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Bone Marrow Transplantation (2008), 1–7

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ORIGINAL ARTICLE

Graft-versus-adult T-cell leukemia/lymphoma effect following allogeneic hematopoietic stem cell transplantation

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Allogeneic hematopoietic stem cell transplantation (allo-HSCT) has proven effective in adult T-cell leukemia/lymphoma (ATL) patients. To study the graft-versus-ATL (Gv-ATL) effects after allo-HSCT, we analyzed 21 ATL patients who had been treated at our hospital. Of these, 18 had acute-, 2 had lymphoma- and 1 had chronic-type ATL; at allo-HSCT, seven patients were in CR, one was in PR, five had stable disease (SD) and eight had progressive disease (PD). Disease state after allo-HSCT was CR in 14, PR in 3, SD in 1 and PD in 3 patients. Among 15 patients who survived longer than 100 days, ATL relapsed in 10 patients, skin relapsed in 9 patients and 5 had relapsed on the skin alone. After we discontinued immunosuppressant therapy in these 10 patients, 8 manifested GVHD; ATL was ameliorated to CR in 6 patients. Donor lymphocytes were infused into two patients who did not show GVHD; one obtained CR. In five patients with skin relapse alone, four patients achieved CR following the discontinuation of the immunosuppressants. Our results demonstrate that relapse of ATL after allo-HSCT tends to develop on skin, and Gv-ATL effects played a critical role in the outcome of allo-HSCT for ATL.

Bone Marrow Transplantation advance online publication, 10 March 2008; doi:10.1038/bmt.2008.39

Keywords: adult T-cell leukemia/lymphoma; graft-versus-ATL effect; allogeneic hematopoietic stem cell transplantation; GVHD; GVL effect

Introduction

Adult T-cell leukemia/lymphoma (ATL), one of the most aggressive hematological malignancies, is caused by a specific retrovirus, human T-cell lymphotropic virus type I (HTLV-1). It is endemic in southwestern Japan where our institutes are located. ATL manifests systemic symptoms such as lymph node swelling, hepatosplenomegaly, abnormal peripheral blood lymphocytes with lobulated nuclei, the so-called flower cells, hypercalcemia and skin lesions that are seen in about 50% of ATL patients.[1–3] As the results of therapeutic trials using different combination chemotherapies to improve the poor prognosis of ATL are unsatisfactory,[4,5] an alternative therapeutic approach, hematopoietic stem cell transplantation (HSCT), has been tried. Although many ATL patients treated by autologous HSCT experienced recurrence,[6] a better therapeutic outcome was obtained with allogeneic HSCT.[7–10] As allo-HSCT may induce GVHD by eliciting cellular immune responses of donor against recipient cells, graft-versus-ATL (Gv-ATL) effects may play a beneficial role. To assess whether Gv-ATL effects after allo-HSCT contribute to the eradication of ATL cells, we performed a retrospective study of 21 ATL patients who had undergone allo-HSCT.

Patients and methods

Diagnosis and classification of clinical subtypes of ATL

The diagnosis of ATL was based on the following criteria: (1) anti-HTLV-1 positive serum, (2) abnormal PBLs with convoluted or lobulated nuclei or histological findings of ATL in biopsy specimens from the lymph nodes, digestive tract or skin, (3) tumor cells with the mature $CD4^+$ phenotype and (4) clonal integration of HTLV-1 provirus in the DNA of tumor cells as determined by Southern blotting.[11] ATL was classified into four clinical subtypes, that is, acute-, lymphoma-, chronic- and smoldering type, according to the criteria of the Japanese Lymphoma Study Group.[11]

Patients

Based on our inclusion criteria, we enrolled 21 ATL patients who had undergone allo-HSCT at Imamura Bun-in Hospital between June 1998 and March 2005. Their median age at the time of allo-HSCT was 49 years (range, 37–62 years). The study population included 18 patients with acute-, 2 with lymphoma- and 1 with chronic-type ATL. Prior informed consent for inclusion in this study was

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Received 18 July 2007; revised 13 December 2007; accepted 5 February 2008

obtained from all patients. Clinical data in their medical records were reviewed and their clinical profiles are summarized in Table 1.

Response to treatment

Responses to induction chemotherapy and allo-HSCT were evaluated before starting allo-HSCT and at the time when the engraftment was achieved. They were recorded as CR, PR, stable disease (SD) or progressive disease (PD) according to the criteria of the Japan Clinical Oncology Group. CR was defined as the disappearance of all clinical and radiographic evidence of disease and the normalization of lactate dehydrogenase (LDH) for at least 4 weeks. As HTLV-1 carriers frequently harbor a small percentage of abnormal lymphocytes, CR was recorded when less than 5% of such cells were present if the absolute lymphocyte count was less than 4×10^9 per liter. PR was defined as a reduction in measurable disease indices by at least 50% with a more than 75% reduction in the absolute abnormal lymphocyte count for at least 4 weeks without the development of new lesions or disease progression. We also required that LDH was decreased to <1.5 of the normal upper limit. PD was defined by a $\geqslant 25\%$ increase in measurable disease indices or the appearance of new lesions during treatment. Lastly, SD was defined by a response between PR and PD.[5]

Conditioning regimen, source of transplanted cells and GVHD prophylaxis

The patients underwent conventional myeloablative HSCT (CST) and reduced-intensity HSCT (RIST). Allogeneic stem cells were obtained from bone marrow, peripheral blood or umbilical cord blood.

For GVHD prophylaxis, CYA alone or short-term MTX (sMTX) and CYA were used for HSCT from HLA-matched siblings; for patients received from unrelated- or HLA-mismatched related donors, we used sMTX and CYA or sMTX and tacrolimus.

Assessment of ATL relapse

We evaluated patients with successful HSCT who survived for more than 100 days for ATL relapse or regrowth. In patients who underwent a second transplant because of graft failure or rejection, survival was counted from the first transplant. Relapse was assessed based on the pre-HSCT conditioning regimen, the ATL disease state at/after HSCT and the relapse site.

Diagnosis of GVHD

GVHD was diagnosed based on quantification or histological assessment of skin rash, serum bilirubin, and diarrhea and recorded according to the standard criteria as Grade 0–IV.[12]

Donor lymphocyte infusion

Donor lymphocyte infusion (DLI) was carried out to induce GVHD in patients who did not show GVHD after the cessation of immunosuppressant therapy because of ATL relapse. $CD3^+$ lymphocytes (10^7–10^8 cells per kg) from the peripheral blood of donors were infused.

Table 1 Summary of transplantation

	Patients	
	(No.)	*(%)*
Total	21	100
Sex		
Male	13	61.9
Female	8	38.1
Clinical subtype of ATL		
Acute	18	85.7
Lymphoma	2	9.5
Chronic	1	4.8
Age (years)		
Median (range)	49 (37–62)	
Disease status at SCT		
CR	7	33.3
PR	1	4.8
SD	5	23.8
PD	8	38.1
Conditioning regimen		
Conventional myeloablative HSCT	10	47.6
Reduced-intensity HSCT	11	52.4
Source of stem cell		
Bone marrow	5	23.8
Peripheral blood	14	66.7
Cord blood	2	9.5
HLA		
Match	14	66.7
Mismatch	7	33.3
Duration from the first chemotherapy to SCT (months)		
Median (range)	5.7 (3.1–27.0)	

Abbreviations: ATL = adult T-cell leukemia/lymphoma, HSCT = hematopoietic stem cell transplantation; PD = progressive disease; SD = stable disease.

Immunohistochemical staining

Tissue sections, 4 μm in thickness, were prepared from formalin-fixed, paraffin-embedded specimens. Each section was deparaffinized in xylene; antigenic activity was retrieved by incubation in 0.01 M citrate buffer (pH 6.0) in an autoclave (121 °C, 10 min). The sections were incubated with 3% H_2O_2 in methanol for 10 min to block endogenous peroxidase activity. Each section was subsequently incubated (room temperature, overnight) with the primary antibody and biotinylated horse anti-mouse immunoglobulin G antibody (Vector Laboratories, Burlingame, CA, USA) diluted 1:200 with Tris. After 30-min reaction with avidin–biotin-peroxidase complex (Vector Laboratories), the samples were incubated for 10 min with diaminobenzidine (brown) (Vector Laboratories) and counterstained with Carrazzi's hematoxylin. The expression of CD4 and 8 was examined in all the cases who suffered skin relapse of ATL or skin GVHD after HSCT

using monoclonal antibody to human CD4 and 8 (CD4: Novocastra Laboratories, Newcastle upon Tyne, UK; CD8: Medical and Biological Laboratories, Nagoya, Japan) diluted 1:50 with PBS.

Statistical analysis
Overall survival (OS) from the day of allo-HSCT was analyzed by the Kaplan–Meier method. The log-rank test was used to compare OS among the subgroups.

Results

Transplant conditions
The ATL disease state at the time of allo-HSCT was CR in seven, PR in one, SD in five and PD in eight patients. Of the 21 patients, 10 underwent CST and 11 received RIST, 5 received a bone marrow transplant, 14 a peripheral blood SCT and 2 a cord blood transplant (Table 1). Of the four patients who failed or rejected the primary transplant, three underwent a second allo-HSCT procedure.

