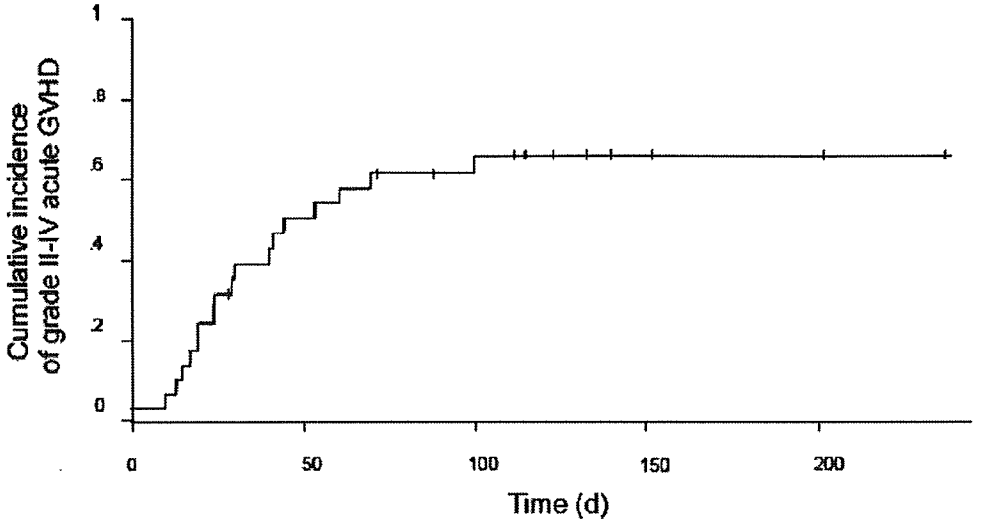

Figure 1. Cumulative incidence of grade II–IV acute GVHD in patients who achieved neutrophil engraftment.

day 14. Acute GVHD developed in 25 of the 28 patients who achieved engraftment (89%): grade I GVHD in 8 patients, grade II in 12 patients, grade III in 3 patients, and grade IV in 2 patients. The cumulative incidence of grade II–IV acute GVHD was 61% (Figure 1). Chronic GVHD developed in 4 of 18 patients, with limited disease in 1 patient and extensive disease in the other 3 patients.

Survival and disease progression

The 1-year OS and PFS were 49.5% (95% confidence interval [CI], 31.2%–78.5%) and 49.2% (95% CI, 33.6%–72.1%), respectively (Figure 2). Disease progression was observed in 5 patients, and the median number of days from transplantation to disease progression was 122 (range, 61–223 days). As of the last follow-up, 14 deaths had been reported. Primary cause of death was disease progression in 2 patients and was not described in 3 patients, but the other 9 deaths were not due to disease progression (see Table 3). Primary causes of transplantation-related death within 100 days after transplantation were late graft failure in 1 patient, GVHD in 1 patient, infection in 3 patients (with methicillin-resistant *Staphylococcus aureus*–positive sepsis in 1 patient and pulmonary infection in 2 patients), thrombotic microangiopathy (TMA) in 2 patients, veno-occlusive disease (VOD) in 1 patient, and arrhythmia in 1 patient.

Univariate and Multivariate Analyses for OS

Pretransplantation and posttransplant factors were calculated for OS (Table 4). In univariate analyses, OS was not significantly associated with sex, duration from diagnosis to transplantation, ECOG performance status, conditioning regimen, number of bone marrow cells transplanted, or presence of grade II–IV acute GVHD. On the other hand, patient age and disease status at transplantation were identified as significant independent risk factors. In multivariate analyses, only patient age at transplantation was identified as exerting a significant independent risk impact on OS (≥50 years vs <50 years; relative risk, 3.47; 95% CI, 1.03–11.6; $P = .044$). Disease status at transplantation exerted a marginally significant impact on OS (NR vs CR or PR; relative risk, 3.17; 95% CI, 0.96–10.5; $P = .059$) (Figure 3).

Influence of Pretransplantation Factors on Disease Progression and Progression-Free Mortality

The cumulative incidence of disease progression and progression-free mortality at 1 year were 18.6% and 32.3%, respectively (Figure 4). To clarify how age and disease status at transplantation affected OS, we evaluated the relationship between these factors and the incidence of progression-free mortality. The cumulative incidence of progression-free mortality was significantly higher in patients age ≥50 years at transplantation (50% vs 18%; $P = .048$; Figure 5A). NR at transplantation exerted a marginally significant effect on increased progression-free mortality (54% vs 20%; $P = .070$; Figure 5B).

DISCUSSION

This study analyzed the data and evaluated treatment outcomes for 33 patients with ATLL who received UBMT. Two important findings were identified regarding UBMT for ATLL. First, UBMT from HTLV-I–negative donors for ATLL represents a feasible treatment. Second, recipient age (≥50 years) and NR disease status at transplantation were independent risk factors for OS, and patients with ATLL displaying these risk factors tended to exhibit higher frequencies of treatment-related mortality.

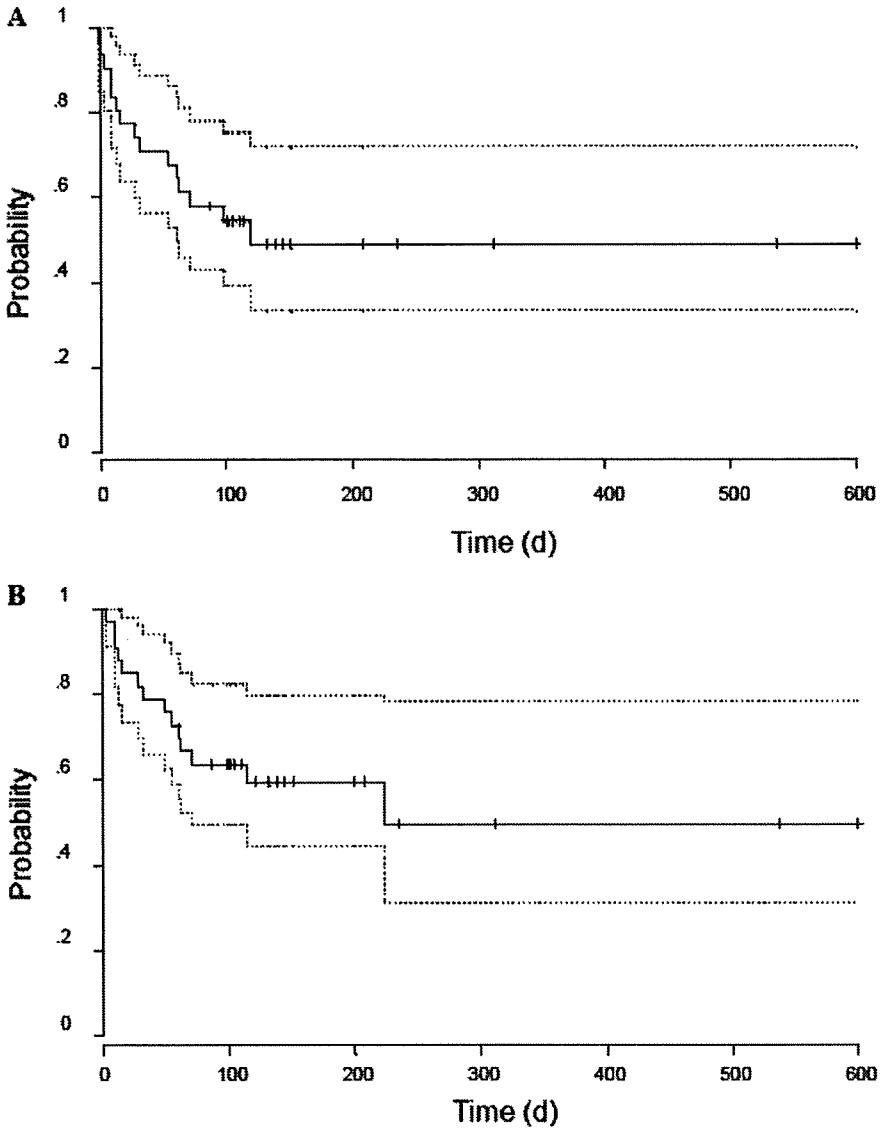

Figure 2. Probability of progression-free survival (A) and overall survival (B) after unrelated bone marrow transplantation for adult T-cell leukemia/lymphoma. Dashed lines represent 95% confidence intervals.

Table 4. *Prognosis factors in univariate and multivariate analyses*

	Univariate		Multivariate	
	Relative risk (95% CI)	*P*	Relative risk (95% CI)	*P*
Age ≧50 versus <50 years	4.03 (1.23–13.3)	.022	4.03 (1.23–13.3)	.022
Male versus female	0.97 (0.34–2.80)	.95		
PS 0–1 versus 2–4	0.44 (0.11–1.70)	.23		
NR versus CR or PR	3.37 (1.03–11.0)	.044		.059
UBMT within 1 year versus beyond 1 year	0.54 (0.15–2.00)	.35		
RIST versus CST	0.71 (0.19–2.59)	.60		
TBI versus non-TBI	1.35 (0.45–4.04)	.59		
Cell dose < 3.0 × 10^8/kg versus ≧ 3.0 × 10^8/kg	0.98 (0.31–3.05)	.97		
GVHD II–IV present versus absent	1.91 (0.50–7.26)	.34		

CI indicates confidence interval; PS, performance status; NR, nonremission; CR, complete remission; PR, partial remission; UBMT, unrelated bone marrow transplantation; RIST, reduced-intensity stem cell transplantation; CST, conventional stem cell transplantation; TBI, total body irradiation; GVHD, graft-versus-host disease.

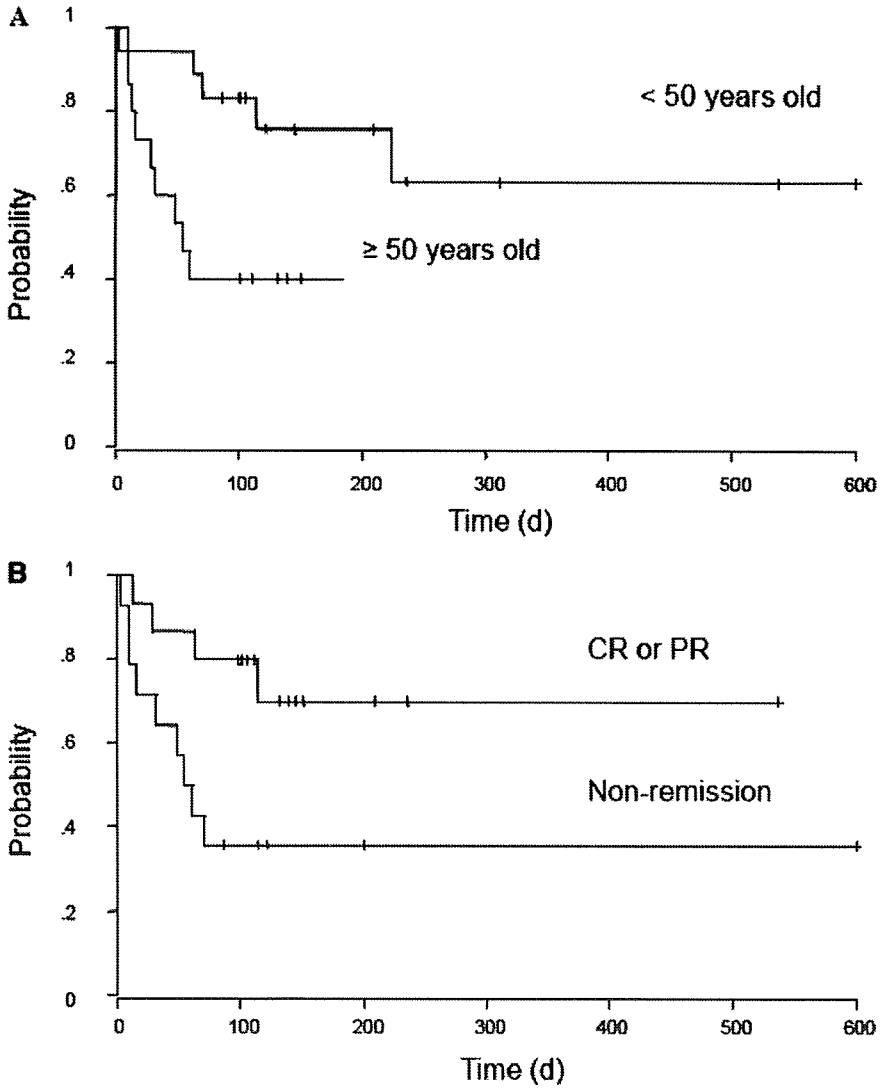

Figure 3. Overall survival according to pretransplantation factors, age (A) and disease status at transplantation (B).

