沖縄県におけるhuman T-cell leukemia virus type I (HTLV-1)のtax genotype解析

Title

Human T-cell Leukemia Virus Type I *Tax* genotype analysis in Okinawa, the southernmost and remotest islands of Japan: Different distributions compared with mainland Japan and the potential value for the prognosis of aggressive adult T-cell leukemia/lymphoma

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Tax genotyping of HTLV-1 in Okinawa, Japan

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Abstract

Okinawa, comprising remote islands off the mainland of Japan, is an endemic area of human T-cell leukemia virus type I (HTLV-1), the causative virus of adult T-cell leukemia-lymphoma (ATL) and HTLV-1-associated myelopathy (HAM). We investigated the *tax* genotype of HTLV-1 among 29 HTLV-1 carriers, 74 ATL patients, and 33 HAM patients in Okinawa. The genotype distribution—60 (44%) *tax*A cases and 76 (56%) *tax*B cases—differed from that of a previous report from Kagoshima Prefecture in mainland Japan (*tax*A, 10%; *tax*B, 90%). A comparison of the clinical outcomes of 45 patients (*tax*A, 14; *tax*B, 31) with aggressive ATL revealed that the overall response and 1-year overall survival rates for *tax*A (50% and 35%, respectively) were lower than those for *tax*B (71% and 49%, respectively). In a multivariate analysis of two prognostic indices for aggressive ATL, Japan Clinical Oncology Group-Prognostic Index and Prognostic Index for acute and lymphoma ATL, with respect to age, performance status, corrected calcium, soluble interleukin-2 receptor, and *tax* genotype, the estimated hazard ratio of *tax*A compared with *tax*B was 2.68 (95% confidence interval, 0.87–8.25; $P = 0.086$). Our results suggest that the *tax* genotype has clinical value as a prognostic factor for aggressive ATL.

Keywords

HTLV-1, *tax* genotype, Okinawa, aggressive ATL, prognostic factor

1. Introduction

Human T-cell leukemia virus type I (HTLV-1) is the causative virus of adult T-cell leukemia/lymphoma (ATL) [1–4], a distinct peripheral T cell malignancy, and HTLV-1-associated myelopathy (HAM) [5, 6], a chronic inflammatory disease. Aggressive ATL (i.e., acute, lymphoma, or unfavorable chronic type) has a very poor prognosis, with a median survival time (MST) of 8–13 months [7–9].

Southwest Japan, particularly Okinawa Prefecture comprising remote islands off mainland Japan and the Kyushu region of mainland Japan, has the highest prevalence of HTLV-1 in the world. The other main endemic areas are Central Africa, the Caribbean coast, South America, and Melanesia. HTLV-1 is transmitted by cell-cell contact via breastfeeding [10] and sexual intercourse [11]. The lifetime risks of developing ATL and HAM from HTLV-1 carriers (HCs) have been estimated at approximately 5% [12] and 0.25% [13] respectively, and some HCs are asymptomatic throughout their lifetime. Based on a recent nationwide survey, the prevalence of HTLV-1 is approximately 1.08 million in Japan [14].

HTLV-1 exists in a provirus form, consisting of a 5′ LTR, *gag*, *pro*, *pol*, *env*, *pX*, and a 3′ LTR region within the host genomic DNA [4, 15]. Tax, a transcriptional regulator encoded by *pX* [16], is associated with ATL development via the transcriptional regulation of HTLV-1 provirus genes [17] and cellular genes. Multiple functions of Tax have been reported, including resistance to apoptosis by NF-κB activation [18, 19], repression of tumor suppresser p53-dependent transcription [20] impediment of DNA repair [21] and obstruction of cell cycle check points [22]. In the pathogenesis of ATL, these molecular mechanisms are thought to lead to the immortalization and genome instability of HTLV-1-infected CD4-positive T cells, resulting in ATL development. Inactivation of the *tax* gene in fresh ATL cells has been reported [23], despite the association between Tax and the onset of ATL. It is possible that *tax* expression in ATL cells is suppressed to allow the cells to evade

CD8-positive cytotoxic T lymphocytes [24, 25]. However, the mechanisms leading to the malignant transformation of HTLV-1-infected cells are not completely clear.

HTLV-1 is classified into three major phylogenetic subtypes—Melanesian, Central African, and cosmopolitan—based on the 5′ LTR sequence [26–28]. The cosmopolitan subtype, which is widely disseminated worldwide, is further divided into four subgroups: transcontinental, Japanese, West African and North African [29, 30]. Among them, the Japanese and the transcontinental subgroups have been isolated in Japan, and the former is the dominant subgroup in mainland Japan (9/10 in Hokkaido, 9/9 in Honshu, and 41/54 in Kyushu, Fig1) [31]. However, a large-scale nationwide Japanese study of HTLV-1 genotypes has not yet been conducted.

