matsumura T, Kami M, et al. Bloodstream infection after umbilical cord blood transplantation using reduced-intensity stem cell transplantation for adult patients. Biol Blood Marrow Transplant 2005; 11: 429–436.
15. Ljungman P, Griffiths P, Paya C. Definitions of cytomegalovirus infection and disease in transplant recipients. Clin Infect Dis 2002; 34 (8): 1094–1097.
16. Nagafuji K, Aoki K, Henzan H, et al. Cidofovir for treating adenoviral hemorrhagic cystitis in hematopoietic stem cell transplant recipients. Bone Marrow Transplant 2004; 34 (10): 909–914.
17. Ascioglu S, Rex JH, de Pauw B, et al. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. Clin Infect Dis 2002; 34 (1): 7–14.
18. Oken MM, Creech RH, Tormey DC, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 1982; 5 (6): 649–655.
19. Classen DC, Burke JP, Ford CD, et al. *Streptococcus mitis* sepsis in bone marrow transplant patients receiving oral antimicrobial prophylaxis. Am J Med 1990; 89: 441–446.
20. Collin BA, Leather HL, Wingard JR, Ramphal R. Evolution, incidence, and susceptibility of bacterial bloodstream isolates from 519 bone marrow transplant patients. Clin Infect Dis 2001; 33 (7): 947–953.
21. Bilgrami S, Feingold JM, Dorsky D, Edwards RL, Clive J, Tutschka PJ. *Streptococcus viridans* bacteremia following autologous peripheral blood stem cell transplantation. Bone Marrow Transplant 1998; 21 (6): 591–595.
22. Razonable RR, Litzow MR, Khaliq Y, Piper KE, Rouse MS, Patel R. Bacteremia due to viridans group Streptococci with diminished susceptibility to levofloxacin among neutropenic patients receiving levofloxacin prophylaxis. Clin Infect Dis 2002; 34 (11): 1469–1474.

23. Gafter-Gvili A, Fraser A, Paul M, Leibovici L. Meta-analysis: antibiotic prophylaxis reduces mortality in neutropenic patients. Ann Intern Med 2005; 142 (12, Part 1): 979–995.
24. Radice C, Munoz V, Castellares C, et al. *Listeria monocytogenes* meningitis in two allogeneic hematopoietic stem cell transplant recipients. Leuk Lymphoma 2006; 47 (8): 1701–1703.
25. Sullivan KM, Dykewicz CA, Longworth DL, et al. Preventing opportunistic infections after hematopoietic stem cell transplantation: the Centers for Disease Control and Prevention, Infectious Diseases Society of America, and American Society for Blood and Marrow Transplantation Practice Guidelines and beyond. Hematology Am Soc Hematol Educ Program 2001; 392–421.
26. Bilgrami S, Aslanzadeh J, Feingold JM, et al. Cytomegalovirus viremia, viruria and disease after autologous peripheral blood stem cell transplantation: no need for surveillance. Bone Marrow Transplant 1999; 24: 69–73.
27. Nagafuji K, Eto T, Hayashi S, Tokunaga Y, Gondo H, Niho Y. Fatal cytomegalovirus interstitial pneumonia following autologous peripheral blood stem cell transplantation. Fukuoka Bone Marrow Transplantation Group. Bone Marrow Transplant 1998; 21 (3): 301–303.
28. Frere P, Pereira M, Fillet G, Beguin Y. Infections after CD34-selected or unmanipulated autologous hematopoietic stem cell transplantation. Eur J Haematol 2006; 76 (2): 102–108.
29. La Rosa AM, Champlin RE, Mirza N, et al. Adenovirus infections in adult recipients of blood and marrow transplants. Clin Infect Dis 2001; 32 (6): 871–876.
30. Chakrabarti S, Mautner V, Osman H, et al. Adenovirus infections following allogeneic stem cell transplantation: incidence and outcome in relation to graft manipulation, immunosuppression, and immune recovery. Blood 2002; 100 (5): 1619–1627.
31. Symeonidis N, Jakubowski A, Pierre-Louis S, et al. Invasive adenoviral infections in T-cell-depleted allogeneic hematopoietic stem cell transplantation: high mortality in the era of cidofovir. Transpl Infect Dis 2007; 9 (2): 108–113.
32. Takeshima M, Takamatsu H, Iida M, Mochizuki Y, Okumura H, Yoshida T. Frequent viral infections and delayed CD4 + cell recovery following CD34 + cell-selected autologous peripheral blood stem cell transplantation. Int J Hematol 1999; 70 (3): 193–199.
33. Moore J, Brooks P, Milliken S, et al. A pilot randomized trial comparing CD34-selected versus unmanipulated hemopoietic stem cell transplantation for severe, refractory rheumatoid arthritis. Arthritis Rheum 2002; 46 (9): 2301–2309.
34. Kanda Y, Mineishi S, Saito T, et al. Long-term low-dose acyclovir against varicella-zoster virus reactivation after allogeneic hematopoietic stem cell transplantation. Bone Marrow Transplant 2001; 28 (7): 689–692.
35. Asano-Mori Y, Kanda Y, Oshima K, et al. Long-term ultra-low-dose acyclovir against varicella-zoster virus reactivation after allogeneic hematopoietic stem cell transplantation. Am J Hematol 2008; 83 (6): 472–476.