ATLL has an extremely poor prognosis, with projected 2- and 4-year survival rates of 16.7% and 5.0% for the acute type and 21.3% and 5.7% for the lymphoma type, respectively. [3] Neither intensified chemotherapy nor autologous stem cell transplantation have improved the prognosis. Encouraging results for allo-HSCT for ATLL from HLA-matched related donors have been reported by several groups; thus, allo-HSCT may improve the poor prognosis of ATLL. However, the number of patients in most reports has been too small to allow evaluation of the efficacy of allo-HSCT for ATLL. The present results were derived from a large number of patients who underwent transplantation (33 patients) performed through the JMDP. Longer follow-up is, of course, needed to confirm the curative potential of allo-HSCT for ATLL. However, the good survival rates noted here suggest that allo-HSCT is an effective treatment for ATLL, and that patients with ATLL will benefit from allo-HSCT through HTLV-I–negative unrelated donors, because the OS and PFS rates at 1 year after UBMT were 49.5% and 49.2%, respectively. Compared with the results for patients with non-Hodgkin's lymphoma in the National Marrow Donor Program, the incidence of grade III–IV acute GVHD in the present study was low (18% vs 30%). [26] The outcome in the present study appears to be favorable, possible due to the lower incidence of grade III–IV acute GVHD. This observation is compatible with previous studies showing a lower incidence of acute GVHD in Japanese patients compared with Western patients, which might reflect the less diverse genetic background of in the Japanese population. [27,28]

Frequency of relapse after transplantation differs between autologous and allo-HSCT for ATLL. The use of high-dose chemotherapy with autologous HSCT has been reported in only 9 patients, all of whom relapsed or died from transplantation-related mortality. [8] In contrast, the cumulative incidence of

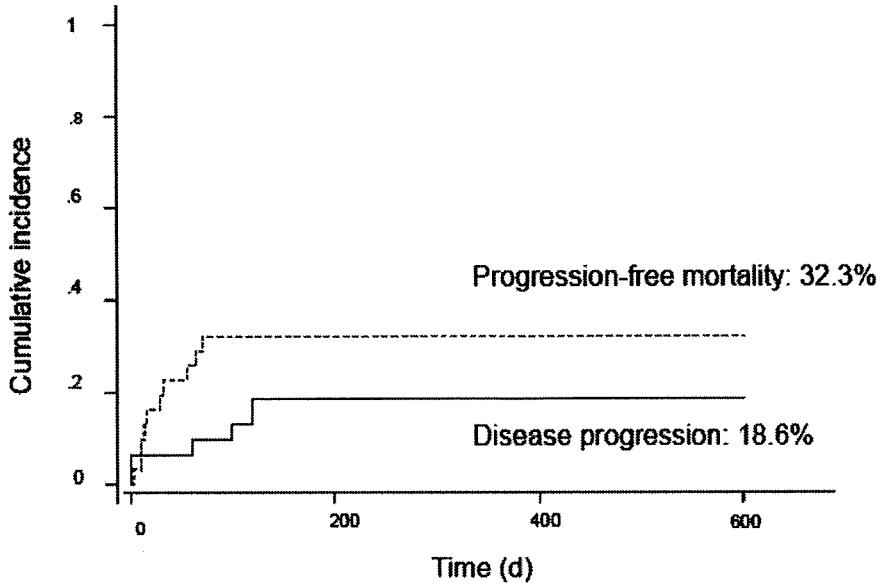

Figure 4. Cumulative incidence of disease progression (—) and progression-free mortality (---) after transplantation.

disease progression was lower after UBMT in this study. Interestingly, patients with ATLL displaying acute or chronic GVHD reportedly did not relapse. [9] In another report, patients with ATLL who relapsed after allo-HSCT reachieved CR after tapering or discontinuation of immunosuppressive agents and donor lymphocyte infusions. [10,11] Reactivation in tax-specific CD8-positive cytotoxic T lymphocytes (CTLs), which has been recently shown in patients with ATLL after allo-HSCT, may indicate a potential contribution of CTLs to anti-ATLL immunity and induction of a GvATLL effect. [29] These results strongly suggest that a GvATLL effect could work on some patients with ATLL to prevent relapse after allo-HSCT. In the present study, neither univariate nor multivariate analysis showed a survival benefit for acute GVHD. We were unable to analyze the relationship between chronic GVHD and relapse, because of the low number of patients with chronic GVHD. In fact, the number of patients may have been insufficient to confirm GvATLL in this study. On the other hand, the absence of benefit from GVHD in preventing relapse suggests that a GvATLL effect could occur in patients with ATLL after allo-HSCT without clinically obvious GVHD. [11]

Transplantation-related mortality was a significant problem in this study. Five patients (15%) died within 20 days, from infection in 3 patients and TMA in 2 patients. Nine patients (27%) died within 100 days, due to infection in 3 patients, TMA in 2 patients, and VOD in 1 patient. Patients with ATLL might have an increased risk of frequent opportunistic infection, because they have an associated T-cell immunodeficiency. Furthermore, ATLL is usually systemic in distribution, and the accumulated organ damages as a result of repeated cytotoxic chemotherapy seen in patients before transplantation may have contributed to the onset of TMA. In univariate and multivariate analysis, recipient age (≥50 years) and NR disease status at transplantation represented significant risk factors for OS. The multivariate analyses were limited by the small number of patients in each subgroup; however, patients displaying these risk factors tended to have a higher rate of treatment-related mortality than patients without these factors, and it can be assumed that these risk factors have a significant relationship with outcome clinically. In this study, mostly myeloablative conditioning regimens were used before transplantation. Given that conventional allo-HSCT is designed to eradicate tumor cells with myeloablative intensity using maximally tolerated doses of high-dose chemotherapy and radiotherapy, the desirable effects often may be offset by overwhelming toxicity in patients age ≥50 years. Moreover, the number of patients with ATLL who are eligible for allo-HSCT with myeloablative conditioning is limited, because the typical patient with ATLL has a relatively advanced age at presentation (about 60 years). To reduce treatment-related mortality, allo-HSCT with reduced-intensity conditioning offers a new treatment option for patients with ATLL who are ineligible for allo-HSCT with myeloablative conditioning due to advanced age or medical infirmity. [30,31] Okamura et al [32] reported on 16 patients age > 50 years with ATLL who underwent allo-HSCT with reduced-intensity conditioning from HLA-matched related donors and found that treatment-related mortality was acceptable and that allo-HSCT with reduced-intensity conditioning was a feasible treatment for ATLL. Given these findings, UBMT

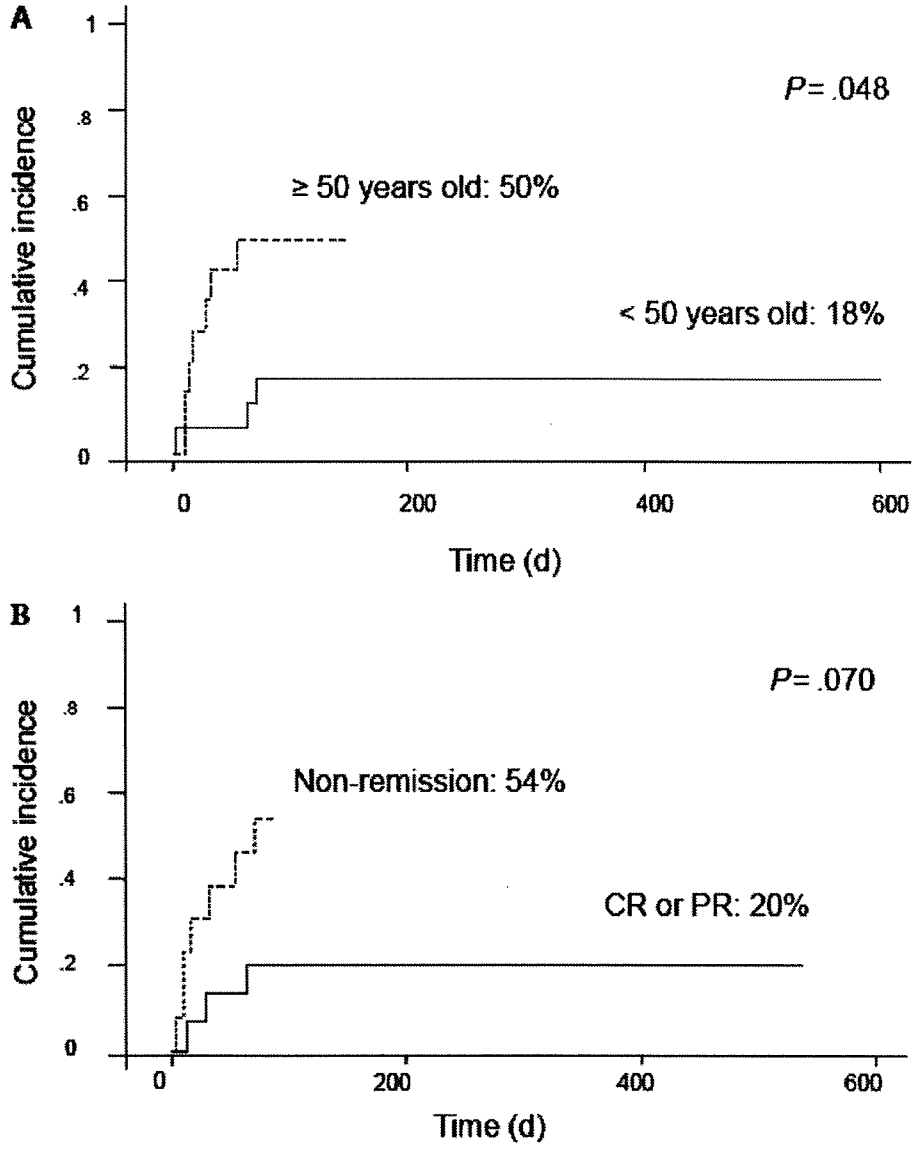

Figure 5. Cumulative incidence of progression-free mortality grouped according to pretransplantation factors, age (A) and disease status at transplantation (B).

with reduced-intensity conditioning should be considered for elderly patients with ATLL.