Furukawa *et al*. analyzed the *tax* gene by sequencing 61 HAM, 55 ATL, and 62 HCs samples in Kagoshima Prefecture (in the Kyushu region of mainland Japan), and found 4 specific nucleotides substitutions (positions 7897, 7959, 8208, and 8344) in 20 cases, termed *tax*A, and consensus sequences at these 4 nucleotide positions in all other cases, which were termed *tax*B. They phylogenetically compared the *tax* gene (all 20 *tax*A and 21 representative *tax*B samples) with other HTLV-1 genes (ATK, ATL-YS, BOI, H5, Rk13, and TSP-1), for which the full sequences are known. The phylogenetic tree clearly showed that HTLV-1 genomes with *tax*A and *tax*B belonged to the transcontinental and the Japanese subgroups, respectively. To confirm this correlation between the *tax* subgroups and LTR classification, they also sequenced the LTR region of 4 and 3 HTLV-1 specimens with *tax*A and *tax*B genotypes, respectively, and showed that they belong to the transcontinental and the Japanese subgroups, respectively. The *tax*B genotype is dominant (>90%) in Kagoshima Prefecture, and the *tax*A genotype is more frequent in patients with HAM than HCs [32].

Okinawa Prefecture is located in the subtropical southernmost point of Japan and is an important HTLV-1 endemic area. Recently, we conducted a large-scale retrospective study of 659 patients with aggressive ATL in Okinawa Prefecture and demonstrated poorer prognosis and a greater number of patients older than 90 years compared with patients in mainland Japan [33], as well as a high rate of *Strongyloides stercoralis* infection [34]. Based on these observations, we hypothesized that the genetic background of HTLV-1 differs between ATL patients in Okinawa Prefecture and mainland Japan. Hence, in this study, we examined the *tax* genotypes of HCs, ATL patients, and HAM patients in the prefecture to clarify the distribution. In addition, we explored the association between the *tax* genotype and prognosis in patients with aggressive ATL.

2. Methods

2. 1. Patients and specimens

A total of 103 samples were obtained from 29 HCs and 74 ATL patients (45 acute, 12 lymphoma, 7 chronic, 10 smoldering) at four institutions (University of the Ryukyus Hospital, Heartlife Hospital, Nakagami Hospital, and Naha City Hospital) in Okinawa Prefecture from January 2013 to October 2015. HCs were checked using particle agglutination tests with the anti-HTLV-1 antibody and ATL patients were diagnosed based on the criteria proposed by the Japan Clinical Oncology Group (JCOG) [7]. Peripheral blood mononuclear cells (PBMCs) were separated from peripheral blood of all HCs and 63 ATL patients using the Ficoll-Hypaque method. Eight lymph nodes and 3 skin lesions were harvested from among the ATL patients. Specimens were stored at -80°C until use. Collection of PBMCs and subsequent analyses of 33 patients with HAM in Okinawa Prefecture were conducted by multiple collaborating laboratories at the Department of

Microbiology, Kawasaki Medical School, Okayama Prefecture.

The study protocol was approved by the Institutional Review Board of each institution. All patients provided prior written informed consent to participate in the study.

2. 2. Analysis of HTLV-1 tax

Genomic DNA was extracted from cryopreserved specimens (PBMCs, lymph nodes, and skin lesions) using the QIAGEN Blood/DNA Mini Kit (Hilden, Germany) according to the manufacturer's protocol. To identify the *tax* genotype, nested polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) was performed, as previously reported [32]. Briefly, 30–50 ng of DNA was amplified by PCR using 0.2 μM *tax* gene-specific primers (PXO1^{+}, 5′-TCGAAACAGCCCTGCAGATA-3′ [7257–7267]; PXO2^{-}, 5′-TGAGCTTATGATTTGTCTTCA-3′ [8447-8467]). The following cycling conditions were used: initial denaturation at 95°C for 30 s, 20–30 cycles of denaturation at 95°C for 15 s, annealing at 62°C for 1 min, and extension at 72°C for 1.5 min, followed by a final extension at 72°C for 5 min. Then, a 1-μL aliquot of the PCR product was used as a template for the second PCR with 0.2 μM nested primers (PXI4^{+}, 5′-CACGCTAACAGCCTGGCAAAA-3′ [7972–7992]; PXI3^{-}, 5′-AGACGTCAGAGCCTTAGTCT-3′ [8374–8393]) under the following conditions: denaturation at 95°C for 30 s, 20–30 cycles of denaturation at 95°C for 15 s, annealing at 62°C for 30 s, extension at 72°C for 30 s, and a final extension at 72°C for 5 min.