Response to allo-HSCT
Disease state after allo-HSCT was CR in 14, PR in 3, SD in 1 and PD in 3 patients. In 14 patients not in CR at the time of allo-HSCT, 7 patients achieved CR. Survival after allo-HSCT ranged from 1.4 to 83.7+ months; median survival was 8.4 months. At the time of data collection, seven patients were alive; the median observation period was 28.0+ months (range, 4.3+ to 83.7+ months). The 3-year survival rate of the 21 patients was 33.2 ± 10.9% (Figure 1); it was 12.5% in patients with PD and 47.0% in patients with CR, PR or SD at the time of allo-HSCT. The difference was statistically significant ($P=0.02$, Figure 2). After allo-HSCT, 15 patients survived for more than 100 days, 7 had undergone RIST and 8 had received CST.

ATL relapse
ATL relapsed in 10 of 15 patients who survived more than 100 days (cases 1–10 in Table 2) during the administration of immunosuppressants; of these, 6 had undergone RIST and 4 had received CST. With respect to the disease state at allo-HSCT, two patients with CR and eight patients not in CR relapsed. Of these eight patients not in CR at allo-HSCT, four patients achieved CR after allo-HSCT. The site of relapse was the skin in 9, the peripheral blood in 4 and the lymph nodes in 4 patients. In the representative case (case 4), the axillar, supraclavicular and inguinal lymph nodes were enlarged and indurated erythematous plaques appeared on the chest 28 days after HSCT. A biopsy specimen of a chest lesion revealed a Pautrier's microabscess and dense dermal infiltration by lymphoid cells with nuclear atypia. They were positive for CD4 and negative for CD8 (Figure 3). We concluded that ATL had relapsed and discontinued the administration of CYA.

Graft-versus-ATL effect
Immunosuppressant therapy for GVHD prophylaxis was discontinued in all 10 patients who suffered a relapse (cases 1–10 in Table 2), 8 developed GVHD (grade I, $n=1$; grade II, $n=6$; grade IV, $n=1$). In case 4, within a few days of cessation of CYA administration, the skin lesions disappeared and the lymph node size decreased. After 2 weeks, erythematous plaques reappeared on the trunk. Histologically, these lesions revealed spongiosis, hydropic basal cell degeneration and dermal edema. Infiltrating lymphocytes were predominantly positive for CD8 and the lesion was diagnosed as GVHD (Figure 4). Immunosuppressant therapy was restarted and the patient survived for more than 4 years without ATL relapse. In this case, the cessation of immunosuppressant therapy resulted in the development of acute GVHD which induced the remission of ATL. In six of these eight patients (cases 1–6 in Table 2), ATL improved and they ultimately achieved CR. For pre-HSCT conditioning, three patients each had undergone RIST and CST. All six patients suffered ATL relapse in the skin and were monitored closely. One patient (case 4 in Table 2) who initially experienced ATL improvement after immunosuppressant therapy was halted and who achieved CR, developed acute skin GVHD. In

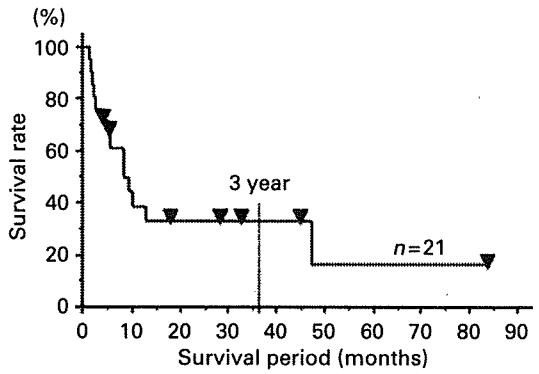

Figure 1 Kaplan–Meier plot of overall survival following allo-HSCT for adult T-cell leukemia/lymphoma (ATL) The median survival period after allo-HSCT was 8.4 months (range, 1.4 to 83.7+ months). In seven surviving patients, the observation period (triangles) ranged from 4.3+ to 83.7+ months (median 28+ months). The 3-year survival rate of the 21 patients was 33.2%.

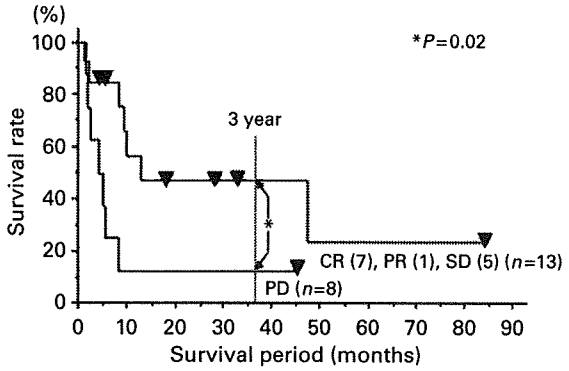

Figure 2 Comparison of the overall survival rate in patients with progressive disease (PD) and patients with CR, PR and stable disease (SD). The 3-year survival rate was 12.5% in PD patients and 47.0% in patients with CR, PR and SD ($P=0.02$).

Table 2 Patient characteristics and graft-versus-ATL effect of 10 patients with ATL relapse after hematopoietic stem cell transplantation

Case	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
Patient status										
Age(years)/sex	46/M	45/M	49/M	62/M	55/M	61/F	49/M	60/M	51/F	51/M
Clinical subtype of ATL	Acute	Acute	Acute	Acute	Lymphoma	Acute	Acute	Acute	Acute	Acute
Disease state at HSCT	CR	CR	SD	PD	PD	PD	PR	SD	PD	PD
Involved organ at ATL	PB, LN, lung, liver	PB, skin, BM, DT	PB	PB, skin, LN, BM	PB, skin, LN	PB, skin, BM	PB, LN, DT	PB, skin, BM	PB, LN, DT	PB, LN
Conditioning regimen	CST	CST	CST	RIST	RIST	RIST	CST	RIST	RIST	RIST
Disease state after HSCT[a]	CR	CR	PR	CR	CR	CR	CR	SD	PR	PR
Involved organ at ATL relapse	Skin	Skin	PB, skin	PB, skin, LN	Skin	Skin	LN	Skin	PB, skin, LN	PB, skin, LN
Gv-ATL effect										
Acute GVHD after the cessation of ISA (Grades)	II	I	II	II	IV	II	II	II	0	0
DLI (CD3+ cells per kg)	None	None	None	None	None	None	None	None	5.26×10^7 (day 55)	1.46×10^8 (day 104)
Acute GVHD after DLI (Grade)	—	—	—	—	—	—	—	—	III	0
Outcome of ATL after Gv-ATL effect	CR	CR	CR	CR	CR	CR	SD	SD	CR	PD
Chronic GVHD	Limited	Extensive	Not evaluable	None	Not evaluable	Not evaluable	Not evaluable	Not evaluable	Not evaluable	Not evaluable
Overall survival from HSCT (months)	32.4+	17.4+	9.4	44.8+	4.2	8.4	8.4	5.7+	5.3	5.7
Cause of death	Alive	Alive	Pneumonia	Alive	Acute GVHD	second relapse of ATL[b]	IPD	Alive	Acute GVHD	ATL

Abbreviations: ATL = adult T-cell leukemia/lymphoma; CST = conventional myeloablative HSCT, DLI = donor lymphocyte infusion; DT = digestive tract; F = female; Gv-ATL effect = graft-versus-ATL effect; IPD = interstitial pulmonary disease; LN = lymph nodes; M = male; PB = peripheral blood; PD = progressive disease; RIST = reduced-intensity HSCT; SD = stable disease.

[a]Disease status after HSCT was evaluated at the time when the engraftment was achieved.

[b]This patient died after a second ATL relapse in the central nervous system. She achieved CR after the cessation of immunosuppressant therapy administered after the occurrence of a dermal ATL relapse.

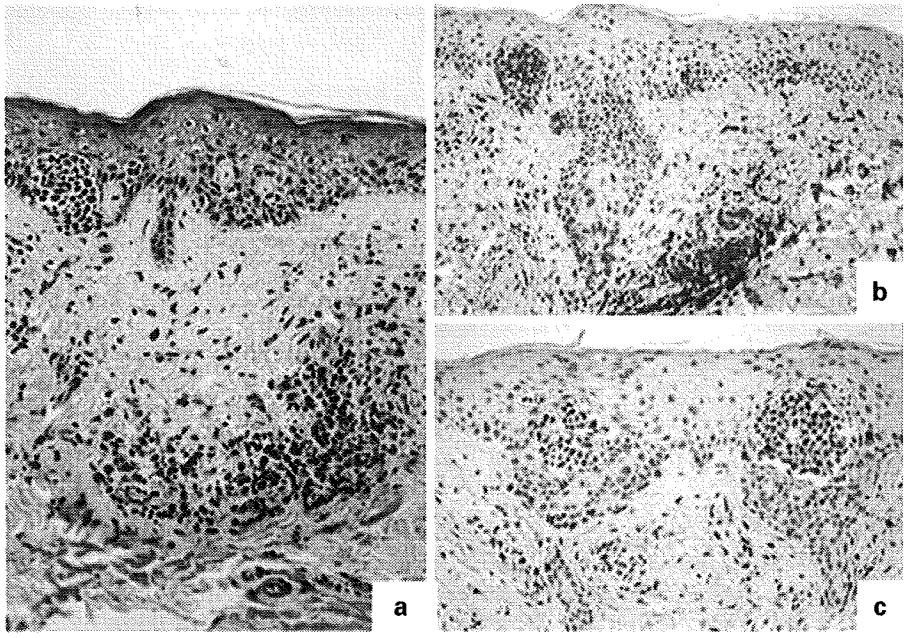

Figure 3 Case 4: skin biopsy at the time of adult T-cell leukemia/lymphoma (ATL) relapse. (**a**) Hematoxylin-eosin, (**b**) CD4, (**c**) CD8 (original magnification × 100). A biopsy specimen of a chest lesion at 28 days after allo-HSCT revealed a Pautrier's microabscess and dense dermal infiltration by lymphoid cells with nuclear atypia (**a**). They were positive for CD4 (**b**) and negative for CD8 (**c**).