Another concern related to allo-HSCT for ATLL involves the use of HTLV-1–positive carrier donors. About 2/3 of siblings of patients with ATLL are HTLV-I carriers. From the perspective of HTLV-I–positive donor risk, granulocyte colony–stimulating factor (G-CSF) can reportedly stimulate the proliferation of ATLL cells [33], and HTLV-I–positive donors may be at increased risk of developing ATLL due to the administration of G-CSF in the setting of allogeneic peripheral blood stem cell transplantation. From the perspective of patients with ATLL, allo-HSCT from an HTLV-I–positive donor may carry a risk of HTLV-I–associated disease after allo-HSCT [34] or a risk of promoting the future development of ATLL due to the new HTLV-I load on immunocompromised recipients [13,14]. On the other hand, to date there is no evidence in the JMDP or the literature that ATLL can develop from infected HTLV-I–negative donor cells due to the HTLV-I load of the recipient. The HTLV-I proviral load dramatically decreased to an undetectable level after transplantation, especially after transplantation from HTLV-I–negative donors. [18, 32] This decreased HTLV-I proviral load was observed after both myeloablative and reduced-intensity conditioning. Transplantation from an HTLV-I–positive donor is reportedly associated with a higher frequency of relapse compared with transplantation from an HTLV-I–negative donor. [11] Therefore, the uninfected normal donor T cells might overwhelm infected HTLV-I recipient T cells due to a GvATLL response and might act as an antiviral therapy. However, an HTLV-I–positive do-

nor might avoid clonal expansion of HTLV-I–infected T lymphocytes after allo-HSCT through the provision of cytotoxic T cells. Thus, it is currently difficult to determine whether an HTLV-I–positive or–negative donor should be selected. Longer follow-up is needed to resolve this issue. In the meantime, a prudent clinical attitude toward both HTLV-I–positive donors and recipients with ATLL is warranted.

In conclusion, allo-HSCT from an HTLV-I–negative unrelated donor appears to be an feasible alternative treatment for patients with ATLL for whom an HLA-matched related donor is unavailable. Further prospective controlled studies are needed to assess the efficacy of allo-HSCT for ATLL and to define the clinical indications of allo-HSCT for ATLL, taking into account donor selection, the conditioning regimen, and the prognostic factors identified in this study.

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APPENDIX: PARTICIPATING INSTITUTIONS

The following centers in Japan participated in this study: Hokkaido University Hospital, Sapporo University Hospital, Sapporo Hokuyu Hospital, Japanese Red Cross Asahikawa Hospital, Asahikawa Medical College Hospital, Hirosaki University Hospital, Tohoku University Hospital, Yamagata University Hospital, Akita University Hospital, Fukushima Medical College, National Cancer Center Central Hospital, Institute of Medical Science at the University of Tokyo, Toho University Hospital, Omori Hospital, Tokyo Metropolitan Komagome Hospital, Nihon University Hospital, Itabashi Hospital, Jikei University Hospital, Keio University Hospital, Tokyo Medical College Hospital, Tokyo Medical and Dental University Hospital, Tokyo University Hospital, Yokohama City University Hospital, Kanagawa Children's Medical Center, Kanagawa Cancer Center, Tokai University Hospital, St Marianna University Hospital, Chiba University Hospital, Chiba Children's Hospital, Matsudo Municipal Hospital, Kameda General Hospital, Saitama Children's Medical Center, Saitama Cancer Center Hospital, Saitama Medical School Hospital, Ibaraki Children's Hospital, Jichi Medical School Hospital, Dokkyo University Hospital, Fukaya Red Cross Hospital, Saiseikai Maebashi Hospital, Gunma University Hospital, Niigata University Hospital, Niigata Cancer Center Hospital, Shinshu University Hospital, Saku Central Hospital, Hamamatsu University Hospital, Hamamatsu Medical Center, Shizuoka General Hospital, Shizuoka Children's Hospital, Japanese Red Cross Nagoya First Hospital, Nagoya Daini Red Cross Hospital, Meitetsu Hospital, Nagoya University Hospital, Nagoya Ekisaikai Hospital, National Nagoya Hospital, Aichi Medical School Hospital, Nagoya City University Hospital, Showa Hospital, Anjo Kousei Hospital, Fujita Health University Hospital, Mie University Hospital, Kanazawa University Hospital, Kanazawa Medical University Hospital, Toyama Prefectural Central Hospital, Fukui Medical School Hospital, Shiga University of Medical Science, Center for Adult Disease in Osaka, Kinki University Hospital, Osaka University Hospital, Osaka Medical Center and Research Institute for Maternal and Child Health, Matsushita Memorial Hospital, Hyogo College of Medicine Hospital, Hyogo Medical Center for Adults, Kobe City General Hospital, Kobe University Hospital, Kyoto University Hospital, Kyoto Prefectural University of Medicine Hospital, Social Insurance Kyoto Hospital, Tottori Prefectural Central Hospital, Tottori University Hospital, Hiroshima Red Cross Hospital and Atomic-Bomb Survivors Hospital, Yamaguchi University Hospital, Ehime Prefectural Central Hospital, Okayama National Hospital, Kurashiki Central Hospital, Kyushu University Hospital, Harasanshin General Hospital, Hamanomachi General Hospital, National Kyushu Cancer Center, St Mary's Hospital, Kokura Memorial Hospital, Saga Prefectural Hospital, Nagasaki University Hospital, Miyazaki Prefectural Hospital, Kumamoto National Hospital, Kumamoto University Hospital, Oita Medical University Hospital, Kagoshima University Hospital, and Imamura Bun-in Hospital.

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ORIGINAL ARTICLE

Small number of HTLV-1-positive cells frequently remains during complete remission after allogeneic hematopoietic stem cell transplantation that are heterogeneous in origin among cases with adult T-cell leukemia/lymphoma

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Allogeneic hematopoietic stem cell transplantation (allo-HSCT) can provide long-term remission for patients with adult T-cell leukemia/lymphoma (ATLL) caused by human retrovirus, human T-lymphocyte virus (HTLV-1). To understand how HTLV-1-positive cells including ATLL cells were suppressed by allo-HSCT, we examined HTLV-1 provirus load and residual ATLL cells in peripheral blood of transplant recipients using PCR-based tests. We found that the copy number of HTLV-1 genome, called provirus, became very small in number after allo-HSCT; however, in most cases, provirus did not disappear even among long-term survivors. Tumor-specific PCR tests demonstrated that most of HTLV-1-positive cells that remained long after transplantation were not primary ATLL cells but donor-derived HTLV-1-positive cells. We also found a case having very low amount of residual disease in peripheral blood even long after transplantation. There was only one recipient in whom we failed to show the presence of HTLV-1 genome and antibody against HTLV-1 even with an extensive search, which strongly suggested the elimination of HTLV-1 after allo-HSCT. These results demonstrated that after allo-HSCT the small amount of residual HTLV-1-positive cells were heterogeneous in origin and that long-term disease control for ATLL could be obtained without the complete elimination of HTLV-1.
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Keywords: ATLL; transplantation; MRD; HTLV-1

Introduction

Adult T-cell leukemia/lymphoma (ATLL) is a peripheral T-cell lymphoma caused by a retrovirus, human T-lymphocyte virus (HTLV-1), which randomly integrates into the genome of infected T cells.[1–3] The HTLV-1 genome in T cells, called provirus, has been utilized for the diagnosis of the disease caused by or the carrier state of HTLV-1. For example, Southern blot analysis of HTLV-1, when it demonstrates a monoclonal proliferation of cells infected with HTLV-1, provides the strongest evidence for the diagnosis of ATLL.[4] Southern blot analysis usually detects a monoclonal population composed of 3–5% of total cells, which is generally enough to diagnose ATLL. On the other hand, polymerase chain reaction (PCR)-based tests detect HTLV-1 genome with much higher sensitivity than Southern blot analysis, allowing us to monitor a small amount of HTLV-1 provirus load.[5,6]

The clinical course of ATLL widely differs by clinical subtypes (acute, lymphoma, chronic and smoldering). The prognoses of acute and lymphoma types are very poor when treated with conventional or even high-dose chemotherapy;[7,8] however, with allogeneic hematopoietic stem cell transplantation (allo-HSCT), a long-term clinical remission (CR) is achievable as reported from several groups including ours.[9–11] For example, among cases with acute ATLL, allo-HSCT reduced the volume of tumor cells in the peripheral blood to undetectable level when tested by morphological examination or Southern blot analysis, suggesting that the reduction of ATLL cells was less than 5% of WBC, as we reported previously.[11]

In this study, as an extension of our previous report, to understand how small the population of HTLV-1-positive cells would become after allo-HSCT and to test whether HTLV-1 could be eradicated, we investigated HTLV-1 provirus load and the minimum residual disease (MRD) in 22 cases of ATLL using PCR-based gene amplification. Since PCR for HTLV-1 provirus picked up not only ATLL cells, but also all cells infected with HTLV-1, including polyclonal non-ATLL cells, we introduced a specific PCR method to detect ATLL cells utilizing a unique integration site of HTLV-1 in each ATLL case.

We found that cells carrying HTLV-1 existed at the very low level in peripheral blood of long-term survivors after allo-HSCT. Most of them were donor-derived cells, but MRD was simultaneously present only in one case. We also experienced a single case in which anti-HTLV-1 antibodies became negative with no HLTV-1 genome amplified with PCR-based tests, suggesting the eradication of HTLV-1.

Patients and methods

Clinical features of patients with ATLL

The diagnosis and classification of ATLL was based on the criteria proposed by the Lymphoma Study Group of Japan.[12] Twenty-two patients with the diagnosis of acute or lymphoma type ATLL who received allo-HSCT in three hospitals in Nagasaki, an endemic area of HTLV-1 in Japan, between September 1997 and May 2004 were included in this study.

Table 1 summarizes the clinical characteristics of these patients. Median age of the patients was 48 years. In 21 of all 22 cases, donor-derived hematopoiesis was obtained (Table 2).