The second PCR product (3 μL) was digested with 0.5 U/μL *Acc*II restriction enzyme (Takara, Kusatsu, Japan) and subsequently separated by electrophoresis on 2% agarose gel. Four nucleotide positions (7897, 7959, 8208, and 8344) were simultaneously substituted between the *tax*A and *tax*B genotype (deposited in the DDBJ/Genbank/EMBL

database under accession number J02029) [32] and the nucleotide at position 8344 was replaced with an *Acc*II restriction site in the *tax*A genotype. After *Acc*II digestion, the *tax*A fragment should be shorter than the *tax*B fragment (theoretical sizes, 372 and 422 bp, respectively). HTLV-1-infected T cell lines MT1 and MT2 were used for references.

2. 3. Follow-up study

Clinical data, including age, sex, performance status (PS), Ann-Arbor stage, white blood cell count, ATL cell subset, serum albumin, corrected calcium, soluble interleukin 2 receptor (sIL-2R), and lactate dehydrogenase were obtained for 45 patients with aggressive ATL (35 acute, 10 lymphoma) who received initial chemotherapy and for whom adequate judgment of the response criteria were obtained at the final follow-up (among 57 patients with aggressive ATL). The chemotherapy regimen was chosen by attending physicians based on the general condition of the patient, except one patient with *tax*A who was enrolled single-arm confirmatory trial using allogeneic hematopoietic stem cell transplantation for aggressive ATL (JCOG0907, UMIN000004147). We regarded VCAP-AMP-VECP (cyclophosphamide, doxorubicin, vincristine, ranimustine, vindesine, carboplatin, etoposide, and prednisone) and CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) as adequate intensity chemotherapies based on a JCOG phase III randomized trial for aggressive ATL (JCOG9801) (MST: 13 months in the VCAP-AMP-VECP arm, and 11 months in the CHOP arm) [9]. To the adequate intensity chemotherapy, we added THP-COP (cyclophosphamide, pirarubicin, vincristine, and prednisone), which can achieve a 42.5% of complete response (CR) rate equivalent to 41.4% in CHOP in elderly patients with non-Hodgkin lymphoma [35].

2. 4. Statistical analysis

Fisher's exact test was used to compare the distributions of *tax* genotypes among patients. The Kaplan–Meier method was applied to calculate the OS of patients with aggressive ATL and the log-rank test was used to assess the discrepancy between *tax* genotypes. Data obtained for patients who were alive at the time of the last follow-up were censored. The Cox proportional hazard model was used to estimate the effect of *tax* genotype on OS. All statistical analyses were conducted using STATA (Version 13, Stata Corporation, College Station, TX, USA).

3. Results

3. 1. Distribution of tax genotypes in Okinawa Prefecture

We detected PCR-RFLP products as fragments of 422 bp (*tax*B) and 372 bp (*tax*A), as expected (Fig 2). We successfully amplified all DNA samples and identified the *tax* genotypes by nested PCR-RFLP.

Table 1 shows the distribution of *tax* genotypes categorized according to clinical diagnosis and ATL subtype. Among the 136 cases, there were 60 (44%) *tax*A and 76 (56%) *tax*B genotype cases (Fig 1). According to clinical diagnosis, we observed 13 (45%) patients with *tax*A genotype and 16 (55%) patients with *tax*B genotype among 29 HCs, 26 (35%) and 48 (65%) among 74 patients with ATL, respectively, and 21 (64%) and 12 (36%) among 33 patients with HAM, respectively. Among the 74 patients with ATL, we observed 12 (27%) of 45 acute type, 5 (42%) of 12 lymphoma type, 3 (43%) of 7 chronic type, and 6 (60%) of 10 smoldering type with the *tax*A genotype.

3. 2. Comparison of clinical characteristics of patients with aggressive ATL between

the two tax genotype groups

We compared the clinical characteristics of 45 patients with adequate follow-up data (among 57 patients with aggressive ATL) with respect to the *tax* genotype (Table 2). Among the patients, 14 (10 acute, 4 lymphoma) and 31 (25 acute, 6 lymphoma) belonged to the *tax*A and *tax*B groups, respectively. The median ages of the *tax*A and *tax*B groups were 67 (53–82) and 69 years (42–90), respectively. According to the two prognostic indices for aggressive ATL, Japan Clinical Oncology Group-Prognostic Index (JCOG-PI) [36] and Prognostic Index for acute and lymphoma ATL (ATL-PI) [37], we divided the patients into two groups (moderate and high-risk) and three groups (low, intermediate, and high-risk), respectively. The moderate and high-risk groups for JCOG-PI each had 7 patients with the *tax*A genotype and 7 and 24 patients with the *tax*B genotype, respectively ($P = 0.088$). We did not detect disparity among the distributions (low, intermediate, high; 3, 6, and 5 in the *tax*A group and 5, 15, and 11 in the *tax*B group, respectively) for ATL-PI ($P = 0.915$).