Longstanding Remission of Adult Onset Still's Disease Under Imatinib Therapy in a Patient with Chronic Myelogenous Leukemia

To the Editor:

Imatinib mesylate is a potent and selective inhibitor of tyrosine kinases such as Bcr-Abl, platelet-derived growth factor (PDGF) receptor, and c-Kit, and it is widely used to treat chronic myeloid leukemia (CML) and c-Kit-positive gastrointestinal stromal tumors. Imatinib also exerts potent immunomodulatory effects *in vitro* and *in vivo*[1]. Recent reports described the efficacy of imatinib in treating patients with various autoimmune and inflammatory diseases such as rheumatoid arthritis (RA), systemic scleroderma, pulmonary arterial hypertension, and lupus nephritis[2-5].

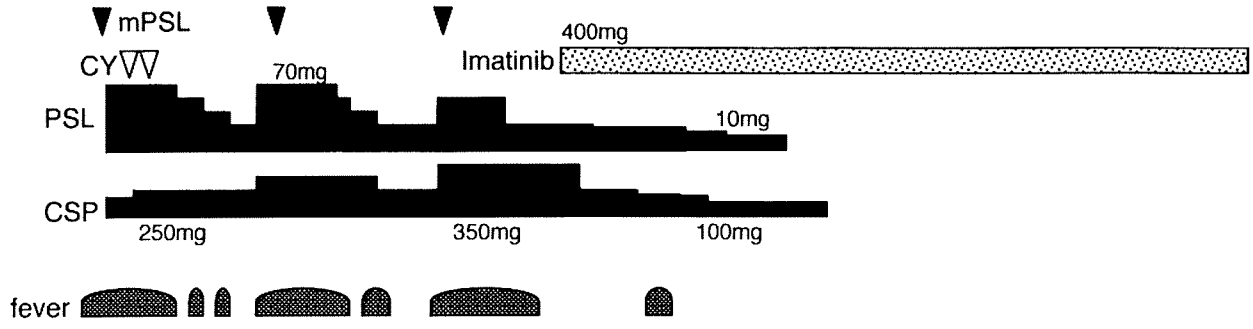
We describe a patient with steroid-resistant adult onset of Stills disease (AOSD), who incidentally developed CML. Treatment with imatinib induced hematological remission of CML as well as improvement of symptoms of AOSD. Immunosuppressants were tapered and discontinued 2 years after the initial imatinib treatment. The patient has maintained remission of AOSD for 5 years under imatinib treatment.

In August 2000, a 25-year-old Japanese man was admitted to the Department of General Medicine, Kyushu University Hospital, due to fever of unknown origin. He exhibited symptoms of pharyngitis, arthralgia, typical rash, leukocytosis, high level of C-reactive protein (CRP; 33.0 mg/dl), and hyperferritinemia (19,777 ng/ml). A diagnosis of AOSD was made based on his symptoms and these results. He was treated with prednisolone (PSL; 1 mg/kg daily). However, the disease proved to be steroid-resistant. Treatment with methylprednisolne pulse therapy (1000 mg for 3 days) in combination with cyclosporine (CSP) and cyclophosphamide resulted in gradual clinical improvement (Figure 1). Cyclophosphamide was discontinued, and PSL (0.25 mg/kg/day) and CSP (5 mg/kg/day) were taken daily as outpatient medication. However, he was frequently readmitted because of high fever, rash, and arthralgia. He experienced frequent AOSD flares, for which he eventually received treatment with pulse methylprednisolne along with increased doses of PSL and CSP (Figure 1).

In July 2002, he was referred to the Department of Hematology, Kyushu University Hospital, for evaluation of leukocytosis. A peripheral blood test revealed a white blood cell count of 69.9 × 10^9/l (0.4% blasts, 0.4% promyelocytes, 14.4% myelocytes, 5.4% metamyelocytes, 70.3% neutrophils, 7% eosinophils, 1.7% basophils, and 3.8% lymphocytes). A bone marrow aspirate showed hypercellular marrow with marked myeloid predominance. Chromosomal and molecular analyses revealed 46,XY, t(9;22)(q34;q11) in all metaphases and the presence of major-BCR/ABL fusion transcript. He was diagnosed with chronic-phase CML. Treatment with imatinib 400 mg daily was initiated, resulting in a prompt decrease in the number of white blood cells; he exhibited a hematological and molecular response to CML. Following treatment with imatinib, he experienced no more high fevers, rashes, and arthralgia as in previous episodes. In July 2003, he was finally cured of these symptoms and had a marked decrease in serum levels of ferritin and CRP (Figure 1). Immunosuppressants were tapered, and PSL and CSP were discontinued in January and July 2004, respectively (Figure 1). Thereafter, he has maintained a molecular remission of CML. Since imatinib treatment he has completed 4 years with no symptoms of AOSD.

To our knowledge, this is the first report of the efficacy of imatinib in a patient with AOSD. Despite the standard immunosuppressive treatment for AOSD, our patient exhibited frequent flares that required increased dosage of corticosteroids and calcineurin inhibitors, in addition to methylprednisolne pulse therapy and cyclophosphamide therapy. Alternative therapy of biological agents such as interleukin 1 (IL-1) receptor antagonist, IL-6 receptor antibody, and tumor necrosis factor (TNF) inhibitors might have been considered for this patient. However, 2 years after the diagnosis of AOSD, he incidentally developed CML. Treatment with imatinib 400 mg daily resulted in a hematological and molecular remission of CML as well as a gradual clinical improvement of AOSD, allowing tapering of CSP and PSL. One year after imatinib treatment, AOSD symptoms had completely disappeared, and after 2 years of treatment, immunosuppressants were finally discontinued with no signs of AOSD flares.