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Table 1 Characteristics of patients and transplantation

Case no.	*Age at HSCT /sex*	*Disease status at HSCT*	*Donor α HTLV-1 Ab*	*Donor*	*Source*	*Conditioning Regimen*
1	44/M	NC	–	Related	BM	TBI-VP16-CA
2	48/M	PD	–	Related	BM	TBI-VP16-CA
3	43/F	CR	–	Unrelated	BM	TBI-VP16-CA
4	51/M	PR	–	Related	BM	BU-CY2
5	30/F	PR	+	Related	BM	BU-CY3
6	54/F	PR	+	Related	BM	BU-CY2
7	44/F	PR	–	Unrelated	BM	TBI-CY
8	48/F	CR	+	Related	BM	BU-CY3
9	35/M	PD	–	Related	PB	BU-CY2
10	39/M	PD	–	Related	PB	TBI-CY
11	41/F	NC	–	Unrelated	BM	TBI-CY
12	48/M	PR	–	Related	PB	TBI-CY
13	46/M	PR	+	Related	BM	TBI-CY
14	50/F	PR	–	Unrelated	BM	TBI-CY
15	63/M	PD	+	Related	PB	FLU-BU-ATG (RIST)
16	53/M	CR	–	Related	PB	FLU+L-PAM (RIST)
17	55/M	NC	–	Related	PB	FLU-BU (RIST)
18	63/M	NC	–	Related	PB	FLU-CY (RIST)
19	48/F	PR	+	Related	BM	FLU+L-PAM (RIST)
20	53/M	CR	+	Related	BM	FLU-CY (RIST)
21	56/M	PD	+	Related	PB	FLU+L-PAM+TBI (RIST)
22	62/M	PR	–	Related	PB	FLU-BU (RIST)

Abbreviations: BM, bone marrow; BU, busulfan; CA, Cytarabine; CR, complete response; CY, cyclophosphamide; FLU, fludarabin; HSCT, hematopoietic stem cell transplantation; L-PAM, melphalan; NC, no change; PB, peripheral blood; PD, progressive disease; PR, partial response; RIST, reduced-intensity conditioning transplantation; TBI, total body irradiation; VP16, Etoposide.

Table 2 Results of transplantation

Case no.	*Engraftment*	*Relapse*	*aGVHD*	*cGVHD*	*Outcome*
1	+	Day 3074	I	—	Alive with ATLL (day 3094+)
2	+	—	IV	NE	Died of GVHD on day 123
3	+	Day 144	II	—	Died of ATLL on day 165
4	+	Day 169	I	—	Died of ATLL on day 237
5	+	—	0	—	Alive in CR (day 1756+)
6	+	Day 833	II	—	Alive in 2nd CR (2nd CR after local irradiation, day 1679+)
7	+	Day 262	0	Extensive	Died of ATLL on day 1310
8	+	—	0	Extensive	Alive in CR (day 1497+)
9	+	—	III	—	Died of infection on day 137
10	+	—	0	Limited	Alive in CR
11	+	Day 78	I	Extensive	Died of ATLL on day 218
12	+	—	III	—	Alive in CR (day 254+)
13	+	—	IV	—	Died of TRM on day 120
14	+	—	0	—	Died of cerebral haemorrhage on day 216
15	+	—	0	Extensive	Died of GVHD on day 167
16	+	—	II	Limited	Alive in CR (day 1138+)
17	+	—	I	Extensive	Alive in CR (day 1087+)
18	+	—	III	Extensive	Died of infection on day 370
19	+	—	II	—	Alive in CR (day419+)
20	–	—	NE	NE	Alive in CR with recipient-derived hematopoiesis (day 580)
21	+	NE	0	NE	Died of infection on day 41
22	+	Day 73	I	Extensive	Alive with ATLL (day 184+)

Abbreviation: aGVHD, acute GVHD; cGVHD, chronic GVHD; NE, not eligible.

Only one patient (case 21) did not achieve CR after allo-HSCT and seven patients experienced a relapse of ATLL. At the time of analysis, 11 patients were alive and nine of these patients remained in CR.

Quantitative measurement of HTLV-I provirus load in peripheral blood

Peripheral blood samples were collected from the patients after they gave a written informed consent. Genomic DNA was extracted from mononuclear cells (MNC) of peripheral blood using the QIAGEN DNA Midi Kit (QIAGEN, Hiden, Germany) and from paraffin-embedded sample using DEXPAT (TAKARA BIO INC, Shiga, Japan). Quantitative measurement of HTLV-1 provirus was performed with real-time quantitative PCR (RQ-PCR) using the LightCycler System and DNA Master Syber Green I (Roche diagnostics, Mannheim, Germany) as reported previously.[13] In brief, 30 ng of genomic DNA was used as a template and the copy number of HTLV-1 provirus was assessed by the ratio of the amount of tax region of HTLV-1 and that of beta globin gene (tax copies/MNC = 2 × copy number of tax/copy number of beta-globin gene). The mean value of two

experiments was shown as the copy number of HTLV-1 provirus load. Figure 1 shows the correlation between the ratios of the positive control plasmid containing tax region in the irrelevant plasmids and the results of RQ-PCR tests in a log-scale graph. A statistically significant correlation was found ($r=0.89$, $P<0.001$). This system could quantify one copy of the tax gene in 5000 cells.

Detection of primary ATLL cells with inverse PCR

To detect the residual ATLL cells, we performed an inverse PCR as reported by Takemoto *et al.*[14] that amplified the integration site of HTLV-1 in the genome of tumor cells whose sequence

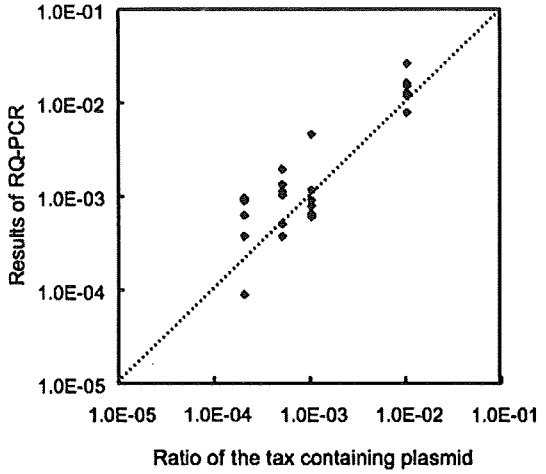

Figure 1 Correlation of the ratio of tax copy number between control plasmid and the quantification using RQ-PCR. Control plasmids containing the tax region of HTLV-1 were serially diluted with plasmids containing irrelevant sequence (beta-globin) and the ratio of target plasmid was quantified using the RQ-PCR method.

was then utilized to establish case-specific PCR primers that amplified a part of HTLV-1 (LTR) and the franking region. Each PCR in this study could at least detect one primary ATLL cell among 10 000 normal cells. PCR condition and the DNA sequence of the primer sets in nine cases tested are available upon request.

Colony formation and the expansion of HTLV-I-infected cells to test the origin of those cells

Previously, we established a method to clonally amplify HTLV-I-infected cells.[15] In brief, MNC in the peripheral blood were cultured in semisolid media containing 0.93% methylcellulose dissolved in Iscove's modified Dulbecco's medium (IMDM) supplemented with 20% fetal calf serum (FCS) and 200 ng/ml of recombinant human interleukin (rhIL)-2 (TECHNE Corp., Minneapolis, MN, USA). After three weeks of culture, each colony grown in the semisolid media was picked up individually and transferred to liquid culture (IMDM with 20% FCS and 20 ng/ml of rhIL-2) for clonal expansion. All cell culture was performed at 37°C with 5% CO_2. The origin of cells (donor or recipient) was assessed by means of sex mismatch (using Y chromosome specific SRY gene detection) or the difference of the number in short tandem repeat (STR method).

Results

Quantitative measurement of HTLV-I provirus after allo-HSCT

A total of 86 samples in 22 patients were collected; samples per patient were from 1 to 10 (median 3.5 samples) with median sampling time of 6 months from transplant (0.5 month to 8.3 years). The copy numbers of HTLV-1 provirus in each case are shown in Figure 2a and b. Most of the samples contained a low amount of HTLV-1 provirus, except for two conditions: (1)

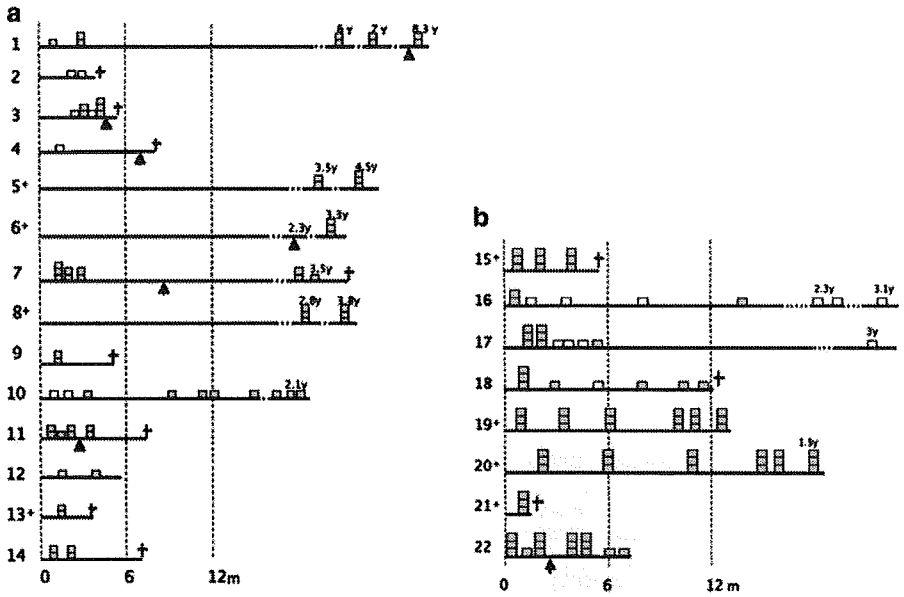

Figure 2 Quantification of HTLV-1 provirus load in the peripheral blood of recipients. Case number is on the left side of the figures. Case number with plus mark represents transplantation from a carrier donor. Copy number of provirus is shown as a gray or white box: three gray boxes represent virus load $\geq 10^{-2}$; two gray boxes, $10^{-2} \sim 10^{-3}$; one gray box, $10^{-3} \sim 10^{-5}$; white box, below detection level. Time after transplantation is described as month (m) or year (y). Cross mark represents death of the case and arrow indicates the time of relapse of ATLL. Cases treated with myeloablative conditioning are shown in (**a**) and those received RIST are in (**b**).

transplantation from a carrier donor and (2) right before (about 2 weeks) or after the clinical relapse of ATLL. In 22 samples transplanted from carrier donors, the provirus load was always 500 copies/10^5 cells or more despite the clinical disease status at sampling. The average copy number of HTLV-1 was significantly higher in patients transplanted from a carrier donor than from a noncarrier donor (mean value, 15 000 and 760 copy/10^5 cells, respectively, $P<0.0001$).

Within 6 months from transplantation, the provirus load became undetectable at least once in eight out of 15 cases (case numbers 2, 3, 4, 10, 12, 16, 17 and 18). However, in all seven cases tested later, the copy number of HTLV-1 provirus became detectable again. At the time of the last follow-up, provirus load was below the detection level in only two cases (case numbers 16 and 17). The provirus load during the early period following transplantation was not related to the type of conditioning regimen, disease status before the transplantation or the duration of survival. There was no statistically significant association between provirus loads and the development of severe acute GVHD (data not shown). No specific pattern in the kinetics of virus load was noticed among long-term survivors or among patients that experienced relapse.

Analysis of MRD in the peripheral blood

As a low level of HTLV-1 provirus load was detected in the peripheral blood of most patients, we tested whether primary ATLL cells remained as MRD using specific PCR for primary ATLL cells, which amplified a unique franking genomic region of the HTLV-1 integration site in each case. In nine cases (cases 1, 5, 9, 10, 15, 18, 19, 21 and 22), 34 samples were analyzed with this method (Table 3 and Figure 3). Although the sensitivity of the inverse PCR varied from case to case, the amount of MRD that could be detected by this method was always below the provirus load quantified by RQ-PCR in every sample (data not shown).