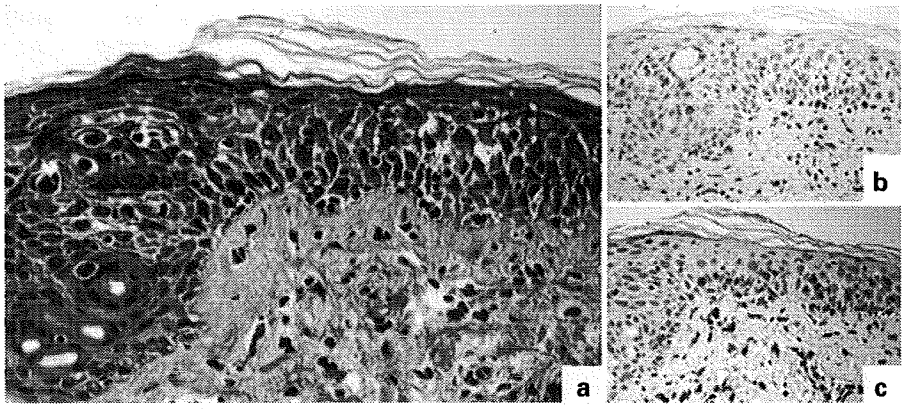

Figure 4 Case 4: skin biopsy at the manifestation of acute GVHD. (**a**) Hematoxylin-eosin, (**b**) CD4, (**c**) CD8 (original magnification × 400). A biopsy specimen of the erythematous plaques on the trunk. Histologically, these lesions revealed spongiosis, hydropic basal cell degeneration and dermal edema (**a**). Infiltrating lymphocytes were predominantly positive for CD8 (**b**, **c**).

cases 1–3, 5, 6 (Table 2), biopsy of skin erythema that appeared after immunosuppressant therapy was stopped led to a diagnosis of acute skin GVHD. Their ATL skin lesions improved gradually and they achieved CR with the development of acute skin GVHD. Among two patients, who did not manifest GVHD after the cessation of immunosuppressant therapy, DLI was carried out to induce acute GVHD; one patient (case 9 in Table 2) achieved CR.

Discussion

We studied the outcome of allo-HSCT in 21 ATL patients to examine the possible contribution of Gv-ATL effects to its clinical effectiveness. After allo-HSCT, 14 patients achieved CR. The median survival was 8.4 months and the 3-year survival rate was 33.2 ± 10.9%. Our data are comparable to previously reported results.[7–10] However, we delivered allo-HSCT even in patients whose disease state at HSCT was PD ($n = 8$) or SD ($n = 5$); CR was achieved in four of the PD and three of the SD patients, suggesting that even patients with advanced ATL might benefit from allo-HSCT.

We used RIST or CST for pre-HSCT conditioning and found that among 15 patients who survived more than 100 days, 7 had undergone RIST and 8 had received CST. Among 10 patients with ATL recurrence, 6 had undergone RIST and 4 CST, and of the six patients who achieved CR after the cessation of immunosuppressant therapy, RIST

and CST were used in three each. Thus, although the difference between RIST and CST was not significant, we prefer RIST in patients scheduled for allo-HSCT because it is safe and simpler to administer compared to CST.

We carefully examined the clinical course of the 10 patients with relapse who survived more than 100 days. ATL relapsed in two of six patients in status CR at the time of allo-HSCT versus eight of nine who were not in CR at that point. Of these eight patients not in CR at allo-HSCT, four patients achieved CR after allo-HSCT. Not unexpectedly, the incidence of relapse was lower in the patients who were in CR at the time of allo-HSCT. In 9 of 10 patients the skin was the relapse site, suggesting that relapse of ATL after allo-HSCT tends to develop on skin. Uchiyama *et al.*[1] reported that cutaneous manifestations were seen in at least 50% of ATL patients. Chemokines and their receptors may play a critical role in cellular recruitment and tissue homing and CCR4 and CXCR4 molecules specifically regulate the skin homing of cutaneous T-cell lymphoma cells (CTCL).[13–15] The expression of molecules responsible for skin homing of ATL cells may be altered after allo-HSCT.

Graft-versus-leukemia effects have been demonstrated after HSCT for other types of leukemia. The minor histocompatibility antigen (mHA) of the recipient is thought to be the target of GVHD; Gv-L or graft-versus-lymphoma (Gv-Ly) effects are mediated by mHA on the recipient leukemia cells.[16] The likelihood of leukemia recurrence after allo-HSCT was increased when the T cells were removed from the graft or the donor was a genetically identical twin,[17] indicating that Gv-L effects play an important role in the prevention of leukemia recurrence. We discontinued immunosuppressant therapy in 10 patients with ATL relapse because we suspected the involvement of Gv-ATL effects. GVHD developed in eight patients and ATL was ameliorated to CR in six of these eight patients. The other two patients received DLI to induce GVHD; one patient obtained CR. These results demonstrate that Gv-ATL effects played a critical role in eradicating the ATL lesions. While the Gv-ATL effects are beneficial in the treatment of ATL, possible toxicity of the procedure should be concerned in the clinical settings because 9 of 21 patients died of GVHD or thrombotic microangiopathy in this study.

In our study population, ATL relapsed on the skin of 9 of 10 patients. Others[18–21] reported Gv-Ly effects in CTCL. Our review of the literature uncovered 21 CTCL patients who underwent allo-HSCT, 11 had mycosis fungoides and 10 had Sézary syndrome;[18–26] 6 suffered relapse and 1 patient each achieved CR and PR after the cessation of immunosuppressant therapy. The Gv-ATL effects noted in our study appear to be more profound than those reported in the literature. In five patients with ATL relapse on the skin alone (Table 2), four patients achieved CR after acute GVHD following the discontinuation of the immunosuppressants. Since skin is one of the major target organs of acute GVH reaction,[27] Gv-ATL effect might be more significant in patients with skin relapse alone.

Although the number is limited, our retrospective study demonstrated the Gv-ATL effects of allo-HSCT particularly in patients with skin lesions. Studies are underway in our laboratory to determine the optimal conditions for allo-HSCT in patients with ATL.

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Biology of Blood and Marrow Transplantation 14:702-708 (2008)

1083-8791/08/1406-0001$32.00/0
doi:10.1016/j.bbmt.2008.03.010

Allogeneic Hematopoietic Stem Cell Transplantation Using Reduced-Intensity Conditioning for Adult T Cell Leukemia/Lymphoma: Impact of Antithymocyte Globulin on Clinical Outcome

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Received February 25, 2008; accepted March 25, 2008

ABSTRACT

Allogeneic hematopoietic stem cell transplantation (HSCT) is an effective treatment for adult T cell leukemia/lymphoma (ATLL), but shows high mortality. We evaluated the feasibility of reduced-intensity transplantation using fludarabine and busulfan, with particular focus on the clinical impact of antithymocyte globulin (ATG) in the conditioning regimen. Fourteen elderly patients with aggressive ATLL were enrolled in the current study without ATG, and were compared to those in 15 patients who were treated similarly, but with ATG, in our previous study. Engraftment was prompt, and treatment was tolerable. Overall (OS) and progression-free survival (PFS) at 3 years were 36% and 31%, respectively. HTVL-1 proviral load became undetectable by the polymerase chain reaction in 62% of patients. Compared to the previous study with ATG, complete donor chimera was significantly delayed. Although early relapse tended to be decreased, OS or PFS was not improved significantly. Analysis of combined data from both our current and previous studies disclosed that grade I-II acute GVHD was the only factor that favorably affected OS and PFS. These data suggested the presence of a graft-versus-ATLL effect and the feasibility of a transplant procedure without ATG in elderly ATLL patients, but could not demonstrate the clinical benefit of incorporating ATG.

KEY WORDS

Adult T cell leukemia/lymphoma • Hematopoietic stem cell transplantation • Allogeneic • Reduced-intensity transplantation • Antityymocyte globulin • Graft-versus-ATLL effect

INTRODUCTION

Adult T cell leukemia/lymphoma (ATLL) is a peripheral T cell malignancy that is associated with human T cell leukemia virus type 1 (HTLV-1) and a dismal prognosis [1]. It is classified into 4 subtypes according to its clinical features: acute, lymphoma, chronic, and smoldering. The former 2 subtypes have the worst prognosis, with a median survival of approximately 1 year [2-6]. On the other hand, recent reports have suggested that about 40% to 45% of patients with

Table 1. *Patient Characteristics in the Current Study in Comparison with those in the Previous Study*

Characteristic	ATG − Current Study (ATL-NST-2)	ATG + Previous Study (ATL-NST-1)	P
Number of patients	14	15	
Age, years			.15
Median	56	57	
Range	50-64	51-67	
Patient sex			1.00
Male	9	8	
Female	5	7	
Subtype of ATLL			1.00
Acute	10	10	
Lymphoma	4	5	
Initial chemotherapy			.65
CHOP-based	9	12	
LSG 15	3	2	
Modified EPOCH	2	2	
Status at transplantation			.59
CR	4	3	
PR	10	10	
Refractory	0	2	
Donor HTLV-1 serology			1.00
Positive	7	7	
Negative	7	8	
CD34+ cells infused, $\times 10^6$/kg pt weight			.39
Median	3.6	4.5	
Range	1.3-9.4	1.6-8.0	

ATLL indicates adult T cell leukemia/lymphoma; CHOP, cyclophosphamide (Cy); doxorubicin (DXR); vincristine (VCR); prednisone (Pred); LSG 15, Cy, DXR, VCR, Pred, MCNU, carboplatin (CBDCA), etoposide (VP-16); Modified EPOCH, VP-16, VCR, Pred, CBDCA, DXR; CR, complete remission; PR, partial remission; HTLV-1, human T cell leukemia virus type-1; ATG, antithymocyte globulin.

these subtypes could survive without disease after allogeneic hematopoietic stem cell transplantation (allo-HSCT) using a conventional conditioning regimen [7-10]. However, treatment-related mortality (TRM) after conventional allo-HSCT is still as high as 40% to 45% because most ATLL patients are over 50 years of age and have poor morbidity, and this limits the wider application of this procedure.