Our case suggests that, in contrast to PSL and CSP, imatinib might be useful for treatment of AOSD. Selective inhibition of tyrosine kinases could provide a potent therapeutic option for a variety of autoimmune and inflammatory disorders[1]. Imatinib mesylate is a signal transduction inhibitor that targets Bcr-Abl, c-Kit, PDGF receptor, c-fms, and LCK. Recent reports have shown that imatinib proved beneficial in patients with various autoimmune disorders, including RA, systemic scleroderma, pulmonary arterial hypertension, lupus nephritis, ankylosing spondylitis, psoriasis, and Crohn's disease[1-5]. Consistent with the clinical findings, *in vitro* studies demonstrated that imatinib can inhibit multiple signaling pathways implicated in the pathogenesis of various autoimmune disorders, including T-cell proliferation, macrophage c-fms activation and cytokine production, c-Kit-mediated mast-cell release of TNF-α and IL-6, and synovial fibroblast PDGF receptor signaling and proliferation[3,7-10]. AOSD is a systemic inflammatory disease that includes the participation of various cytokines such as IL-1, IL-6, IL-18, TNF-α, macrophage colony-stimulating factor, PDGF, and interferon-γ. Biological agents such as anti-TNF-α



Year	2000/8	2001	2002	2003	2004	2005	2006	2007	2008
WBC(/μL)	31,320	23,420	18,200	69,900	4,900	8,740	6,370		4,210
Ferritin(ng/mL)	19,777	1,297	248	125.3	54.1	78.2	52.3		102.4
CRP(mg/dL)	33.0	17.6	14.8	0.8	0.4	0.3	0.3		0.3
PCR for BCR-ABL				+	-	-	-		-

Figure 1. Clinical course of AOSD before and after treatment with imatinib mesylate. mPSL: methylprednisolne; CY: cyclophosphamide; CSP: cyclosporine; CRP: C-reactive protein; WBC: white blood cell count.

and anti-IL-1 have been successfully used in cases of refractory AOSD[6]. Therefore, as in our case, imatinib could prove beneficial in patients with AOSD, an inflammatory disorder involved with various cytokines and multiple signaling pathways; the precise etiology of AOSD remains unclear[1,9].

Imatinib shows promise for treatment of AOSD and other autoimmune and inflammatory diseases. However, to clarify the efficacy of imatinib as well as other tyrosine kinase inhibitors, it is necessary to define which kinases and cellular responses mediate the disease pathogenesis. Moreover, since the effect of imatinib on autoimmune disorders has been observed in patients with CML treated with 400 mg imatinib per day, it is critical to define the therapeutic dosages suitable for treatment of these disorders[2,3,5].

CML arises from primary mutations in Bcr-Abl, and relatively high doses of imatinib are required to inhibit proliferation of the leukemic cells[1]. In contrast, autoimmune diseases are not associated with mutations in kinases, and wild-type kinases participate in the dysregulated cellular responses that mediate tissue injury. Therefore, lower doses of imatinib may prove beneficial in autoimmune diseases[1]. Further studies are needed to define the course of therapy required for imatinib treatment, and the influence of treatment, in autoimmune and inflammatory diseases.

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REFERENCES

1. Paniagua RT, Robinson WH. Imatinib for the treatment of rheumatic diseases. Nat Clin Pract Rheumatol 2007;3:190-1.
2. Miyachi K, Ihara A, Hankins RW, Murai R, Maehiro S, Miyashita H. Efficacy of imatinib mesylate (STI571) treatment for a patient with rheumatoid arthritis developing chronic myelogenous leukemia. Clin Rheumatol 2003;22:329-32.
3. Eklund KK, Joensuu H. Treatment of rheumatoid arthritis with imatinib mesylate: clinical improvement in three refractory cases. Ann Med 2003;35:362-7.
4. Baroni SS, Santillo M, Bevilacqua F, et al. Stimulatory autoantibodies to the PDGF receptor in systemic sclerosis. N Engl J Med 2006;354:2667-76.
5. Ames PR, Aye WW, Beatty C, O'Reilly D. Imatinib treatment of seropositive arthritis in a young woman with chronic myeloid leukemia. J Rheumatol 2008;35:1682.
6. Pouchot J. How can we improve the management of adult-onset Still's disease? Joint Bone Spine 2007;74:117-9.
7. Dietz AB, Souan L, Knutson GJ, Bulur PA, Litzow MR, Vuk-Pavlovic S. Imatinib mesylate inhibits T-cell proliferation in vitro and delayed-type hypersensitivity in vivo. Blood 2004;104:1094-9.
8. Sandler C, Joutsiniemi S, Lindstedt KA, Juutilainen T, Kovanen PT, Eklund KK. Imatinib mesylate inhibits platelet derived growth factor stimulated proliferation of rheumatoid synovial fibroblasts. Biochem Biophys Res Commun 2006;347:31-5.
9. Paniagua RT, Sharpe O, Ho PP, et al. Selective tyrosine kinase inhibition by imatinib mesylate for the treatment of autoimmune arthritis. J Clin Invest 2006;116:2633-42.
10. Koyama K, Hatsushika K, Ando T, et al. Imatinib mesylate both prevents and treats the arthritis induced by type II collagen antibody in mice. Mod Rheumatol 2007;17:306-10.