Eighteen out of 19 samples collected after this period were negative for MRD regardless of the presence of HTLV-1 provirus. An exception was the sample taken at the time of relapse that took place 8.3 years after transplantation in case 1. CR was continuously maintained in this case and the peripheral blood samples at 6 and 7 years from transplantation were negative in the MRD test. A subcutaneous tumor, which developed at relapse, consisting mostly of CD4-positive cells, had the same integration site of HTLV-1 as primary ATLL cells, demonstrating that the primary ATLL cells had persisted for more than 8 years as MRD.

Analysis of the origin of cells carrying HTLV-1 provirus

Although most of the cells carrying provirus were HTLV-1-infected cells and were not derived from ATLL clones, these findings raised the question of whether these infected cells derived from recipients or donors. To answer this question, we cultured peripheral blood MNC in semisolid media in the presence of rhIL-2 to clonally expand cells infected with HTLV-1. Among 10 cases that maintained CR more than a year, samples were obtained from eight cases. In five out of eight cases, we could establish 30 cell lines (Table 4). Each cell line contained HTLV-1 provirus (data not shown).

In case 20, in which the graft was rejected after transplantation, all eight cell lines were derived from the recipient cells. Among other four cases, 22 out of 23 cell lines were found to originate from the donor cells including one cell line of case 1 that received transplantation from a noncarrier donor. In case 5, despite long-term CR (4.5 years) and complete donor chimerism in the peripheral blood, there was one cell line (one of seven cell lines) that derived from a recipient. By using the established cell line of recipient origin, we determined the franking genomic sequence of HTLV-1 integration site and set up the inverse-PCR. It was applied retrospectively to the genomic DNA extracted from a paraffin-embedded lymph node, which was a biopsy sample for the initial diagnosis in case 5. The lymph node

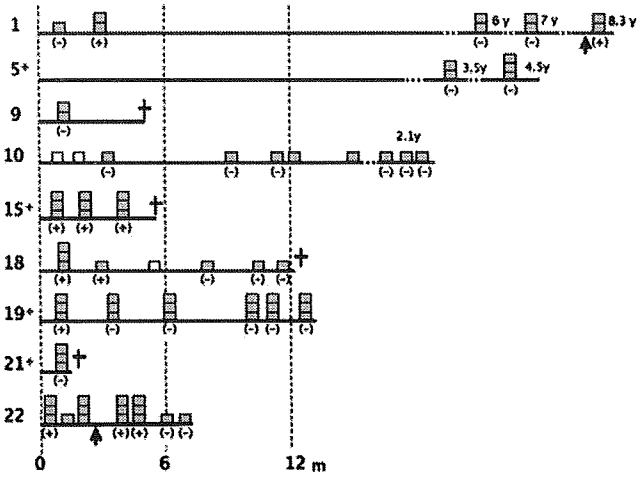

Figure 3 MRD of ATLL after transplantation. MRD of ATLL was assessed using case-specific inverse PCR method. Results of the inverse PCR are shown under the boxes that represent the copy number of provirus. Marks in this figure are the same as in Figure 2.

Table 3 DNA sequence of the franking region of HTLV-1 integration site

Case no.	*DNA sequence of the integration site*
1	AAATTTAGTACACAatatactatgacatataaagtatatgaggt...
5	AAATTTAGTACACAcagatctttccaggaaagataactttaaaa...
9	AAATTTAGTACACAtgcattaagttgaaagctggaaaaattaaa...
10	AAATTTAGTACACAaaaatgtacccaggatttgttttaatcagt...
15	AAATTTAGTACACAaggcataagccagatttacattataaatgc...
18	AAATTTAGTACACAaaatgtaaaaagcctcaagaaattgtaagc...
19	AAATTTAGTACACAgtttctaatcttattttgctgtgcaagctg...
21	AAATTTAGTACACAcatatgaactttaaagtagtttttccaat...
22	AAATTTAGTACACAggcacccagcctaaacccactgctacctga...

DNA sequence of the part of the 3′ region of HTLV-1 integration sites are shown.
Upper cases indicate the sequence of LTR.

Table 4 Origin of colony-forming cells in recipients

Case no.	*Anti-HTLV-1 Ab in the donor*	*Time (year) after HSCT at sampling*	*Number of IL-2-dependent cell lines*		
			Total	*Donor derived*	*Recipient derived*
1	–	7	1	1	0
5	+	4.5	7	6	1
8	+	3.8	5	5	0
10	–	2.1	0	—	—
16	–	2.3 and 3.1	0	—	—
18	–	1	0	—	—
19	+	1.1	9	9	0
20*	+	1.5	8	0	8

*Graft was rejected in case 20.

Table 5 Serial tests for anti HTLV-1 antibody and provirus in case 16

Time after transplantation	*3 weeks*	*1.5 months*	*0.5 year*	*1 year*	*1.6 years*	*2.3 years*	*3.1 years*
Anti-HTLV-1 antibody (PA assay)	NT	NT	x 16	UD	NT	UD	UD[a]
Proviral load	1.86×10^{-3}	UD	UD	UD	UD	UD	UD
Nested PCR test for pX region	NT	UD	UD	Positive	UD	UD	UD
PCR test for gag region	NT	NT	NT	NT	UD	UD	UD
PCR test for env region	NT	NT	NT	NT	UD	UD	UD
IL-2-dependent CFC	NT	NT	NT	NT	2 colonies	0	0

Abbreviations: CFC, colony-forming cell; NT, not tested; UD, undetectable.
[a]Undetectable with three different methods; Western blotting, particle agglutination and fluorescent antibody test.

sample had the same integration site of HTLV-1 as the cell line established 4.5 years after transplantation. Although two peripheral blood samples taken 4.5 years after transplantation were negative for this inverse-PCR, the colony-formation method could detect MRD in the same sample in case 5.

Negative results in the tests for HTLV-1 infection in case 16

In cases 16 and 17, at the time of the last follow-up, HTLV-1 provirus load was below the sensitivity of PCR (1 provirus/10^5 cells). However, the test for antibody against HTLV-1, which is widely used to demonstrate the infection with HTLV-1, was found to be negative only in case 16 (Table 5). Three different methods (Western blotting, particle agglutination and fluorescent antibody test) failed to demonstrate antibodies against HTLV-1 in this case. PCR tests for other parts apart from tax of HTLV-1, gag and env regions, were also negative. All extensive searches for HTLV-1 infection became negative 2.3 years after transplantation and remained negative 8 months later, 3.1 years from transplantation when this manuscript was written.

Discussion

In the present study, we measured HTLV-1 provirus load, detected MRD and determined the origin of HTLV-1 positive cells in the peripheral blood in 22 cases with ATLL treated with allo-HSCT. The HTLV-1 provirus load was reduced at least once to low levels (less than 1000 copies/10^5 cells) in most cases even among those who were transplanted in the status other than CR or those who received a reduced-intensity conditioning. These results showed a strong anti-ATLL effect of allo-HSCT in the short period after transplantation. The average dose of HTLV-1 provirus was significantly higher among cases transplanted from HTLV-1 carrier donors, suggesting the carryover of the virus positive cells from the donors. However, the level of provirus load after transplant did not always correlate to the final clinical outcome. Surprisingly, among most of the patients who survived more than 2 years, HTLV-1 provirus was detectable, although at a lower level, by PCR in their peripheral blood. Contrary to our results, Hishizawa *et al.*[16] using a quantitative PCR method similar to ours, reported the kinetics of HTLV-1 provirus load after allo-HSCT in five cases with ATLL, and they showed that HTLV-1 provirus load was undetectable in two cases in continuous CR. Major differences between their report and ours are the length of the follow-up period (1–15 and 1–84 months) and the number of patients (five and 22 cases). The longer observation periods and larger case number in our study might have facilitated the notice of the reappearance of HTLV-1-positive cells after allo-HSCT.

In contrast with the frequent positive results of provirus load, MRD of primary ATLL was rarely detectable after transplantation. In particular, after 6 months from transplantation, all samples of five cases tested during remission were negative for the MRD test despite the detectable level of provirus load, clearly demonstrating the presence of HTLV-1-positive cells other than ATLL in the peripheral blood of these patients.

HTLV-1-positive cells present in the recipients after allo-HSCT could be theoretically categorized into four groups: (1) MRD of primary ATLL cells, (2) non-ATLL cells of a recipient carrying HTLV-1 (e.g. T lymphocytes at the carrier state), (3) donor-derived cells infected with HTLV-1 in the host after transplant and (4) infused donor cells in the case of transplantation from a carrier of HTLV-1. Based on the results of colony-formation experiments, although the number of clones tested was not large, we demonstrated that there was difference in the origin of cells with HTLV-1 provirus. We found MRD in case 5 (as defined in group 1), donor-derived HTLV-1-positive cells in case 1 (group 3) and examples of group 4 in cases 5, 8 and 19. Non-ATLL cells of recipients were shown in case 20 (group 2). In some cases, we assumed that donor CD4-positive T cells were infected *de novo* with HTLV-1 in the recipient's body after transplantation as observed in case 1. Virus transmission into donor lymphocytes was described previously and our observation supported this report.[17]

In case 1, the MRD tests in the peripheral blood were negative in both samples taken at 6 and 7 years from transplantation; however, ATLL relapsed clinically as a subcutaneous tumor after 8 years of continuous CR. With the same integration sites of HTLV-1 in the primary and relapsed tumor cells, it was apparent that the primary ATLL cells remained somewhere in the body for more than 8 years after allo-HSCT and that negative tests for MRD in the peripheral blood did not necessarily indicate eradication of ATLL even long after transplantation.

On the other hand, in case 16, even with the extensive search for HTLV-1 provirus by PCR for various parts of HTLV-1 genome, we failed to demonstrate its presence in the peripheral blood. The antibody against HTLV-1 also became negative only in this case. So far, there has been no evidence to show the presence of HTLV-1 in this case for more than 8 months. There was a previous report of the eradication of HTLV-1 from a carrier who received allo-HSCT for pure red cell aplasia.[18] The tests for the virus performed in case 16 were almost the same as used in this report, suggesting that HTLV-1 was cleared off from the body after allo-HSCT in this case, indicating eradication of both ATLL cells and carrier T cells of HTLV-1 simultaneously by allo-HSCT.

Recently, we reported that allo-HSCT would bring about graft-versus-ATLL (GvATLL) effect even without clinically obvious graft-versus-host disease (GVHD).[10] GvATLL could be achieved when a specific immune response targeting HTLV-1 was initiated, such as cytotoxic T cells for tax protein as Harashima

et al.[19] reported. It is also possible that allogeneic immune reaction against recipient cells contributed to GvATLL effect even without HTLV-1-specific immune reactions as seen in transplantations from carrier donors. As most long-term survivors were positive for HTLV-1 provirus and anti-HTLV-1 antibody, our observation suggested that GvATLL had an effect on ATLL cells but not HTLV-1 provirus in most cases. Allogeneic immune reaction without clinically apparent GVHD might be enough to suppress ATLL cells in these situations.

In summary, allo-HSCT for ATLL profoundly reduced provirus load of HTLV-1 in recipients; however, small amounts of HTLV-1-positive cells that remained in long-term survivor were heterogeneous in origin. We also experienced the single case in which HTLV-1 seemed to be eradicated with allo-HSCT. Thus, it was suggested that the way allo-HSCT suppressed and controlled ATLL and HTLV-1 itself was not simple but heterogeneous from case to case. Further analysis is necessary to understand how ATLL is controlled by allo-HSCT through GvATLL effect, and to find how this effect be controlled and enhanced.