To overcome this limitation, we previously conducted a phase I study of reduced-intensity allogeneic transplantation (ATL-NST-1) to examine its feasibility and safety in the treatment of 15 patients with ATLL who were aged 50 years or more [11]. The conditioning regimen consisted of fludarabine (180 mg/m^2), busulfan (8 mg/kg), and low-dose (5 mg/kg) antithymocyte globulin (ATG, Fresenius AG, Bad Homburg, Germany), as modified from a previous report [12]. Although this study confirmed its feasibility and safety, 9 of the 15 patients (60%) relapsed, and 7 of these relapsed within 100 days. This observed high rate of early relapse raised the possibility that ATG, which was used to enhance engraftment and suppress a graft-versus-host reaction, may have negatively suppressed a graft-versus-lymphoma effect to lead to the high relapse rate. Alternatively, the rapid proliferation of ATLL cells may outpace a developing donor-derived anti-ATLL activity. Therefore, in the present modified phase I study (ATL-NST-2), we eliminated ATG from the original conditioning regimen to compare the results with the previous study. Because ATLL is a rare endemic hematologic malignancy, to the best of our knowledge, these are the only on-going prospective trials that focus on reduced-intensity allo-HSCT for this disease [13].

PATIENTS AND METHODS

Patient and Donor Characteristics

Eligibility criteria for this study were identical to those in the first trial. Briefly, patients with ATLL of acute or lymphoma type [2], who were aged between 50 and 70 years, were eligible. Patients were required to be in either complete remission (CR) or partial remission (PR) at the time of registration, and to have an HLA-identical sibling donor. Patients did not have an active uncontrolled infection. Those who had a past history of CNS invasion were included. Written informed consent was obtained from all patients and donors before enrollment into the study, which was approved by the institutional review board of each participating hospital.

For this modified study, 14 patients were registered between March 2003 and February 2006 at 7 institutions. Their characteristics are given in Table 1 in comparison with those of the 15 patients treated in the first study (ATL-NST-1), who were enrolled between April 2001 and November 2002 and treated with low-dose (5 mg/kg) ATG.

Treatment

Donors received granulocyte-colony stimulating factor (G-CSF) 5 μg/kg twice daily, beginning 4 days before leukapheresis and continuing until collections were complete. The conditioning regimen consisted of fludarabine 30 mg/m^2 per day infused over 30 minutes on days −8 to −3, and busulfan 1 mg/kg orally every 6 hours for 4 times on days −6 and −5. For prophylaxis of graft-versus-host disease (GVHD), cyclosporine 3 mg/kg per day was infused continuously starting on day −1, and the dose was adjusted to between 250 and 400 ng/mL. The dose was tapered after 50 days if there was no sign of acute GVHD (aGVHD). G-CSF 5 μg/kg was administered to recipients on day 6 and thereafter until the absolute neutrophil count was $>1 \times 10^9$/L for 2 consecutive days.

Study Endpoints

The primary objective of the study was to evaluate feasibility and safety in terms of the achievement of complete donor chimera before 91 days, defined as 90% or more peripheral blood mononuclear cells (PBMNC) of donor origin, and the occurrence of TRM within 100 days after transplantation. The degree of donor-recipient chimerism in PBMNC, CD3-positive or negative fraction, was examined after 14, 30, 60, and 90 days according to the previously published method [14]. The day of sustained engraftment was defined as the first of 3 consecutive days with the absolute neutrophil count exceeding 0.5×10^9/L.

The secondary objectives were toxicities, occurrence of aGVHD and chronic GVHD (cGVHD), overall (OS) and progression-free survival (PFS), and HTLV-1 proviral load as a surrogate marker for anti-HTLV-1 effect. Toxicities were graded using the National Cancer Institute Common Toxicity Criteria (version 3.0) [15]. Acute GVHD was diagnosed and graded according to the standard criteria [16,17]. Chronic GVHD was assessed according to the standard criteria in patients who survived >100 days after transplantation [18]. OS was defined as the duration from transplantation to death from any cause. PFS was defined as the duration from transplantation to disease progression or death from any cause. The HTLV-1 proviral load was estimated by taking blood samples before and 1, 2, 3, 6, 12, 18, and 24 months after transplantation, and HTLV-1 proviral DNA was measured by quantitative PCR amplification of HTLV-1 pX DNA using a Light Cycler System. The detection limit of the HTLV-1 proviral load was 0.5 copies/1000 cells [19].

Statistical Analysis

We hypothesized that 80% of patients were expected to survive >100 days with engraftment of 90% or more of donor chimerism after this modified treatment. Simon's 2-stage design was used [20]. In the first stage, 7 patients were to be assessed and we expected that >3 patients would fulfill the criteria. Next, 9 additional patients were to be assessed. If >10 of the total 16 patients met the criteria, we considered that our hypothesis would have been shown to be true.

Patients and disease characteristics were compared by either Fisher's exact test or the Wilcoxon rank-sum test. Estimates of time to event, including neutrophil recovery, complete donor chimera, death, relapse, and GVHD, were made by the Kaplan-Meier method. All P values are reported as 2-sided, with $P < .05$ indicating statistical significance. The hazard ratio of death or relapse with respect to clinical features was estimated by a Cox proportional hazard model. All analyses were performed using SPSS 15.0 or SAS (ver. 9) for Windows.

RESULTS

Engraftment and Toxicities

Because 12 of the 14 patients (86%) survived >100 days with complete donor chimera after transplantation, this treatment was demonstrated to be successful, and the enrollment of patients was stopped at that point. Neutrophil engraftment and complete donor chimera in PBMNC were achieved in all 14 patients before 91 days. The median number of days until engraftment and the achievement of complete donor chimera was 11 days (range: 9 to 16 days) and 60 days (14 to 90 days), respectively. TRM occurred in 3 patients; 1 subdural hemorrhage on day 21, 1 interstitial pneumonia syndrome on day 32, and 1 bronchiolitis obliterans on day 630.

In comparison with the previous study, where ATG was included in the preparatory regimen, complete donor chimera was significantly delayed in PBMNC (Figure 1; $P = .001$) and CD3-positive T cell fractions ($P = .038$). On the other hand, the speed of neutrophil recovery and the achievement of complete donor chimera in CD3-negative myelomonocytic cell fraction were almost identical between the 2 studies.

Concerning nonhematologic toxicities, no patients experienced grade 3 or 4 toxicities, except 3 TRM as described above. CMV antigenemia was observed in 11 patients (79%) at least once within 100 days after transplantation, although none developed a CMV disease. Other infectious complications within 100 days after transplantation included mild hemorrhagic

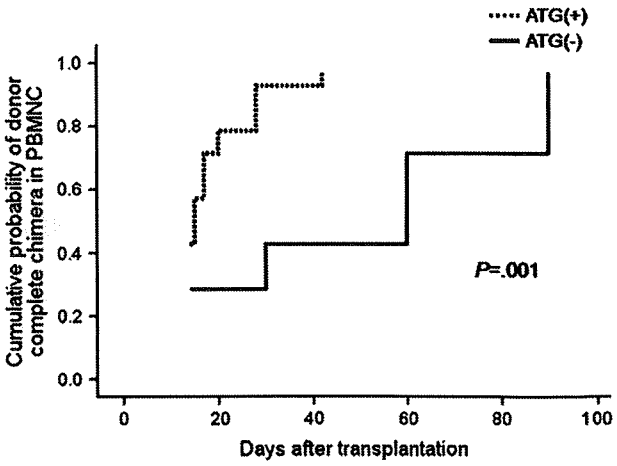

Figure 1. Kaplan-Meier estimate of the cumulative probability of achieving complete donor chimera in the mononuclear cell fraction in peripheral blood. PBMNC indicates peripheral blood mononuclear cell; ATG, antithymocyte globulin; ATG(+), patients treated with a conditioning regimen that included ATG in the previous study (n = 15); ATG(−), patients treated without ATG in the current study (n = 14). The achievement of complete donor chimera was significantly delayed in the current study compared to the previous study ($P = .001$).

Table 2. *Clinical Outcomes of the Current and the Previous Studies*

	ATG				
	−		+		
	Current Study (n = 14)		Previous Study (n = 15)		
Characteristic	No.	%	No.	%	P
Complete donor chimera≤90 days	14	100	14	93	.78
Death					
Overall	8	57	10	67	.88
All TRM	3	21	4	27	.60
TRM ≤100 days	2	14	1	7	1.00
ATLL	5	36	6	40	.88
Relapse					
Overall	6	43	9	60	.45
≤100 days	3	21	7	47	.25
Acute GVHD	12	86	10	67	.27
I	4	29	1	7	
II	5	36	4	27	
III	3	21	3	20	
IV	0	0	2	13	
Chronic GVHD*	10/12	83	6/13	46	.063
CMV antigenemia	11	79	13	87	.65
EB-LPD	0	0	2	13	
No. of pts with undetectable PCR for HTLV-I*†	8/13	62	8	53	.96

ATLL indicates adult T-cell leukemia/lymphoma; TRM, treatment-related mortality; GVHD, graft-versus-host disease; CMV, cytomegalovirus; EB-LPD, Epstein-Barr virus-associated lymphoproliferative disorders; PCR, polymerase chain reactions; HTLV-1, human T cell leukemia virus type-1; ATG, antithymocyte globulin.