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ORIGINAL ARTICLE

Long-term outcomes of autologous PBSCT for peripheral T-cell lymphoma: retrospective analysis of the experience of the Fukuoka BMT group

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Peripheral T-cell lymphoma (PTCL) is generally characterized by poor prognosis after conventional chemotherapy compared with aggressive B-cell lymphoma. To elucidate the role of high-dose chemotherapy (HDCT) with auto-SCT, we retrospectively analyzed the outcomes of 39 patients with PTCL who received HDCT and auto-SCT between 1990 and 2005. Eleven patients were histologically typed as angioimmunoblastic, nine as anaplastic large-cell lymphoma, seven as natural killer/T-cell lymphoma and twelve as PTCL unspecified. Clinical conditions at transplantation were complete response (CR) in 27 patients and non-CR in 12 patients. Thirty-two patients received a pre-transplant conditioning regimen (MCEC) comprising ranimustine, carboplatin, etoposide and CY, and seven did other TBI-based regimens. Rapid engraftment was obtained in all cases, and transplant-related death was not seen. An estimated 5-year OS was 62.1% with a median follow-up of 78 months. The 5-year OS was significantly higher in patients transplanted during complete response than in those during other disease status (71.4% vs 27.3%, $P=0.046$). HDCT supported by auto-SCT may therefore be effective as consolidation in CR for PTCL treatment.
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Keywords: PTCL; autologous PBSCT; HDCT

Introduction

Peripheral T-cell lymphomas (PTCLs) are neoplasms derived from mature T cells and natural killer (NK) cells. They account for <10% of non-Hodgkin's lymphoma (NHL) cases. PTCLs are divided into several subtypes such as PTCL unspecified (PTCL-U), systemic anaplastic large-cell lymphoma (ALCL), angioimmunoblastic T-cell lymphoma and NK/T-cell lymphoma.[1] PTCLs show distinct variations in different geographic regions and races; a higher proportion of NHL is seen in Asia.[2]

Novel and effective agents such as rituximab and yttrium-90 ibritumomab tiuxetan have benefited patients with aggressive B-cell NHL.[3] Several studies have showed the superiority of high-dose chemotherapy (HDCT) with auto-SCT to conventional chemotherapy for patients with aggressive B-cell NHL as consolidation after the initial response.[4,5] Compared with aggressive B-cell NHL, the prognosis of PTCL patients is considerably poorer if they are treated with CY, doxorubicin, vincristine and prednisone (CHOP) or CHOP-like regimens.[6,7] Survival advantage of HDCT with auto-SCT for PTCL patients is contentious because most studies have involved small series and short-term follow-up.[8–10]

Since 1990, in Fukuoka Blood and Marrow Transplantation Group (FBMTG), HDCT with autologous PBSCT (auto-PBSCT) has been evaluated as consolidation for patients in CR1 or partial response (PR) 1, and as salvage for patients with relapsed and refractory disease, to improve PTCL outcomes. In this study, we retrospectively analyzed the results of 39 patients with PTCL who received HDCT with auto-PBSCT to clarify the efficacy of this treatment.

Patients and methods

Patients

Between January 1990 and June 2005, 39 patients who received HDCT with auto-PBSCT for PTCL were enrolled into this study at four institutions of FBMTG in Japan. Histology revealed that nine patients had ALCL, eleven

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Table 1 Patient characteristics

Disease status at auto-SCT	*PTCL*			*DLBL*
	CR1/PR1	*Sensitive relapse*	*Refractory*	*CR1/PR1*
No. of cases	23	9	7	64
Age (range) at transplantation	55 (16–66)	52 (27–68)	45 (21–51)	53 (21–67)
Sex, M/F	18/5	6/3	7/0	36/28
Histology (n)				
Unspecified	7	4	1	
Angioimmunoblastic T-cell lymphoma	6	3	3	
Anaplastic large-cell lymphoma	5	2	2	
Natural killer/T-cell lymphoma	5	0	1	
Ann Arbor stage				
I–II	4	2	1	8
III–VI	19	7	6	55
NA	0	0	0	1
Age-adjusted IPI score at diagnosis				
0–1	10	2	1	38
2–3	13	6	3	24
NA	0	1	2	2
Conditioning regimen				
MCEC	20	5	7	60
TBI-containing regimen	3	2	0	2
Others	0	2	0	2
Months from diagnosis to auto-SCT (range)	6 (2–12)	12 (9–29)	5 (4–7)	7 (2–37)

Abbreviations: CR1/PR1 = first complete or partial response; DLBL = diffuse large B-cell lymphoma; F = female; IPI = International Prognostic Index; M = male; MCEC = pre-transplant regimen, consisting of ranimustine, carboplatin, etoposide and CY; PTCL = peripheral T-cell lymphoma.

had angioimmunoblastic T-cell lymphoma, seven had NK/T-cell lymphoma and twelve had PTCL-U. Clinical characteristics at diagnosis are shown in Table 1. Stage at diagnosis was defined according to the Ann Arbor staging system.[11] The age-adjusted International Prognostic Index (aa-IPI) at diagnosis was retrospectively evaluated in 36 of 39 patients (92%).[12]

Exclusion criteria were involvement of the central nervous system, inadequate major organ functions, concomitant malignancy and active viral infection (for example, hepatitis B, hepatitis C, human immunodeficiency virus).