Acknowledgements

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Allogeneic Stem Cell Transplantation for Adult T-Cell Leukemia/Lymphoma

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Abstract

Adult T-cell leukemia/lymphoma (ATLL) develops in elderly individuals who have been infected with human T-cell leukemia virus type 1 (HTLV-1), and the prognosis for patients with ATLL has been extremely poor. Retrospective studies of allogeneic stem cell transplantation (alloSCT) for selected populations of patients have achieved several encouraging results; however, the reported incidence of transplantation-related mortality (TRM) have been high, even though more than 80% of patients received stem cells from related donors and the patients were relatively young for ATLL. This report documents a prospective feasibility study of alloSCT with reduced-intensity conditioning (RIST) for elderly ATLL patients (>50 years). Regimen-related toxicities and nonhematologic toxicities were acceptable. Fourteen of 15 evaluable patients achieved complete donor chimerism within 90 days, and 1 patient had early TRM after RIST. The HTLV-1 proviral load became undetectable in 8 of 15 patients, suggesting that RIST has potential as an antiviral treatment. The results of alloSCT are promising, and 30% to 40% of patients who achieve remission and have suitable donors can now become long-term survivors with either conventional alloSCT or RIST. It is clear that a graft-versus-ATLL effect is present after alloSCT, regardless of the conditioning regimen or the stem cell source.

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Key words: ATLL; alloSCT; RIST; HTLV-1 proviral load

1. Introduction

Despite the remarkable progress in the treatment of hematologic malignancies during the last decade, adult T-cell leukemia/lymphoma (ATLL) in elderly persons infected with human T-cell leukemia virus type 1 (HTLV-1) still remains one of the most dismal lymphoid malignancies, and no safe or effective therapy is currently available. During a 10-year period (between January 1991 and December 2000), 296 patients newly diagnosed with acute or lymphoma-type ATLL were treated with chemotherapy alone at 2 institutions (Imamura Bun-in Hospital in Kagoshima and National Kyushu Cancer Center in Fukuoka). Both hospitals are located on Kyushu Island, an area in Japan endemic for HTLV-1. After a minimum follow-up more than 6 years for all patients, only 14 (4.7%) of the 296 patients were confirmed to be alive in March 2007. The remaining patients were either dead (275 patients) or lost to follow-up (7 patients) (A.U. and N.U., unpublished data).

Continuing therapeutic trials have been conducted to improve the dismal prognosis of ATLL; however, most of the results have been disappointing [1]. In the best clinical result to date, Yamada et al used combination chemotherapy for ATLL and achieved a complete response rate of 35.5% and an overall survival (OS) rate at 2 years of 31.3% [2]. Recently, encouraging results of retrospective analyses of allogeneic stem cell transplantation (alloSCT) outcomes have been published for selected populations of patients who were younger (less than 50 years) than the mean ATLL patient age [3-5]. In addition, the results of a prospective feasibility study have recently been reported for a new transplantation strategy, alloSCT with reduced-intensity conditioning (RIST), for ATLL patients older than 50 years [6]. According to the Annual Report of Nationwide Survey of the Japan Society for Hematopoietic Cell Transplantation (JSHCT), these encouraging reports have contributed to the rapid increase in the

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number of patients with ATLL who undergo alloSCT with stem cells from various sources. In fact, according to the 2006 report, 325 cases of alloSCT for ATLL have been reported to the JSHCT registry since 1991, and 288 (89%) of these cases were reported between 2000 and 2005 [7]. It may therefore be an appropriate time to review briefly the current status and problems of alloSCT for ATLL.

2. alloSCT with Full, Intensive (Myeloablative) Conditioning Regimens: Results of a Retrospective Analysis

Sobue et al [8] were the first to describe a patient with ATLL for whom successful grafting and full hematologic recovery were documented after bone marrow transplantation (BMT) from an HLA-matched sibling donor (MSD). Unfortunately, however, the patient died 205 days after BMT of interstitial pneumonitis caused by a cytomegalovirus infection. This is a common complication of alloSCT. There have been a few reports on the successful treatment of ATLL with alloSCT, and encouraging results from a retrospective analysis of selected populations of ATLL patients who received alloSCT have been reported more recently. Utsunomiya et al [3] first indicated improved outcomes for ATLL after they reviewed 10 patients who underwent alloSCT at 9 institutions between March 1995 and February 1999 (Table 1). The clinical subtypes of the patients were acute disease (8 patients) and 1 case each of lymphoma and chronic disease. Four patients were in complete remission (CR), 5 patients were in partial remission (PR), and 1 patient experienced no remission (NR). The median age at SCT was 45 years. Nine of 10 donors were MSD, and another was an HLA-matched unrelated donor (MUD). Seven donors were negative for HTLV antibody, and 3 were positive (carrier). The stem cell sources were bone marrow (BM) in 8 cases, peripheral blood (PB) in 1 patient, and combined BM and PB in 1 patient. The conditioning regimens varied with the exception that all received a high dose of total body irradiation (TBI); therefore, all the conditioning regimens were considered myeloablative. The patients tolerated the regimens well, and engraftment occurred in all cases. Six of the 10 patients developed acute graft-versus-host disease (aGVHD), and 3 patients developed extensive chronic GVHD (cGVHD). Four patients died of transplantation-related mortality (TRM), which was due to severe aGVHD, pneumonitis, gastrointestinal bleeding, and renal insufficiency. Two of the 10 patients with no GVHD symptoms relapsed with clinical ATLL. This result suggested that alloSCT can improve survival in ATLL cases if a controllable degree of GVHD develops. In March 2007, the data were updated for the same cohort. After a median follow-up of 111 months, an additional 2 patients have died (from ATLL and TRM), and 4 patients have survived 105, 114, 119, and 120 months after alloSCT and have become long-term survivors. The estimated OS rate at 10 years is 40% (95% confidence interval [CI], 12.3%-67.0%).

Following the report of Utsunomiya et al, 2 additional reports of encouraging results from Japan have appeared in the literature. Kami et al [4] evaluated the feasibility of alloSCT in 11 ATLL patients who received alloSCT between April 1999 and April 2001 at 6 institutions (Table 1). The clinical subtypes of the patients were acute disease (6 cases), lymphoma (4 cases), and 1 case of chronic disease. Six patients were in CR, 1 patient was in PR, and 4 patients had NR. The median age at SCT was 47 years. Nine of the 11 donors were MSD, and the rest were either partially HLA-matched related donors (PMRD) or MUD. Nine patients were negative for HTLV-1 antibody, and 2 were positive. The stem cell source was BM in 7 cases, PB in 3 cases, and combined BM and PB in 1 case. The conditioning regimens were myeloablative for 9 patients, including 8 patients who received TBI. The patients tolerated the regimens well, and neutrophil recovery occurred in all cases. Ten of 11 patients survived beyond 30 days after SCT and achieved CR. Five patients developed aGVHD, and 3 of 8 evaluable patients developed extensive cGVHD. Seven patients died, and all deaths were attributed to TRM (aGVHD in 2 patients, cGVHD in 3 patients, and regimen-related toxicities in 2 patients). Four patients remained alive and disease free at a median follow-up of 2.5 months. The estimated OS rate (mean ± SD) at 1 year was 54.5% ± 30%. From the results of the pilot study, the investigators suggested that alloSCT may offer the best chance of hematologic remission and prolonged survival for ATLL patients and therefore proposed further clinical trials.

To confirm the results of previous reports, Fukushima et al [5] analyzed an additional 40 ATLL patients who underwent alloSCT between 1997 and 2002 in 7 institutions (Table 1). The clinical subtypes of the patients were acute disease (30 patients) and lymphoma (10 patients). Fifteen patients were in the first CR (CR1) or CR2, 13 were in PR, and 12 were in NR. The median age at SCT was 44 years. Of the 40 donors, 27 were MSD, 5 were PMRD, and 8 were MUD. Analyses of the donors for HTLV-1 antibody status revealed that 27 patients were HTLV-1 negative, 9 were positive, and 4 were not evaluable. The stem cell source was BM in 21 cases and PB in 19 cases. According to the authors, the conditioning regimens were myeloablative for all patients, including TBI in 18 patients. aGVHD developed in 26 of 39 patients, and 15 of 31 evaluable patients developed cGVHD. There were 21 deaths after SCT, 16 of which were related to various types of TRM. Nineteen patients were alive at the median observation period of 22.7 months. The estimated OS rate at 3 years was 45.3% (95% CI, 19.7%-58.9%). ATLL relapse occurred in 10 patients. Interestingly, the investigators reported that 5 of the 10 relapsed patients achieved another CR (by reduction or termination of immunosuppressive agents in 3 cases, by standard-dose chemotherapy in 1 case, and by local radiation therapy in 1 case), thus suggesting a graft-versus-ATLL effect induced by modulation of immunosuppression. Univariate and multivariate analyses, however, failed to show any benefit of GVHD on the prevention of relapse in this cohort.

A retrospective analysis of alloSCT administered for ATLL suggested the efficacy of this therapeutic modality, which may contribute to an improved prognosis for ATLL patients; however, the number of ATLL patients who can receive alloSCT is extremely limited because only one third of patients have an MSD or PMRD. The remaining patients are unable to find suitable donors among their family members. Furthermore, usually more than half of the siblings

Table 1.
Results of Allogeneic Stem Cell Transplantation for Adult T-Cell Leukemia/Lymphoma (ATLL)*

Reference	Patients, n	Age, y†	M/F Sex, n	Subtype, n	Donor, n	Donor HTLV-1 Ab, n	Status at SCT, n	Stem Cell Source, n	TBI, n	SCT Type, n	GVHD, n		Outcome	Cause of Death, n		Survival, %‡
											Acute	Chronic		ATLL	Overall TRM	
Utsunomiya, 2001 [3]	10	45 (33-51)	7/3	Acute, 8	MSD, 9	Neg, 7	CR, 4	BM, 8	10	Convt, 10	Yes, 6	Yes, 4	Alive, 4	1	5	40 ± 15 (12.3-67.0), at 10 y
				Lym, 1	MUD, 1	Pos, 3	PR, 5	PB, 1			No, 4	No, 6	Dead, 6			
				Other, 1			Rp/PD, 1	BM + PB, 1								
Kami, 2003 [4]	11	47 (15-59)	7/4	Acute, 5	MSD, 9	Neg, 9	CR, 6	BM, 7	8	Convt, 9	Yes, 5	Yes, 3	Alive, 4	0	7	54.5 ± 30, at 1 y
				Lym, 4	PMRD, 1	Pos, 2	PR, 1	PB, 3		RIST, 2	No, 6	No, 5	Dead, 7			
				Other, 2	MUD, 1	Rp/PD, 4	BM + PB, 1	NE, 3								
Fukushima, 2005 [5]	40	44 (28-53)	22/18	Acute, 30	MSD, 27	Neg, 27	CR, 15	BM, 21	22	Convt, 39	Yes, 26	Yes, 15	Alive, 19	4	17	45.3 (31.8-58.8), at 3 y
				Lym, 10	PMRD, 5	Pos, 9	PR, 13	PB, 19		ND, 1	No, 13	No, 16	Dead, 21			
				MUD, 8	ND, 4	Rp/PD, 12					NE, 9					
Kato, 2007 [11]	33	49 (24-59)	18/15	Acute, 20	MUD, 33	Neg, 33	CR + PR, 15	BM, 33	22	Convt, 27	Yes, 25	Yes, 4	Alive, 19	2§	9§	49.5 (31.2-78.5), at 1 y
				Lym, 7		Pos, 0	Rp/PD, 14			RIST, 6	No, 3	No, 14	Dead, 14			
				ND, 6			ND, 4					NE, 5				
Okamura, 2005 [6]	16	57 (51-67)	9/7	Acute, 11	MSD, 16	Neg, 8	CR, 3	PB, 16	0	RIST, 16	Yes, 10	Yes, 6	Alive, 5	6	4	33.3 ± 12.2 (12.2-56.4), at 5 y
				Lym, 5		Pos, 8	PR, 10				No, 5	No, 6	Dead, 10			
							Rp/PD, 3					NE, 3				