*The number of evaluated patients is shown on the right side of the slash in each column.

†The number of patients whose HTLV-1 provirus could not be detected at lease once was counted.

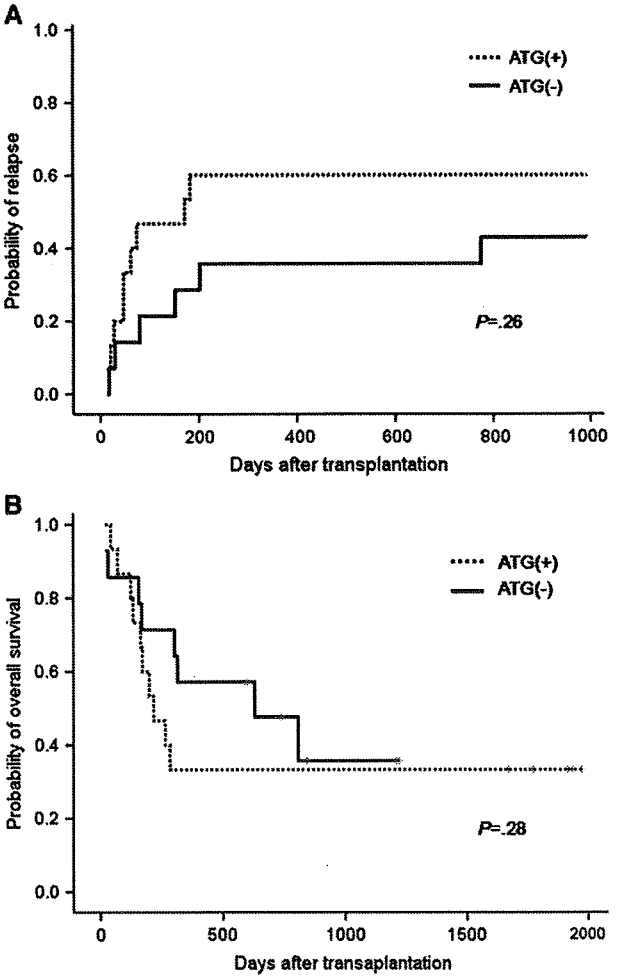

Figure 2. Cumulative incidence estimates of relapse and Kaplan-Meier survival estimates in comparison between the absence (the current study) and presence (the previous study) of ATG in the conditioning regimen. (A) Total and early relapse within 100 days tended to be decreased in the current study, but not significantly different (P = .26). (B) There was no significant difference in overall survival rate between with and without ATG (P = .28).

cystitis because of adenovirus in 3, and mild pneumonia because of pseudomonas aeruginosa in 1 patient.

aGVHD and cGVHD

The incidence of aGVHD was not significantly increased in the current study (P = .27); grade I to IV was 86%, grade II to IV 57%, grade III to IV 21%, whereas in the previous study these values were 67%, 60%, and 34%, respectively. Chronic GVHD developed in 10 of 12 patients (83%) who could be evaluated, and it tended to be increased in the current study compared to the previous study (Table 2; P = .063).

Relapse

Six patients relapsed (within 100 days in 3 [50%]). Total and early relapse within 100 days tended to occur less frequently in the current study than in the first study, but these differences were not statistically significant (Figure 2A; P = .26 and .25, respectively). Two patients relapsed in the CNS on days 78 and 778. Another patient, who relapsed on the skin on day 30, responded to withdrawal of cyclosporine and is currently alive without disease 592 days after transplantation.

Kinetics of HTLV-I Proviral Load

In 13 patients who could be examined serially for HTLV-1 proviral load in the peripheral blood, this value decreased after transplantation and reached an undetectable level within 6 months in 8 patients (62%), including 3 patients who were transplanted from an HTLV-1 carrier donor. There was no significant difference in these kinetics between the 2 studies (Table 2 and Figure 3). In 2 patients, it is still undetectable at 12 and 18 months after transplantation.

OSI and PFS

OS and PFS at 3 years were 36% (95% confidence interval [CI], 21% to 51%) and 31% (95% CI, 17% to

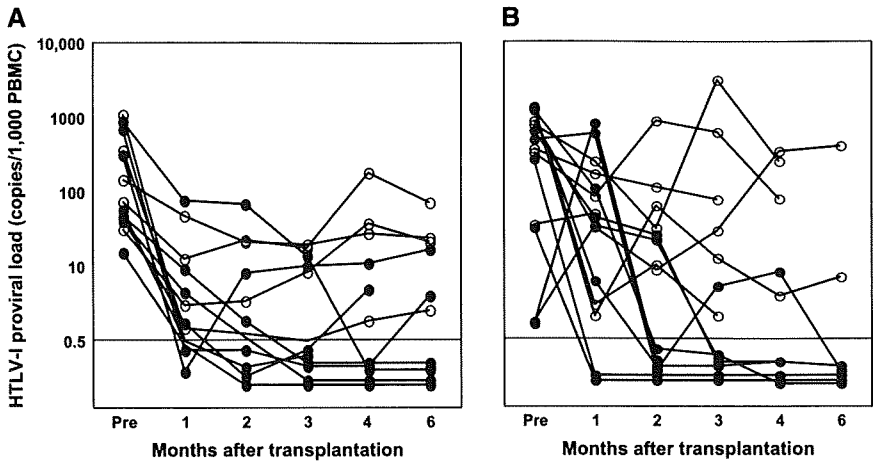

Figure 3. The kinetics of the HTLV-1 proviral load within 6 months after RIST. The panel on the left (A) indicates the kinetics of HTLV-1 proviral load in the current study, whereas the panel on the right (B) shows the kinetics in the previous study. The HTLV-I proviral load was expressed as copy numbers per 1000 mononuclear cells (MNC). A load of <0.5 copies per 1000 MNC was considered to be undetectable. Closed circles indicate patients whose proviral load became undetectable at least once, whereas open circles indicate patients whose proviral load remained detectable.

45%), respectively, with a median follow-up time of 792 days (range: 592 to 1222 days) in surviving patients. There was no significant difference in OS (Figure 2B; $P = .49$) or PFS ($P = .28$) between the 2 studies.

Factors that Affect OS and PFS in a Combined Analysis of Both Studies

We examined the effects of various factors, including the use of ATG in the conditioning regimen, age, gender, type of ATLL, disease status at transplantation, donor's HTLV-1 status, and aGVHD as a time-dependent variable, on OS and PFS in a total of 29 patients who had been enrolled into the 2 studies. The Kaplan-Meier estimate revealed marked differences in OS and PFS among patients who were stratified retrospectively according to the grade of aGVHD (Figure 4). In a univariate analysis, only aGVHD of grade I to II was identified as a prognostic factor with a positive impact for both OS and PFS (Table 3). The use of low-dose ATG in the conditioning regimen did not significantly influence OS or PFS in this particular setting.

DISCUSSION

In our previous study, which incorporated low-dose ATG, we showed that elderly patients with aggressive ATLL could be transplanted safely, and that laboratory-evaluated graft-versus-HTLV-1 activity and clinically observed graft-versus-ATLL (GV-ATLL) effect were important after allo-HSCT [11]. Nevertheless, disease relapse was the main cause of treatment failure, and 9 of the 15 patients (60%) relapsed, 7 of whom relapsed within 100 days after transplantation. The early relapse was considered to be because of the highly resistant nature of ATLL. It was speculated that the disease activity could not be controlled by reduced-intensity conditioning therapy per se, unless a GV-ATLL effect appears earlier. However, GVHD and a related GV-ATLL effect might have been suppressed by ATG included in the conditioning regimen.

In this modified phase I study, we again showed that a reduced-intensity conditioning regimen, regardless of the use of ATG or not, was feasible and safe in elderly patients with ATLL. Total and early relapse within 100 days after transplantation tended to be decreased, although there were no significant differences, as we had not expected. A univariate analysis also failed to show any differences in OS or PFS between patients in the 2 studies treated with and without ATG. It is considered that the reason why we could not show the impact of ATG was because of the small number of patients in these cohorts and the relatively low dose of ATG used in the previous study.

The speed of achieving complete donor chimera was significantly delayed if ATG was not used in the conditioning regimen, as has been reported [21]. It was speculated that, without ATG, host T cells tended to remain reactive to donor-derived allo-antigen and to compete with donor cells in the early phase after transplantation, and would require more time to be completely replaced by donor cells. Because the frequency of aGVHD was not significantly different between with and without ATG in the conditioning regimen, the development of aGVHD may not be associated with the achievement of complete donor chimera.