Treatment

Patients received anthracycline-containing regimens, mainly CHOP regimens (CY, doxorubicin, vincristine and prednisone), as an induction therapy. In addition to chemotherapy, most patients with NK/T-cell lymphoma also received local radiotherapy. Autologous PBSCs were collected during hematological recovery after treatment with a high-dose etoposide (500 mg/sqm for 3 days) or intermediate-dose Ara-C (500 mg/sqm two times daily for 5 days) followed by s.c. injection of G-CSF as previously described.[3] The target cell dose was $>2 \times 10^6$ $CD34^+$ cells/kg in a PBSC harvest. Harvested PBSCs were cryopreserved until use. The median number of infused $CD34^+$ cells was 5.7×10^6 cells/kg (range, 2.4–26.1).

Transplantation procedure

Patient characteristics at transplantation are shown in Table 1. The median age at transplantation was 49 years (range, 16–68 years), and the median time from diagnosis to auto-PBSCT was 7 months (range, 2–29 months). At transplantation, 22 patients were in CR1, 1 in PR1, 9 in chemotherapy-sensitive relapse and 7 in a chemotherapy-resistant state. Thirty-two patients were chemotherapy-sensitive, whereas seven patients were chemotherapy resistant.

The conditioning regimen prepared for auto-PBSCT was referred to as MCEC. It consisted of ranimustine 200 mg/m^2 on days −8 and −3, carboplatin 300 mg/m^2 from days −7 to −4, etoposide 500 mg/m^2 from days −6 to −4 and CY 50 mg/kg on days −3 and −2 in 32 patients (82.1%) as previously described.[3] Five patients were treated with TBI-containing conditioning regimens. On day 0, unpurged PBSCs were reinfused followed by administration of G-CSF. Engraftment was confirmed by granulocyte counts $>0.5 \times 10^9$/l and plt counts $>20 \times 10^9$/l on three consecutive occasions, or independence of plt transfusion.

Statistical methods

OS was defined as days from transplantation to death of any cause. Progression-free survival (PFS) was defined as days from transplantation to disease progression or death of any cause. TRM included all causes of death other than disease progression within 100 days after transplantation. Survival was estimated using the Kaplan–Meier method. Comparisons among those variables of interest at the time of diagnosis were performed by the log-rank test. All *P*-values reported were two-sided and statistical significance was defined at a *P*-value <0.05.

Ethical considerations
This study was conducted in accordance with the ethical guidelines mandated by the Declaration of Helsinki.

Results

Engraftment and treatment-related complications
Engraftment was rapid and documented in all patients. The median days to granulocyte count $>0.5 \times 10^9$/l and a plt count $>20 \times 10^9$/l were 9 (range, 8–11) and 11.5 (range, 7–17), respectively. TRM were not observed. Significant adverse events scored as more than grade 3 according to the National Cancer Institute Common Toxicity Criteria were not seen.

Survival and progression of the disease
With a median follow-up time of 78 months (range, 7–127 months) after auto-PBSCT, CR was achieved and maintained in 23 patients: 17 patients in CR1/PR1, 2 in sensitive relapse and 4 in the chemotherapy-resistant state before transplantation. The 5-year OS and PFS were 62.2% (95% confidence interval (CI), 46.4–77.9%) and 60.6% (95% CI, 45.0–76.2%), respectively (Figure 1a). Progressive disease (PD) was observed in 14 patients: five patients in CR1, three in sensitive relapse and six in the chemotherapy-resistant state before transplantation. Median time to PD was 149 days (range, 28–3815 days) and cumulative incidence of PD at 5 years was 33.8 %. Sixteen patients died. The primary cause of death was PD in 13 patients; the other three patients eventually died of secondary leukemia on day 521, heart failure on day 826 and pneumonia on day 2587, respectively. These three patients died in CR.

To investigate the effect of HDCT with auto-PBSCT in the treatment of PTCL, we compared the outcomes of 23 patients with PTCL, who were in CR/PR1 at transplantation, with those of 64 patients with diffuse large B-cell lymphoma, who were in CR, CR of undetermined significance and PR at transplantation from 1990 to 2005. Patient characteristics were well balanced and not statistically different between the two groups with regard to age, time to transplantation and conditioning regimen. There was no difference in outcomes between these two groups; 5-year OS and PFS were 72.9% vs 75.4% ($P=0.82$) and 73.1% vs 57.1% ($P=0.42$), respectively (Figure 2).

Prognostic factors
Data were evaluated using univariate analysis with the use prognostic factors such as histology, age, disease status at transplantation, stage, aa-IPI and lactate dehydrogenase at diagnosis. Patients having chemotherapy-sensitive disease at the time of transplantation showed significantly better 5-year OS and PFS than those having chemotherapy-resistant disease; OS was 67.2% vs 38.1% ($P=0.014$) and PFS 67.5% vs 28.6% ($P=0.033$). Patients in CR1/PR1 at transplantation showed significantly better 5-year OS and PFS than those with other status; OS was 72.9% vs 45.8% ($P=0.033$) and PFS 73.1% vs 42.2% ($P=0.023$) (Figure 1b). The low score of aa-IPI was also a significant factor influencing survival; OS of patients at low risk by