*GVHD indicates graft-versus host disease; M, male; F, female; HTLV-1 Ab, human T-cell leukemia virus type 1 antibody; SCT, stem cell transplantation; TBI, total body irradiation; TRM, transplantation-related mortality; MSD, HLA-matched sibling donor; Neg, negative; CR, complete remission; BM, bone marrow; Convt, conventional; Lym, lymphoma; MUD, HLA-matched unrelated donor; Pos, positive; PR, partial remission; PB, peripheral blood; Rp/PD, relapse/progressive disease; PMRD, partially HLA-matched related donor; RIST, reduced-intensity SCT; NE, not evaluable; ND, not determined.

†Data are presented as the median (range).

‡Data are presented as the mean ± SD (95% confidence interval).

§Cause of death not determined in 3 cases.

of ATLL patients are HTLV-1 carriers, and alloSCT from an HTLV-1–positive donor may carry the risk of promoting the development of ATLL through the addition of a new HTLV-1 load on the immunocompromised host [9]. Because of these observations, alloSCT using stem cell sources from unrelated donors has increasingly been reported.

3. alloSCT from Unrelated Donors

3.1. Unrelated BM

Several reports have described the efficacy of using BM from unrelated donors obtained from the Japan Marrow Donor Program (JMDP) for ATLL [10,11]. Nakase et al [10] reported the results of unrelated BMT (UBMT) for 8 ATLL patients at a single institution. The conditioning regimens were myeloablative for 5 patients and RIST for 3 patients. Three patients died (2 of TRM and 1 of ATLL), and 5 patients survived at a median follow-up of 20 months. To clarify the feasibility and efficacy of UBMT from an HTLV-1–negative donor for ATLL, Kato et al [11] retrospectively analyzed the registered data and clinical outcomes obtained through the JMDP for 33 ATLL patients who underwent UBMT between September 1999 and January 2004 (Table 1). The clinical subtypes of the patients were acute disease (20 patients), lymphoma (7 patients), and unknown (6 patients). Thirteen patients were in CR, 2 were in PR, and 14 were in NR. The median age at SCT was 49 years. All of the donors tested negative for HTLV-1 antibody. The conditioning regimens were myeloablative for 27 patients (including TBI in 22 patients) and RIST for 6 patients. aGVHD developed in 25 of 28 patients who achieved engraftment. The cumulative incidence of aGVHD of grades II to IV was 61%. cGVHD developed in 4 of 18 patients. There were 14 deaths after UBMT, of which 9 were related to TRM and 2 were due to ATLL; the cause of death was unknown in 3 cases. Nineteen patients were alive at a median follow-up of 139 days. The mean 1-year OS rate was 49.5% (95% CI, 31.2%–78.5%). A multivariate analysis of this cohort identified recipient age as an independent prognostic factor for OS ($P = .044$). Patients ≥50 years old and who presented with nonremission at transplantation tended to have higher TRM rates. These investigators' observations suggest that UBMT could represent a feasible treatment option for ATLL patients.

3.2. Unrelated Cord Blood

Another option for an alternative stem cell source is unrelated cord blood (CB); however, only 1 abstract, for a presentation by Wake et al from an American Society of Hematology meeting [12], has reported on the feasibility of reduced-intensity CB transplantation (RICBT) for ATLL. Eighteen patients with advanced-stage ATLL who underwent RICBT between March 2002 and June 2005 at a single institution were retrospectively analyzed. The pretransplantation conditioning regimen consisted of fludarabine, melphalan, and low-dose TBI (4 Gy). All of the patients received HLA-mismatched CB. Neutrophil and platelet engraftment were observed in 15 and 14 patients, respectively. Although 14 of the 15 patients who survived more than 30 days achieved CR, 6 died of TRM within 100 days after transplantation, and another 6 patients died of relapse. The estimated 1-year OS rate was 27.9% ± 9.0%. This pilot study indicated that RICBT is feasible even for advanced-stage ATLL patients. To further improve the outcome, these investigators suggested that RICBT should be investigated for patients in the early stages of the disease or that a new approach to prevent late relapse should therefore be developed.

4. alloSCT with RIST for ATLL: Prospective Study

If the data of 3 reports are combined [3-5], 29 of 61 patients (48%) died of TRM causes such as GVHD, pneumonitis, and gastrointestinal bleeding, even though more than 80% of the ATLL patients received stem cells from related donors and the patients were relatively young (less than 50 years). Recent developments have allowed alloSCT to be extended to elderly patients through better supportive care and the use of RIST. RIST has been extensively studied for the treatment of hematologic malignancies [13,14]. Therefore, a multi-institutional study of RIST was initiated in 2000 to clarify whether this newly developed procedure is feasible for ATLL patients with a median age of approximately 60 years. The rationale of RIST for ATLL, which is considered an allogeneic immunotherapy, is based on (1) leukemia in elderly persons, (2) the extremely poor outcomes with conventional therapeutic approaches, and (3) the evidence from previous reports suggesting that alloSCT with myeloablative regimens is effective for ATLL and may be associated with a graft-versus-leukemia effect.

4.1. First Phase 1 Study (NST-1 Trial)

4.1.1. Study Design

The eligible patients in this study [6] were 50 to 70 years of age and met the diagnostic and classification criteria for ATLL clinical subtypes. Both acute- and lymphoma-type ATLL patients were eligible. Patients were required to be in CR or PR at the time of registration and to have an MSD as a stem cell donor. The conditioning regimen consisted of 6 daily infusions of fludarabine (30 mg/m^2 for 6 consecutive days on days –8 to –3), oral busulfan (4 mg/kg per day for 2 consecutive days on days –6 to –5), and rabbit antithymocyte globulin (ATG) (2.5 mg/kg per day for 2 consecutive days on days –2 to –1). This regimen was modified from that described by Slavin et al [13]. On day 0, recipients were treated with unmanipulated, granulocyte colony-stimulating factor–mobilized PB stem cells from a sibling donor. Graft rejection and GVHD were prevented by administering cyclosporine (CsA) intravenously to all patients, starting on day –1, and oral CsA was substituted when it could be tolerated. The primary objective of the study was to evaluate the feasibility and safety of RIST, particularly with regard to the occurrence of serious adverse events, in ATLL patients with a median age of approximately 60 years. Serious adverse events were defined as engraftment failure and the occurrence of early TRM. Therefore, the primary end points were engraftment (as judged by the achievement of complete donor chimerism before day 90 after RIST) and the incidence of TRM before day 100 after RIST.

4.1.2. Results

Between March 2001 and December 2002, 16 ATLL patients were consecutively registered and underwent RIST (Table 1). The median patient age was 57 years. Eleven patients had acute ATLL, and 5 had lymphoma-type disease. Eight of the 16 donors were positive for HTLV-1 antibody, and the other 8 patients were negative. Although all patients were in remission (CR or PR) at the time of registration, 3 patients showed disease progression after registration and had active disease (ie, NR) at RIST.

4.1.2.1. Toxicity

All patients tolerated the conditioning regimen. Regimen-related toxicities were relatively mild and nonhematologic toxicity was acceptable. Only 3 of the 15 evaluable patients experienced grade 3 toxicity. No grade 4 nonhematologic toxicity was observed; however, severe transient hematologic toxicity was observed in most of the patients. The median required numbers of red blood cells and platelet transfusions were 1 and 2, respectively.

4.1.2.2. GVHD and Serious Adverse Events

Ten patients developed aGVHD, and 5 had severe aGVHD (grades III and IV). The cumulative percentage of aGVHD of grades II to IV was 63.9% ± 12.9% (mean ± SE). Six of the 13 patients who survived more than 100 days after RIST developed extensive cGVHD. One of these 6 patients subsequently died of infection complications. Episodes of infection were the most frequent complications other than GVHD. Sepsis occurred in 2 patients. Thirteen patients showed reactivation of cytomegalovirus between 6 and 82 days after RIST (median, 19 days), and all 13 patients promptly responded to the preemptive administration of ganciclovir. Two patients developed Epstein-Barr virus–associated lymphoproliferative disorder, which resolved promptly after the discontinuation of CsA treatment. TRM occurred in 4 patients on days 71, 126, 173, and 285 after RIST; TRM was related to aGVHD or cGVHD in all cases.

4.1.2.3. Early Response after RIST

Excluding the 3 patients who were in CR prior to RIST, 12 patients had evaluable disease at the time of transplantation. All 12 patients showed a good clinical response, but prompt reemergence of the disease was noted in 3 patients. As a result, 9 (75%) of the 12 patients exhibited CR by 30 days after RIST.

4.1.2.4. Disease Relapse and Outcome

Disease relapse occurred in 9 patients, and 6 of these relapses were within 100 days after RIST (median, 47 days). Relapse was seen in the lymph nodes (6 patients), lymph nodes and skin (2 patients), and lymph nodes and lung (1 patient). Interestingly, 3 patients who relapsed subsequently achieved a CR2 or PR2 after the rapid discontinuation of CsA treatment. Five patients are alive, and 10 patients have died. The data were updated as of March 2007. At a median follow-up of 61 months for the study cohort, no event has occurred after the second year following alloSCT. Five of 15 patients have survived 56 to 66 months after RIST and are currently off of immunosuppressive therapy. The estimated mean OS rate at 5 years is 33.3% (95% CI, 12.2%-56.40%). The OS rate for patients who developed aGVHD (10 patients) and those who did not (5 patients) was 50% ± 15.8% and 0%, respectively (P = .06) (Figure 1). In this first prospective study of SCT for ATLL, we have shown that RIST is feasible for ATLL.