As shown here, there were no significant differences between the 2 study groups in most patients'

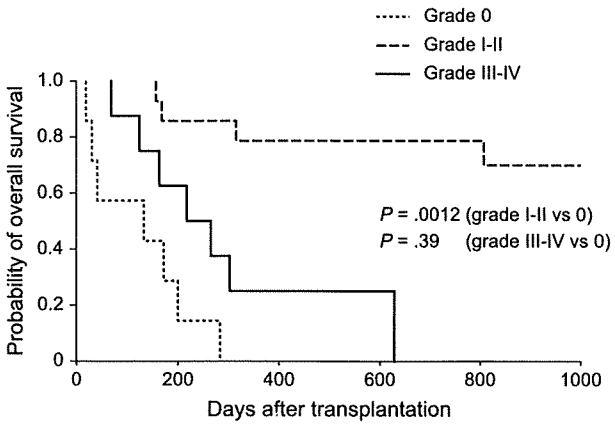

Figure 4. Kaplan-Meier estimate of overall survival for all patients treated in both studies according to the grade of aGVHD. Patients with grade I-II aGVHD had a significantly better overall survival compared to those without aGVHD (P = .0012). Severe aGVHD (grade III-IV) did not affect favorably in respect of overall survival compared to those without aGVHD (P = .39).

characteristics. Therefore, it is reasonable to analyze rare patients in both studies combined together. In the total of 29 patients included in both studies, the median survival time was 304 days, and OS and PFS at 3 years were 36% (95% CI, 27 to 45%) and 25% (95% CI, 16 to 34%), respectively, with a median follow-up of 1222 days (range: 592 to 1973) in the surviving patients. Moreover, our study revealed the characteristics of ATLL in response to transplantation; despite the frequent occurrence of early relapse, a significant number of patients survived thereafter. Four patients who had a relapse subsequently responded to a rapid discontinuation of the immunosuppressive agent and had a sustained remission for 152, 562, 1498, and 1945 days after relapse, suggesting that this disease entity is particularly susceptible to immune modulation [22,23].

Patients who developed grade I-II aGVHD had significantly better OS and PFS than other patients. Because the development of severe GVHD is considered to be too toxic for ATLL patients who are elderly, highly immune-compromised, and/or often have preexisting infectious complications, the regulation of immune reaction should be important for controlling the disease activity. We also confirmed that allogeneic transplantation not only has an anti-ATLL effect, but also anti-HTLV-1 activity. Eleven of the 14 patients (79%) who received graft from an HTLV-1-negative donor and 5 of the 14 patients (36%) from an HTLV-1-carrier donor became negative for HTLV-1 proviral load in the peripheral blood at least once within 6 months after transplantation, with no meaningful difference between the 2 studies.

The frequency of infectious complications in our studies was quite high, regardless of the use of ATG in the conditioning regimen, because 24 of the total

Table 3. *Univariate Analysis of Factors that Influence Overall and Progression-Free Survival*

Parameter and Group	HR	95% CI	P
OS			
ATG			
Absence versus presence	0.72	0.28 to 1.83	.49
Patient age			
>55 versus ≤55	1.92	0.72 to 5.15	.19
Patient sex			
Male versus female	0.67	0.26 to 1.71	.40
Subtype			
Lymphoma versus acute	0.77	0.27 to 2.17	.61
Status at transplantation			
PR versus CR	1.26	0.41 to 3.84	.69
Donor's HTLV-I status			
Carrier versus noncarrier	1.15	0.46 to 2.91	.77
Acute GVHD			
Grade I-II versus grade 0	0.07	0.01 to 0.35	.0012
Grade III-IV versus grade 0	0.59	0.17 to 2.00	.39
PFS			
ATG			
Absence versus presence	0.62	0.26 to 1.48	.28
Patient age			
>55 versus ≤55	2.27	0.85 to 6.12	.10
Patient sex			
Male versus female	0.81	0.32 to 2.07	.66
Subtype			
Lymphoma versus acute	0.70	0.27 to 1.81	.46
Status at transplantation			
PR versus CR	1.22	0.45 to 3.34	.70
Donor's HTLV-I status			
Carrier versus noncarrier	1.06	0.45 to 2.49	.90
Acute GVHD			
Grade I-II versus grade 0	0.15	0.05 to 0.49	.0014
Grade III-IV versus grade 0	0.39	0.13 to 1.13	.08

OS indicates overall survival; ATG, anti-thymocyte globulin; PR, partial remission; CR, complete remission; HTLV-1, human T cell leukemia virus type-1; GVHD, graft-versus-host disease; PFS, progression-free survival; HR, hazard ratio.

29 patients (83%) developed CMV antigenemia, although all were successfully treated with gancyclovir and none progressed to CMV disease. This high rate may reflect profound immunodeficiency underlying the process of ATLL, as has been reported [24,25]. Moreover, 2 patients developed EBV-lymphoproliferative disorders and both were in the first study: there were none in the second study without ATG.

In conclusion, allogeneic reduced-intensity HSCT using fludarabine and busulfan with or without low-dose ATG was shown to be feasible and safe even in elderly patients with ATLL, and the results suggested that its efficacy may be because of a GV-ATLL effect. A combined analysis suggests that the use of ATG could provide too much immune suppression to patients who are already intensely immune-compromised. To confirm these findings, a multicenter phase II study of reduced-intensity HSCT using fludarabine and busulfan without ATG is currently underway.

ACKNOWLEDGMENTS

The first part of this study has been published in the journal *Blood* 105:4143-4145, 2005, by Okamura J, a coauthor of this paper. This was also presented in part at the 13th International Conference on Human Retrovirology, May 22-25, 2007, Hakone, Japan. This work was supported by a grant for Anticancer Project from the Ministry of Health, Welfare, and Labor of Japan. The authors thank Drs. Yutaka Takada and Kaname Miyashita of the Kyushu Cancer Center for performing the chimerism analysis. We also thank Dr. Yoichi Takaue of the National Cancer Center Hospital for his valuable suggestions and constructive comments.

Authors' Disclosures of Potential Conflicts of Interest

The authors have no potential conflicts of interest to disclose.

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Biology of Blood and Marrow Transplantation 14:817-823 (2008)

1083-8791/08/1407-0001$32.00/0
doi:10.1016/j.bbmt.2008.04.014

A Retrospective Analysis of Allogeneic Hematopoietic Stem Cell Transplantation for Adult T Cell Leukemia/Lymphoma (ATL): Clinical Impact of Graft-versus-Leukemia/Lymphoma Effect

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Received March 5, 2008; accepted April 24, 2008

ABSTRACT

Adult T cell leukemia/lymphoma (ATL) is a highly aggressive T cell malignancy, and has a poor prognosis. Recently, allogeneic-hematopoietic stem cell transplantation (allo-HSCT) has been suggested to improve the outcome. We retrospectively analyzed 15 patients with ATL who had received allo-HSCT in 2 institutions in Hokkaido, Japan. The median age of the patients was 57 years. The estimated 3-year overall survival (OS) and progression-free survival (PFS) rates were 73.3% and 66.7%, respectively. Calcineurin inhibitor dosage was reduced and administration was discontinued abruptly in 6 of the 15 patients for disease control; as a result, 4 (66.7%) of the 6 patients achieved complete response (CR) or partial response. Therefore, a graft-versus-leukemia/lymphoma (GVL) effect might be induced by discontinuation of immunosuppression. Thirteen of the 15 patients were followed up by monitoring HTLV-1 proviral DNA levels. In 10 of the 11 patients with positive HTLV-1 proviral DNA before allo-HSCT, HTLV-1 proviral DNA became undetectable at least once after allo-HSCT, and only 1 of the 5 patients in whom HTLV-1 proviral DNA became detectable after allo-HSCT relapsed. Compared to the results of past studies, these results show that allo-HSCT greatly improved the prognosis of ATL and suggest a contribution of the induction of a GVL effect.

KEY WORDS

Adult T cell leukemia/lymphoma • Human T cell lymphotropic virus type 1 • Allogeneic stem cell transplantation • HTLV-1 proviral DNA • GVL effect

INTRODUCTION

Adult T-cell leukemia/lymphoma (ATL) is a highly aggressive T cell malignancy associated with infection of a retrovirus, human T cell lymphotropic virus type 1 (HTLV-1) [1-4]. Acute and lymphoma types of ATL have a particularly poor prognosis because of resistance to conventional chemotherapy or high-dose chemotherapy at an early stage during its clinical course. The median survival periods for patients with these subtypes of ATL who have received chemotherapy are 3 to 13 months [5-8].

Successful allogeneic-hematopoietic stem cell transplantation (allo-HSCT) has recently been reported, and several groups have reported encouraging

results of allo-HSCT for ATL. A graft-versus-leukemia/lymphoma (GVL) effect has also been suggested to improve treatment outcomes.

We retrospectively analyzed 15 patients with ATL who had received allo-HSCT in the Hokkaido University Hospital and the Sapporo Hokuyu Hospital. Compared to results of past studies, our results more strongly suggest that allo-HSCT improves the outcome for ATL, and that a GVL effect improves the clinical outcome after allo-HSCT.

PATIENTS AND METHODS

Diagnosis and Classification of clinical Subtypes of ATL

In all cases, the diagnosis of ATL was based on clinical features, immunophenotype, presence of anti-HTLV-1 antibody, and clonal integration of HTLV-1 proviral DNA. Clinical subtypes of ATL were classified according to the criteria of the Japanese Lymphoma Study Group [9].

Response to Treatment

The response criteria were defined as follows [5]: complete response (CR, resolution of all malignant disease for 4 weeks or more); partial remission (PR, reduction in measurable indices lasting 4 weeks or more, without the development of new lesions or disease progression); and progressive disease (PD, increase in measurable disease or in the number of circulating leukemic cells by 25% or more).

Patients' Characteristics

Fifteen patients with ATL (acute-type, n = 6; lymphoma-type, n = 8; chronic-type, n = 1) received allo-HSCT at the Hokkaido University Hospital and the Sapporo Hokuyu Hospital between 2000 and 2007. These hospitals are located in Hokkaido, an area of Japan in which there is a small number of HTLV-1 infection cases compared to the number of cases in the Shikoku or Kyushu districts of Japan. The patient with chronic type of ATL received allo-HSCT because of poor disease control and the existence of an appropriate HLA-identical sibling donor.