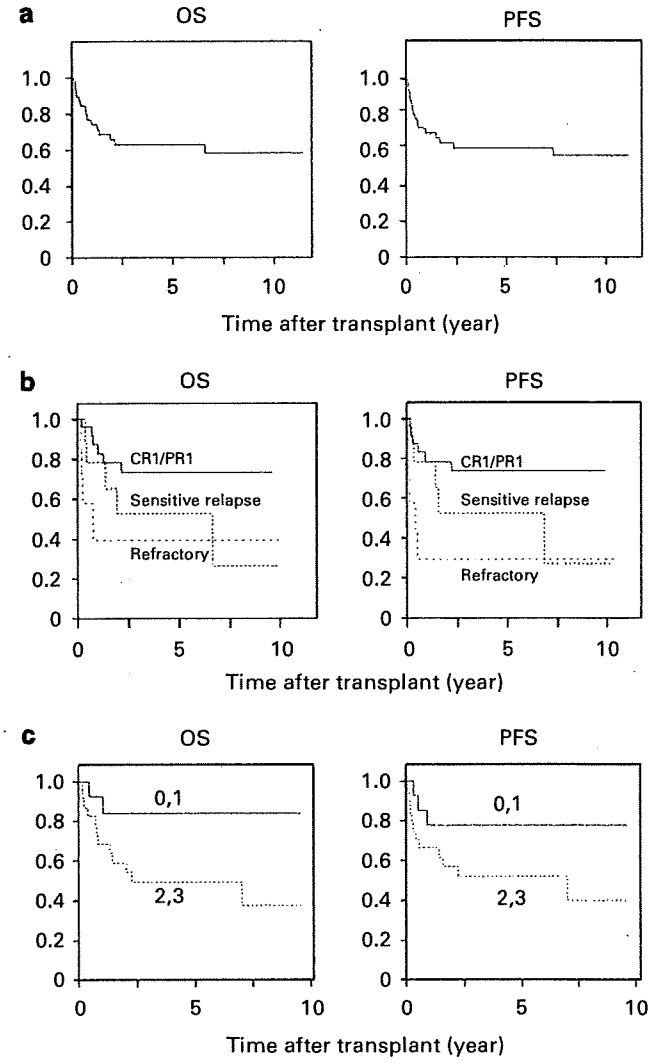

Figure 1 Kaplan–Meier estimates of the OS and progression-free survival (PFS) in all peripheral T-cell lymphoma (PTCL) patients (a). Analyzed according to disease status at transplantation (b). Analyzed according to age-adjusted International Prognostic Index (aa-IPI) at diagnosis (c). Patients who received transplantation as consolidation of initial response and aa-IPI low-risk patients at diagnosis show longer survival by univariate analysis.

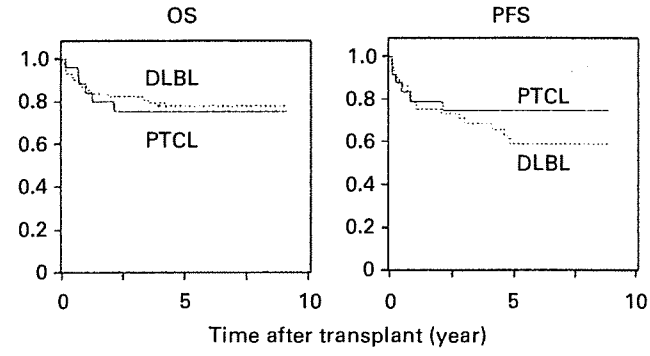

Figure 2 Kaplan–Meier estimates of OS and progression-free survival (PFS) in peripheral T-cell lymphoma (PTCL) and diffuse large B-cell lymphoma (DLBL) transplanted in initial response analyzed according to histology. Histology does not affect survival.

Table 2 Prognostic factors on survival (multivariate analysis)

Parameter	*OS*			*PFS*		
	HR	*95% CI*	P-*value*	*HR*	*95% CI*	P-*value*
Age	1.045	1.002–1.089	0.040	1.043	1.002–1.086	0.040
Histology (ALCL/non-ALCL)	3.973	0.697–0.120	0.120	4.350	0.875–21.62	0.072
aa-IPI score (0, 1/2, 3)	4.691	0.919–23.95	0.063	3.788	0.877–16.36	0.074
Status at transplant (CR/non-CR)	3.117	1.019–9.542	0.046	3.021	1.049–8.700	0.040

Abbreviations: ALCL = anaplastic large-cell lymphoma; CI = confidence interval; HR = hazards ratio; aa-IPI = age-adjusted International Prognostic Index; PFS = progression-free survival.

aa-IPI (score 0 and 1) and at high risk by aa-IPI (score 2 and 3) were 83.9% vs 49.2% ($P=0.035$) and PFS 76.9% vs 50.2% ($P=0.070$) (Figure 1c). Lactate dehydrogenase (<normal vs ⩾normal) also affected OS and PFS; OS was 90.0% vs 49.9% ($P=0.023$) and PFS 90.0% vs 48.4% ($P=0.018$). Neither histological subtypes (ALCL vs non-ALCL), age (<50 years vs ⩾50 years), nor stage (Ann Arbor I–II vs III–IV) showed a significant difference in survival.

We reviewed these univariate analyses by the multivariate method using four parameters: histology, aa-IPI at diagnosis, and age and disease status at transplantation. Despite no significant difference by univariate analysis, we also included histology (ALCL or non-ALCL) as a parameter because it was shown to be a favorable marker in a previous report.[13] Two independent factors (age and disease status at transplantation) were found to significantly influence survival (Table 2).