4.2. Second Phase 1 Study (NST-2 Trial)

Although the results suggested the usefulness of RIST, disease relapse was the main cause of treatment failure in the first study. The addition of ATG to the conditioning regimen to enhance engraftment and suppress GVHD might have contributed to the high relapse rate by excessively suppressing immunologic function. The rapid proliferation of ATLL cells might outpace a developing immune response that is not potent enough to eradicate the residual ATLL cells. Therefore, in the second phase 1 study (the NST-2 trial), ATG was omitted from the conditioning regimen. The rest of the study setting, such as eligibility criteria and end points, was identical to the previous study. Fourteen patients who were registered for NST-2 tolerated the conditioning regimen. The regimen-related toxicities, nonhematologic toxicities, and GVHD incidences were similar to those of the first study. Twelve of 14 patients cleared the primary end points, and the NST-2 trial was thus considered successful. Compared with the first study, only 3 patients relapsed before day 100. This result suggests that removing ATG from the regimen can successfully reduce the incidence of early

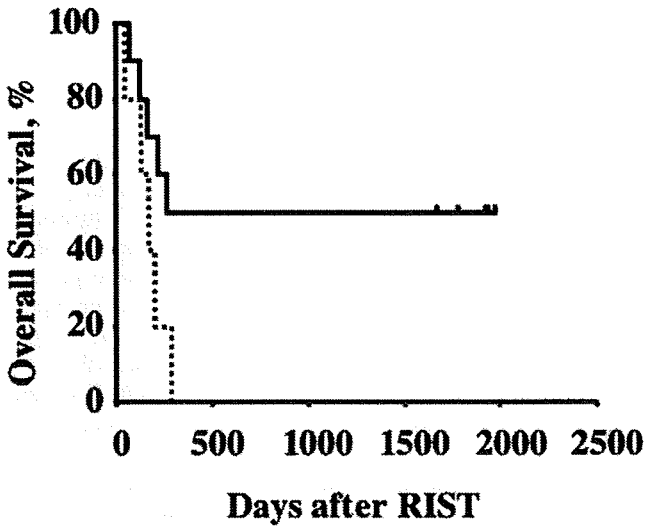

Figure 1. Kaplan-Meier plot of overall survival (OS) following reduced-intensity stem cell transplantation (RIST) for adult T-cell leukemia/lymphoma (NST-1 trial). The solid and dotted lines respectively indicate OS for patients who developed (n = 10) and did not develop (n = 5) acute graft-versus-host disease (P = .06).

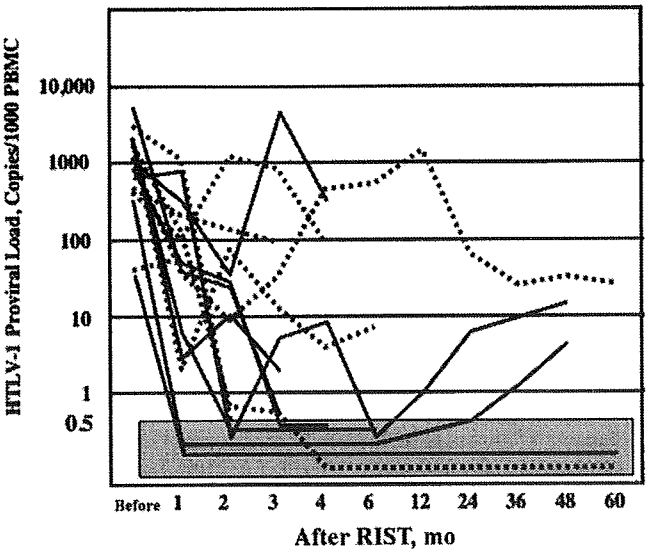

Figure 2. Kinetics of human T-cell leukemia virus type 1 (HTLV-1) proviral load after reduced-intensity stem cell transplantation (RIST) for adult T-cell leukemia/lymphoma. The solid and dotted lines indicate the HTLV-1 proviral load for patients who received peripheral blood stem cells from HTLV-1–negative donors (n = 8) and HTLV-1–positive donors (n = 7), respectively. HTLV-1 proviral load was measured serially after RIST by real-time polymerase chain reaction amplification of pX DNA and was expressed as the number of copies per 1000 peripheral blood mononuclear cells (PBMC). A load of < 0.5 copies/1000 PBMC was considered undetectable. Data are from [6] and updated as of March 2007.

relapse (R.T., unpublished data). The data from the 2 consecutive studies demonstrated the presence of a possible graft-versus-ATLL effect for RIST, which is consistent with the previous reports on alloSCT. A third study (the NST-3 trial), an ongoing phase 2 trial, is designed to confirm the usefulness of RIST for ATLL with the same conditioning regimen as used in NST-2 (fludarabine plus busulfan).

5. Anti–HTLV-1 Activity of alloSCT

ATLL is unique because the causative agent, HTLV-1, has been clearly identified, and ATLL can be monitored by quantifying the HTLV-1 proviral load in infected PB lymphocytes obtained from the patients. Therefore, the kinetics of the HTLV-1 proviral load before and after RIST were prospectively investigated during the trials. A LightCycler System (Roche Diagnostics, Indianapolis, IN, USA) was used to measure HTLV-1 proviral DNA by real-time polymerase chain reaction amplification of HTLV-1 pX DNA. The detection limit of the HTLV-1 proviral load was 0.5 copies/1000 cells [15]. In the first trial, the HTLV-1 proviral load decreased to an undetectable level (ie, <0.5 copies/1000 cells) in 8 patients within 4 months after RIST. The proviral load became undetectable in 6 of 8 patients who received PB stem cells from HTLV-1 antibody–negative healthy sibling donors. In addition, 2 of 7 patients whose donors were virus carriers showed undetectable HTLV-1 proviral levels at 2 months and 3 months after RIST. The long-term follow-up for HTLV-1 proviral levels is shown in Figure 2. Two of the 5 patients who have survived for more than 55 months continue to have an undetectable proviral load. The other long-term survivor whose donor was a carrier showed a high proviral load beyond 1 year without disease relapse. The proviral load gradually returned to the donor level after the second year. A temporary proliferation of HTLV-1–infected (nonleukemic) donor cells, which was confirmed by a chimerism analysis, might have been due to some unknown etiology. The HTLV-1 proviral loads of 2 other patients whose donors were negative for HTLV-1 antibody became detectable again 12 to 24 months after RIST. All 5 patients have continued to exhibit complete chimerism during the course of the trial and remain in CR in the second year after RIST. The dramatic decrease in the HTLV-1 proviral load to an undetectable level after RIST in more than half of the patients was unexpected. Similar results demonstrating an antiviral effect by alloSCT for ATLL have previously been described [10,16]. One of us (R.T.) has proposed a hypothesis regarding the HTLV-1 status after RIST for ATLL (Figure 3). After RIST, a patient first becomes a mixed chimera in which uninfected T-cells and HTLV-1–infected leukemic and nonleukemic cells coexist with donor cells. Then, HTLV-1 is transmitted to donor cells via cell-to-cell contact. However, HTLV-1–infected leukemic cells might be attacked and eliminated by donor-derived effecter cells via the graft-versus-ATLL effect. Moreover, HTLV-1–infected nonleukemic cells of both host and donor origin might also become the target of donor-derived effecter cells via the graft–versus–HTLV-1 effect. Consequently, nonleukemic donor cells that are infected with HTLV-1 and have

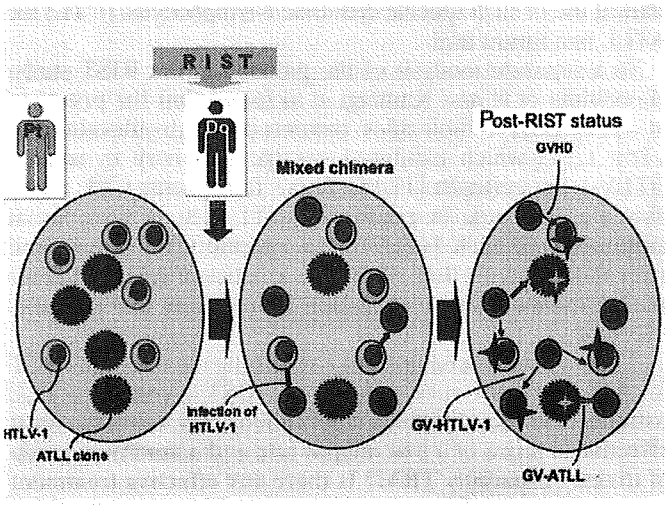

Figure 3. Hypothesis proposed by R.T. for the pathophysiological condition in patients (Pt) with adult T-cell leukemia/lymphoma (ATLL) who undergo reduced-intensity stem cell transplantation (RIST) from donors (Do). Before RIST, ATLL cells and human T-cell leukemia virus type 1 (HTLV-1)-positive and -negative T-cells coexist in patients. After RIST, the patients become mixed chimeras, and the ATLL cells are attacked by donor cytotoxic T-cells. Donor T-cells also remove HTLV-1–negative T-cells. The majority of the transplanted donor lymphocytes are then infected by residual HTLV-1–positive T-cells via cell-to-cell contact, and the patients thereby become HTLV-1 carriers. GVHD indicates graft-versus-host disease; GV-HTLV-1, graft–versus–HTLV-1 effect; GV-ATLL, graft-versus-ATLL effect.

not been completely eliminated may remain after RIST, resulting in some patients demonstrating an HTLV-1 carrier status.

6. Summary

Accumulating data suggest that alloSCT is effective for ATLL, and 30% to 40% of patients who achieve remission (PR or CR) and have suitable donors are now expected to become long-term survivors following either conventional alloSCT with a myeloablative regimen or RIST. It is clear that a graft-versus-ATLL effect is present after alloSCT, because many authors have observed that some patients, even those with refractory disease, can achieve a durable remission after transplantation, regardless of the conditioning regimen or the stem cell source. Furthermore, the observations in several reports that some patients who relapsed after alloSCT responded to a rapid discontinuation of the immunosuppressive agent alone also may support this hypothesis. Many patients still relapse after alloSCT, however, probably because the intensity of the graft-versus-ATLL effect is insufficient to control the aggressive disease. The precise mechanism by which the transplanted graft eradicates the residual leukemic cells is still not fully understood. Possible targets include minor histocompatibility antigens or leukemia-specific antigens. In certain types of leukemia, such as acute and chronic leukemias, several investigators have provided direct evidence that specific T-lymphocytes for minor histocompatibility antigens or peptides participate in the eradication of malignant cells in vivo. It would be interesting to explore the possible clinical use of such specific cytotoxic T-lymphocytes (CTL) for ATLL in a future trial.

In a separate analysis of the patients in the RIST study, Harashima et al and Kannagi et al found that the presence of HLA class I molecules restricted the proliferation of $CD8^+$ CTL, which exhibited specific reactivity to several HTLV-1 Tax epitopes [17,18]. These Tax-specific CTL might play a critical role in eradicating ATL cells in vivo. Serial monitoring of such HTLV-1 Tax–specific CTL before and after RIST may further clarify the kinetics of ATL cell eradication in vivo. This investigation is underway in a prospective study.

There are several questions concerning the use of alloSCT for ATLL. Is it also reasonable to use RIST in younger ATLL patients (<50 years)? Which conditioning regimen is the most effective in terms of a low relapse rate and a minimum grade of toxicity, especially TRM? Is there any effective treatment modality that could be used after alloSCT, such as donor lymphocyte infusion, interferon, or various kinds of immunotherapy using specific CTL, dendritic cells, and so forth, that would ensure the graft-versus-ATLL effect? Because HLA-matched related donors are available for only a limited number of patients, it is necessary to establish alloSCT from alternative stem cell sources, such as unrelated BM and CB. AlloSCT from HLA-haploidentical donors may be another option [19]. Because ATLL is a relatively rare type of leukemia/lymphoma and has an extremely poor prognosis, these questions can be answered only in prospective studies by multi-institutional cooperative groups.

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