Clinical characteristics of the ATL patients are shown in Tables 1 and 2. The median age at the time of diagnosis was 57 years (range: 41-66 years). Disease statuses at SCT were CR in 9 patients (molecular CR in 1 patient), PR in 5 patients, and PD in 1 patient. Eight patients received stem cells from bone marrow (BM), 4 patients received stem cells from peripheral blood (PB), and 3 patients received stem cells from BM and PB. Ten patients underwent transplantation from HLA-identical siblings, 2 donors having anti-HTLV-1 antibodies, and 5 patients underwent transplantation from unrelated HLA-identical donors.

Preconditioning Regimens and Graft-versus-Host Disease (GVHD) Prophylaxis

Five patients received a conventional regimen (etoposide + cyclophosphamide [Cy] + total-body irradiation [TBI]), and 10 patients received a reduced-intensity (RIC) regimen (fludarabine [Flu] + busulphan [Bu] ± TBI for 5 patients and Flu + melphalan ± TBI for 5 patients). GVHD prophylaxis consisted of treatment with cyclosporine (CsA) or tacrolimus (FK) and short-term methotrexate (sMTX): CsA + sMTX in 11 patients and FK + sMTX in 4 patients (Table 2).

Measurement of HTLV-I Proviral DNA

HTLV-1 proviral DNA was measured in mononuclear cells of PB or BM before and after allo-HSCT by Southern blot hybridization, dot blot qualitative analysis using polymerase chain reaction (PCR) amplification of the HTLV-1 pX gene and gag gene, and quantitative real-time PCR amplification of HTLV-1 tax gene, according to the recommendations of the manufacture's (SRL, Inc. and Mitsubishi Chemical Medience Corporation). The quantitative real-time PCR method has been previously described [10].

Statistical Analysis

Overall survival (OS) and progression-free survival (PFS) were analyzed according to the method of Kaplan and Meier. OS was calculated from the day of allo-HSCT until death or last-follow up, and PFS was calculated until disease progression, death, or last follow-up.

RESULTS

Engraftment

All patients tolerated the conditioning regimen and achieved neutrophil recovery ($0.5 \times 10^4/\mu L$) at a median of 17 days (range: 14-20 days) (Table 3), and 13 of the 15 patients achieved platelet recovery exceeding $5.0 \times 10^4/\mu L$ at a median of 29 days (range: 14-46 days).

GVHD

Acute GVHD (aGVHD) developed in 8 (53.3%) of the 15 patients: grade I GVHD in 1 patient, grade II in 5 patients and grade III in 2 patients. Chronic GVHD (cGVHD) developed in 10 (76.9%) of 13 patients followed up over 100 days from transplantation, with limited disease in 3 patients and extensive disease in 7 patients (Table 3). Two patients developed grade III aGVHD after reduction in the dose of and abrupt discontinuation of administration of a calcineurin inhibitor for induction of a GV-ATL effect, and 1 patient (Case 6) also developed severe cGVHD after discontinuation of the immunosuppression.

Table 1. *Patients characteristics*

	Age/Sex	Subtype	WBC (/μL) (Abnormal Lymphocyte(/μL))	LDH(IU/L)	Organ Involvement	Induction Therapy
Case 1	41/F	Acute	27,300 (18,837)	NA	Skin	mNLG-2
Case 2	44/M	Acute	331,000 (304,520)	1611	Liver	CHOP-V-MMV
Case 3	46/F	Chronic	10,900 (0)	245	Skin	PUVA+DCF
Case 4	47/F	Lymphoma	2500 (0)	340	—	CHOP-E
Case 5	49/F	Lymphoma	20,500 (820)	603	Spleen	CHOP→VCAP/AMP/VECP
Case 6	53/F	Lymphoma	6800 (0)	326	—	DCF→CHOP
Case 7	56/F	Lymphoma	5100 (0)	558	—	VCAP/AMP/VECP
Case 8	57/F	Lymphoma	9430 (0)	165	GI tract	VCAP/AMP/VECP
Case 9	58/M	Lymphoma	8050 (0)	208	—	CHOP→VCAP/AMP/VECP
Case 10	60/F	Acute	17,800 (8188)	290	Spleen	VCAP/AMP/VECP
Case 11	60/F	Lymphoma	NA (12,000)	593	—	VCAP/AMP/VECP
Case 12	61/F	Lymphoma	5000 (115)	363	—	CHOP
Case 13	62/F	Acute	39,200 (10,976)	1216	Liver, Skin	VEPA
Case 14	64/F	Acute	15,840 (5544)	418	—	VCAP/AMP/VECP
Case 15	66/M	Acute	26,000 (13,780)	595	Liver, Skin, PE	VCAP/AMP/VECP

GI tract indicates gastrointestinal tract; PE, pleural effusion; mNLG-2, cyclophosphamide, adriamycin, vincristine prednisolone, tetrahydropyranyl adriamycin, ranimustine, vindesine, etoposide, carboplatin; CHOP-V-MMV, cyclophosphamide doxorubicin, vincristine, prednisolone, etoposide, vindesine, ranimustine, mitoxantrone; PUVA, psoralen and ultraviolet light; DCF, deoxycoformycin; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisolone; VCAP, vincristine, adriamycin cyclophosphamide, prednisolone; AMP, adriamycin, ranimustine, prednisolone; VECP, vindesine, etoposide, carboplatin prednisolone; VEPA, vincristine, cyclophosphamide, prednisolone, doxorubicin.

Induction of a GVL Effect

A calcineurin inhibitor dose was reduced and administration was abruptly discontinued in 6 of the 15 patients for induction of a GVL effect because of relapse, regrowth of residual disease, remaining ATLL cells, or remaining recipient-type cells in chimerism analysis. As a result, 3 patients (Cases 2, 7, and 13) achieved CR, 1 patient (Case 10) achieved PR, and 2 patients (Cases 5 and 8) had PD. However, 2 of the 3 patients who achieved CR developed grade III aGVHD and 1 of the 3 patients developed lung cGVHD for which mechanical ventilation was temporarily needed.

Survival, Relapse (or Disease Progression), and Treatment-Related Mortality (TRM)

The median follow-up period after transplantation was 35.5 months (range: 1.5-86.0 months) and the median PFS period after transplantation was 35.5 months (range: 0.4-86.0 months). The estimated 3-year OS and PFS rates were 73.3% and 66.7%, respectively (Figure 1). Four of the 5 patients who received CST are alive and 1 patient (Case 5) died of relapse 477 days after transplantation. Seven of the 10 patients who received RIST are alive. One patient (Case 8) died of disease progression 45 days after transplantation, and 2 patients (Cases 9 and 15)

Table 2. *Transplant characteristics*

	Time from Diagnosis to SCT(Days)	Disease Status	Donor	Stem Cell Source	Conditioning Regimen	GVHD Prophylaxis
Case 1	150	CR	Related	BM+PB	VP-16+Cy+TBI	CsA+sMTX
Case 2	215	PR	Related	BM	VP-16+Cy+TBI	CsA+sMTX
Case 3	825	PR	Related	BM	VP-16+Cy+TBI	CsA+sMTX
Case 4	150	CR	Related	BM+PB	VP-16+Cy+TBI	CsA+sMTX
Case 5	425	PR	Unrelated	BM	VP-16+Cy+TBI	FK+sMTX
Case 6	1275	CR	Unrelated	BM	Flu+L-PAM+TBI	FK+sMTX
Case 7	680	PR	Unrelated	BM	Flu+BU+TBI	CsA+sMTX
Case 8	153	PD	Related	BM	Flu+BU+TBI	CsA+sMTX
Case 9	196	CR	Related	PB	Flu+L-PAM	CsA+sMTX
Case 10	157	CR	Unrelated	BM	Flu+BU+TBI	FK+sMTX
Case 11	232	PR	Related (HTLV-1(+))	PB	Flu+L-PAM	CsA+sMTX
Case 12	371	CR	Related	BM+PB	Flu+L-PAM	CsA+sMTX
Case 13	140	CR	Related	PB	Flu+BU	CsA+sMTX
Case 14	297	CR	Unrelated	BM	Flu+BU+TBI	FK+sMTX
Case 15	127	CR	Related (HTLV-1(+))	PB	Flu+L-PAM	CsA+sMTX

VP-16 indicates etoposide; Cy, cyclophosphamide; TBI, total body irradiation; Flu, fludarabine; L-PAM, melphalan; BU, busulfan; CsA, cyclosporine; FK, tacrolomus; sMTX, short-term methotrexate.