Discussion

Progress in PTCL therapy is slow partly because of the rarity of the disease, variation in incidence according to geographic location and absence of a common marker for therapy using monoclonal antibodies. Consensus regarding optimal treatment for PTCL is therefore lacking, and the prognosis of PTCL is reported to be inferior to that of aggressive B-cell lymphoma. Despite high and good initial response rates, most patients with PTCL fare poorly and develop progression early during or shortly after sequential conventional chemotherapy.[14] The 5-year survival reported is <30% with CHOP regimen or more intensive regimens, such as those used in LNH programs and Hyper CVAD regimen.[15–17] Recent studies have shown the superiority of HDCT with auto-SCT to conventional sequential chemotherapy and salvage chemotherapy for patients with aggressive NHL.[4,5,18] These studies included in part T-cell lymphomas, and have given encouraging retrospective results of PTCL patients treated by HDCT with auto-SCT; estimated OS was reported to be ~30–50%, which was similar to that of aggressive B-cell NHL despite fewer cases analyzed. Thus, these results indicate that HDCT with auto-SCT can benefit some patient populations of PTCL, and the parameters preferentially indicated for HDCT with auto-SCT should be clarified. To address this issue, we retrospectively analyzed our results of 39 consecutive patients with PTCL who underwent HDCT with auto-PBSCT in our institutions (FBMTG) since 1990.

In our study, 5-year OS and PFS in PTCL patients who were autografted in CR1/PR1 were 72.9 and 73.1%, respectively. These values were comparable with the corresponding results in patients with aggressive B-cell lymphoma who underwent HDCT with auto-PBSCT (Figure 2). Detailed analysis suggests that HDCT with auto-SCT may be effective for PTCL patients who have maintained CR1 or PR1 at transplantation, as well those who were chemotherapy sensitive after relapse. aa-IPI and histology type did not affect OS and PFS by multivariate analysis. Of the 39 patients in our study, 23 patients underwent auto-PBSCT in CR1/PR1, 9 patients were in chemotherapy-sensitive relapse and 7 cases in refractory phase. Approximately, 75% of patients in remission and half of those after chemotherapy-sensitive relapse had a durable response. Only two of seven patients transplanted with refractory disease showed a prolonged response. Our results are comparable with previous studies. For example, 5-year OS was reported to be 80% in patients transplanted in CR1 and 45% in other status by the GEL-TAMO group;[19] 5-year OS was reported to be 76 and 30% in patients with CR1/PR1 and refractory disease by Stanford group;[14] and 5-year OS was 80% in CR1 by EBMT group.[20] Therefore, these data strongly indicate that HDCT with auto-SCT can be one of the treatment choices for patients with PTCL as consolidation after achieving initial response, or as salvage after sensitive relapse. This treatment would be less favorable for chemotherapy-resistant patients, with no long-term survivors after HDCT with auto-SCT.[19]

Previous studies have stated the importance of the histological type of PTCL on auto-SCT outcome. Jagasia *et al.*[21] reported improved survival in ALCL type because these studies included relatively more cases of ALK-positive ALCL, which generally confers good prognosis. Corradini *et al.*[10] reported that up-front auto-SCT could induce a high rate of long-term CR only in patients with ALK-positive ALCL. Although number of patients were limited and information about ALK was not available in any of the patients in our study, there was no significant difference in 3-year OS between ALCL and the other subtypes of PTCL (66.7% vs 59.1%; $P=0.12$). Similarly, City of Hope[22] and Stanford series[14] also showed no difference in survival after HDCT with auto-SCT based on histological subtypes of PTCL, although these studies also did not afford a sufficient number of cases to stratify by type and disease status. A large Korean multicenter retrospective analysis including 139 patients also reported no survival difference depending on histological subtypes of

PTCL.[23] Discrepancies in survival data from the several groups mentioned above may in part be due to the heterogeneity of histological subtypes of PTCL. Therefore, larger retrospective and prospective studies are required to evaluate prognostic factors influencing survival in each histological subtype of PTCL.

According to our study and previous reports, the patients who seemed to benefit from HDCT were those in first remission or sensitive relapse at the time of transplantation.[8,10] However, some risk factors, such as prognostic index for PTCL (PIT), IPI and β2-microglobulin, identified some proportion of the patients in remission, who did not benefit from HDCT.[8,24] Therefore, allogeneic SCT as consolidation or salvage for the patients with higher prognostic factors at diagnosis in remission should be investigated. Furthermore, chemotherapies with novel agents that aimed at increasing the CR rate have potential to improve the outcomes of auto-SCT.[25]

In conclusion, our retrospective analyses suggest that HDCT with auto-PBSCT is feasible and safe for the treatment of PTCL. It is necessary to elucidate who could be benefited by this treatment modality depending on clinical features, prognostic markers and histological subtypes. HDCT with auto-SCT after improved initial or salvage chemotherapy must be explored for better outcomes. Prospective clinical trials, including a sufficiently large study population for statistical power, are necessary to define the role of HDCT for this rare disease.

Conflict of interest

The authors declare no conflict of interest.

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References

1 Jaffe ES. *Pathology and genetics of tumours of haematopoietic and lymphoid tissues*. IARC Press: Lyon, Washington DC, 2001, 351P pp.
2 Jaffe ES. Pathobiology of peripheral T-cell lymphomas. *Hematology Am Soc Hematol Educ Program* 2006, 317–322.
3 Kamezaki K, Kikushige Y, Numata A, Miyamoto T, Takase K, Henzan H *et al.* Rituximab does not compromise the mobilization and engraftment of autologous peripheral blood stem cells in diffuse-large B-cell lymphoma. *Bone Marrow Transplant* 2007; **39**: 523–527.
4 Haioun C, Lepage E, Gisselbrecht C, Salles G, Coiffier B, Brice P *et al.* Survival benefit of high-dose therapy in poor-risk aggressive non-Hodgkin's lymphoma: final analysis of the prospective LNH87-2 protocol–a groupe d'Etude des lymphomes de l'Adulte study. *J Clin Oncol* 2000; **18**: 3025–3030.
5 Milpied N, Deconinck E, Gaillard F, Delwail V, Foussard C, Berthou C *et al.* Initial treatment of aggressive lymphoma with high-dose chemotherapy and autologous stem-cell support. *N Engl J Med* 2004; **350**: 1287–1295.
6 Armitage JO, Greer JP, Levine AM, Weisenburger DD, Formenti SC, Bast M *et al.* Peripheral T-cell lymphoma. *Cancer* 1989; **63**: 158–163.
7 Coiffier B, Brousse N, Peuchmaur M, Berger F, Gisselbrecht C, Bryon PA *et al.* Peripheral T-cell lymphomas have a worse prognosis than B-cell lymphomas: a prospective study of 361 immunophenotyped patients treated with the LNH-84 regimen. The GELA (Groupe d'Etude des Lymphomes Agressives). *Ann Oncol* 1990; **1**: 45–50.
8 Rodriguez J, Conde E, Gutierrez A, Arranz R, Leon A, Marin J *et al.* The results of consolidation with autologous stem-cell transplantation in patients with peripheral T-cell lymphoma (PTCL) in first complete remission: the Spanish Lymphoma and Autologous Transplantation Group experience. *Ann Oncol* 2007; **18**: 652–657.
9 Mounier N, Gisselbrecht C, Briere J, Haioun C, Feugier P, Offner F *et al.* All aggressive lymphoma subtypes do not share similar outcome after front-line autotransplantation: a matched-control analysis by the Groupe d'Etude des Lymphomes de l'Adulte (GELA). *Ann Oncol* 2004; **15**: 1790–1797.
10 Corradini P, Tarella C, Zallio F, Dodero A, Zanni M, Valagussa P *et al.* Long-term follow-up of patients with peripheral T-cell lymphomas treated up-front with high-dose chemotherapy followed by autologous stem cell transplantation. *Leukemia* 2006; **20**: 1533–1538.
11 Lister TA, Crowther D, Sutcliffe SB, Glatstein E, Canellos GP, Young RC *et al.* Report of a committee convened to discuss the evaluation and staging of patients with Hodgkin's disease: Cotswolds meeting. *J Clin Oncol* 1989; **7**: 1630–1636.
12 A predictive model for aggressive non-Hodgkin's lymphoma. The International Non-Hodgkin's Lymphoma Prognostic Factors Project. *N Engl J Med* 1993; **329**: 987–994.
13 A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin's lymphoma. The Non-Hodgkin's Lymphoma Classification Project. *Blood* 1997; **89**: 3909–3918.
14 Chen AI, McMillan A, Negrin RS, Horning SJ, Laport GG. Long-term results of autologous hematopoietic cell transplantation for peripheral T cell lymphoma: the Stanford experience. *Biol Blood Marrow Transplant* 2008; **14**: 741–747.
15 Gisselbrecht C, Gaulard P, Lepage E, Coiffier B, Briere J, Haioun C *et al.* Prognostic significance of T-cell phenotype in aggressive non-Hodgkin's lymphomas. Groupe d'Etudes des Lymphomes de l'Adulte (GELA). *Blood* 1998; **92**: 76–82.
16 Escalon MP, Liu NS, Yang Y, Hess M, Walker PL, Smith TL *et al.* Prognostic factors and treatment of patients with T-cell non-Hodgkin lymphoma: the M D Anderson Cancer Center experience. *Cancer* 2005; **103**: 2091–2098.
17 Greer JP. Therapy of peripheral T/NK neoplasms. *Hematology Am Soc Hematol Educ Program* 2006, 331–337.
18 Philip T, Guglielmi C, Hagenbeek A, Somers R, Van der Lelie H, Bron D *et al.* Autologous bone marrow transplantation as compared with salvage chemotherapy in relapses of chemotherapy-sensitive non-Hodgkin's lymphoma. *N Engl J Med* 1995; **333**: 1540–1545.
19 Rodriguez J, Caballero MD, Gutierrez A, Marin J, Lahuerta JJ, Sureda A *et al.* High-dose chemotherapy and autologous stem cell transplantation in peripheral T-cell lymphoma: the GEL-TAMO experience. *Ann Oncol* 2003; **14**: 1768–1775.
20 Fanin R, Ruiz de Elvira MC, Sperotto A, Baccarani M, Goldstone A. Autologous stem cell transplantation for T and null cell CD30-positive anaplastic large cell lymphoma: analysis of 64 adult and paediatric cases reported to the European Group for Blood and Marrow Transplantation (EBMT). *Bone Marrow Transplant* 1999; **23**: 437–442.
21 Jagasia M, Morgan D, Goodman S, Hamilton K, Kinney M, Shyr Y *et al.* Histology impacts the outcome of peripheral