Table 3. *Transplant outcomes*

	Engraftment (Day) Neutrophil/Platelet	aGVHD (Day/Grade)	aGVHD (Day/Type)	Outcome	Cause of Death	Follow-up Time (Month)
Case 1	18/46	None	120/Extensive	CR	Alive	79.0+
Case 2	15/24	31/?	351/Extensive	CR(after d/c CI)	Alive	61.7+
Case 3	16/26	57/?	365/Extensive	CR	Alive	69.1+
Case 4	17/29	26/?	252/Limited	CR	Alive	86.0+
Case 5	15/27	26/?	160/Limited	Dead, d477	Relapse	16.0
Case 6	17/26	None	None	CR	Alive	37.0+
Case 7	14/29	None	None	CR (complete chimera after reducing CI)	Alive	45.3+
Case 8	16/24	8/?	Not evaluable	Dead, d45	Disease progression	1.5
Case 9	14/33	None	None	Dead, d296	TTP	9.7
Case 10	17/30	69/?	210/Extensive	LN relapse,d192 (PR after d/c CI)	Alive	21.2+
Case 11	20/-	None	142/Extensive	CR	Alive	35.5+
Case 12	16/26	None	132/Extensive	CR	Alive	31.0+
Case 13	16/14	29/?	69/Extensive	CR (complete chimera after d/c CI)	Alive	58.9+
Case 14	16/31	None	80/Limited	CR	Alive	4.6+
Case 15	19/-	17/?	Not evaluable	Dead, day46	Bacterial pneumonia	1.5

CI indicates calcineurin inhibitor; TTP, thrombotic thrombocytopenic purpura.

died of TRM because of allo-SCT-related thrombotic thrombocytopenic purpura (TTP) and bacterial pneumonia (Table 3). In 2 patients whose donors had anti-HTLV-1 antibodies, 1 patient (Case 11) is alive and has achieved CR (with negative HTLV-1 proviral DNA), and the other patient (Case 15) died of TRM.

Also, none of the patients were treated with antiretroviral agents after HSCT.

Clinical Course of HTLV-I Proviral DNA

Thirteen of the 15 patients were followed up by monitoring HTLV-1 proviral DNA levels before and after allo-HSCT (Figure 2). In 10 of the 11 patients with positive HTLV-1 proviral DNA before allo-HSCT, HTLV-1 proviral DNA became undetectable more than 1 time after allo-HSCT. (One patient [Case 1] was followed up in another hospital, so the last day for data of HTLV-1 proviral DNA was day 120, and was positive by dot blot qualitative analysis using PCR amplification of the HTLV-1 gag gene.) Although HTLV-1 proviral DNA became detectable in 5 patients again (Cases 4, 5, 7, 10, and 13), 1 of those patients (Case 5) relapsed with leukemia 6 months after allo-HSCT. Case 10 also relapsed in lymph nodes but remained in CR in peripheral blood and bone marrow at that time. In 2 patients with negative HTLV-1 proviral DNA by Southern blot hybridization before allo-HSCT, 1 patient (Case 9) died of TRM and 1 patient (Case 11) is alive, and the status of HTLV-1 proviral DNA remained undetectable in both of those patients.

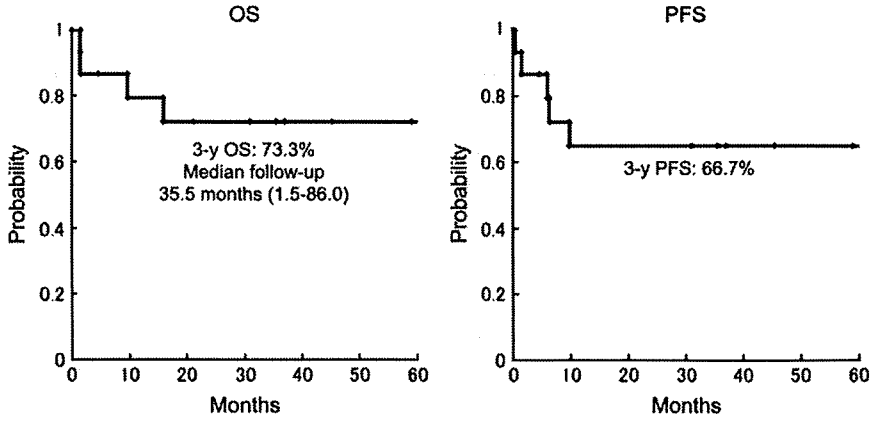

Figure 1. Kaplan-Meier plot of OS and PFS following allogeneic stem cell transplantation for ATL.

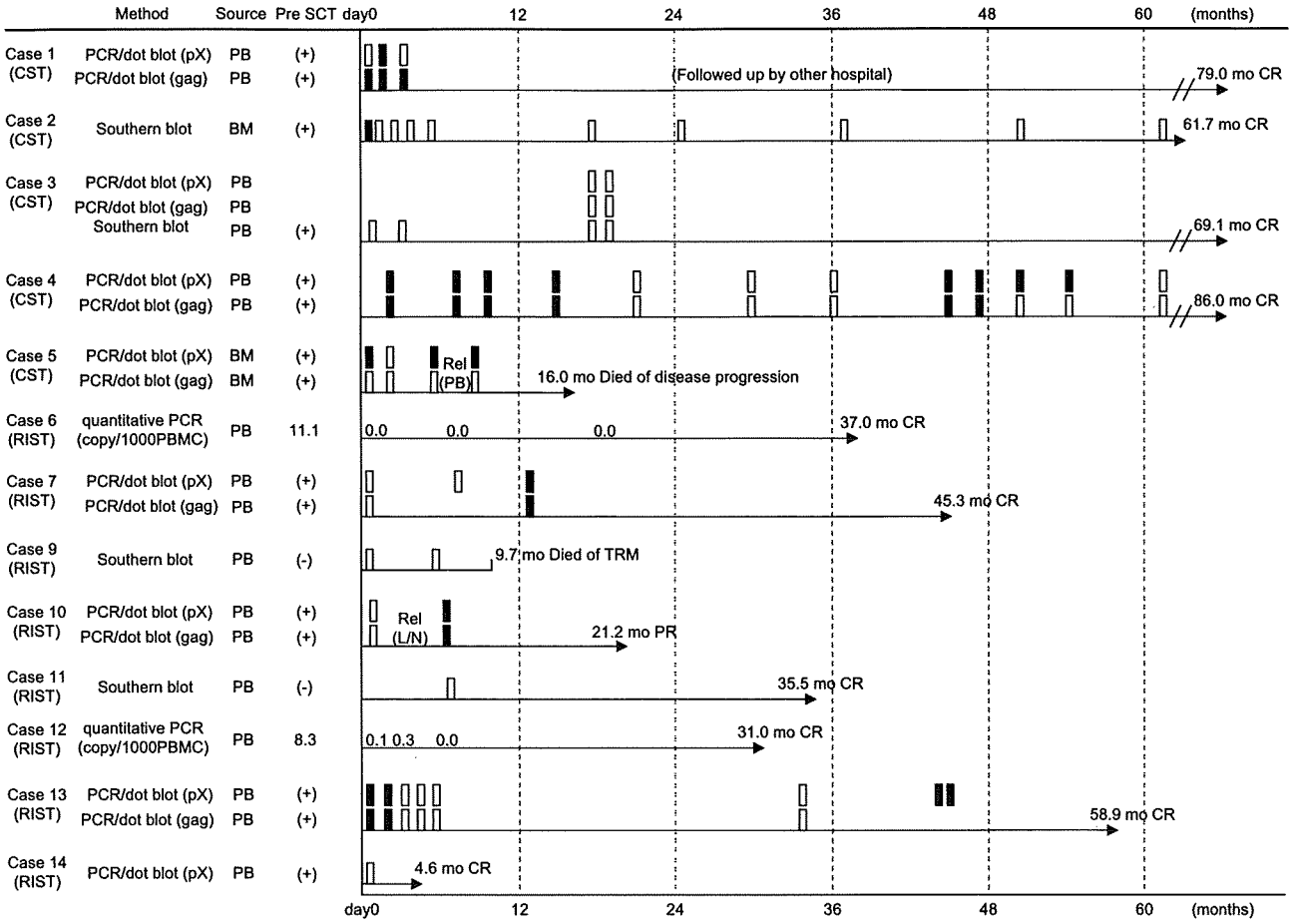

Figure 2. Transition of HTLV-1 proviral DNA before and after allo-HSCT (open squares: negative results, closed squares: positive results).

DISCUSSION

ATL is a highly aggressive hematologic malignancy, and its prognosis is poor because ATL cells are resistant to chemotherapy at an early stage. Cases of treatment with allo-HSCT have been reported since 1987 [11], and since the report of Utsunomiya et al. in 2001 [12], results of several retrospective analyses and a phase I clinical trial of allo-HSCT for ATL have been reported in Japan [13-17]. Survival periods of patients in those studies ranged from 33.3% to 60% for OS and from 20% to 64% for TRM. In our retrospective analysis, 3-year OS was 73.3%, 3-year PFS was 66.7%, and TRM was 20%. These results of allo-HSCT with ATL are much better than those of past studies. Disease progression occurred in only 2 patients (13.3%) after allo-HSCT in this study. The background of this outcome is considered to be that there was only 1 patient with PD before allo-HSCT and better disease control by the induction chemotherapy was obtained in many cases, although these were not greatly different from those in past studies. Also, regarding the lower TRM rate, there was not a great difference in GVHD prophylaxis compared to that in past studies, and special treatment of infection was not done compared to other hematologic malignancies in our hospital. As mentioned above, this study was carried out in Hokkaido, an area of Japan in which there is a small number of HTLV-1 infection cases, and past studies were carried out in Kyushu and Shikoku, areas in which HTLV-1 infection is endemic. Some studies have shown several subtypes of HTLV-1 provirus, and the significance of these subtypes in oncogenesis of ATL has been suggested [18-22]. Although this study was a retrospective analysis and we could not investigate the subtypes of HTLV-1 provirus, 1 reason for the good outcome in this study might be related to the difference in subtypes of ATL. Further studies are needed to clarify the relationship between subtypes of HTLV-1 provirus and prognosis of ATL.

Another reason might be the induction of a GVL effect by reducing the dose of and abrupt discontinuation of administration of a calcineurin inhibitor. A relationship between GVHD and GVL effect has been reported in acute lymphoblastic leukemia (ALL) [23,24]. In ATL, there have also been some reports