3. 3. Comparison of the response to chemotherapy and OS between the tax genotype groups in patients with aggressive ATL

The median and longest follow-up periods for the 45 patients with aggressive ATL were 6 and 22 months, respectively. In total, 79% (11/14) of patients in the *tax*A genotype group and 74% (23/31) in the *tax*B genotype group received adequate intensity chemotherapy with VCAP-AMP-VECP, CHOP, and THP-COP (Table 2). Among the remaining 11 patients, 6 (1 *tax*A, 5 *tax*B) received etoposide monotherapy; 1 (*tax*A) received vincristine monotherapy; 2 (1 each) received mogamulizumab, an anti-C-C chemokine receptor 4 antibody, monotherapy; 1 (*tax*B) received EPOCH (cyclophosphamide, doxorubicin, vincristine, prednisone, and etoposide); and 1 (*tax*B) had no treatment due to

early death.

We found that 7 (50%) of 14 patients in the *tax*A genotype group achieved an overall response (CR or partial response), compared with 22 (71%) of 31 patients in the *tax*B genotype group ($P = 0.296$; Table 3). Seven patients in the *tax*A genotype group and 13 patients in the *tax*B genotype group had died by the last follow-up. The 1-year OS rate was 35% in the *tax*A group and 49% in the *tax*B group ($P = 0.35$, Fig 3).

3. 4. Multivariate analysis of the prognostic impact of tax genotype

We subsequently conducted a multivariate analysis to adjust for confounding factors. We selected four variables—age, PS, corrected calcium, and sIL-2R—that have been previously identified as prognostic factors for aggressive ATL in JCOG-PI [36] and ATL-PI [37] analyses, in addition to the *tax* genotype. We detected significant hazard ratios (HR) for the old age group (≥70 years), high PS group (2–4), and high sIL-2R group (≥20000) compared with each control group, but not for corrected calcium (Table 4). The estimated HR of the *tax*A group compared with the *tax*B group was 2.68 (95% confidence interval (CI), 0.87–8.25; $P = 0.086$; Table 4).

4. Discussion

We revealed that the distribution of HTLV-1 subtypes, in which 44% of patients had the *tax*A genotype and 56% had the *tax*B genotype, in Okinawa Prefecture, was quite different from that found in Kagoshima Prefecture on mainland Japan. In that report, the distribution was 10% (49/447) *tax*A and 90% (398/447) *tax*B among 55 ATL patients, 192 HAM patients, and 200 HCs randomly selected from blood donors (Fig 1) [32]. The present study is the first to show that the *tax* genotype has potential value as a prognostic indicator

of aggressive ATL.

There are three distinct populations of ethnic groups in Japan. These populations represent descendants of the Ryukyuan in the south (Okinawa), descendants of the Ainu in the north (Hokkaido), and those of the Yamato people, who comprise most of the population of Japan. It is thought that the Ryukyuan and Ainu peoples are descendants of aboriginal Japanese populations who have been present since the prehistoric Jomon Age (12,000–2,300 years ago), while the Yamato people are descendants of immigrants from the Asian continent after the Yayoi Age (2,300–1,700 years ago) [38, 39]. In a previous report in the 1980s, an anti-HTLV-1 antibody was more frequently detected in descendants of the Ryukyuan (219/1235, 17.7%) and the Ainu (77/412, 18.4%) than in those of the Yamato people (8/899, 0.89%) [40]. Based on the uneven rates of HTLV-1 infection between aboriginal Japanese and Yamato lineages, Ishida et al. suggested that HTLV-1 infection in Japan may have originated from aboriginal Japanese populations [41]. However, our study revealed different distributions of HTLV-1 genotypes between Okinawa Prefecture and mainland Japan. Furthermore, another previous study demonstrated that the distribution of HTLV-1 subtypes in 22 HCs in Kakeroma Island, which is between Okinawa Prefecture and mainland Japan, was 27.3% transcontinental type and 72.7% Japanese type [42], and these rates were intermediate between those observed in Okinawa Prefecture in our study and those in the Kagoshima Prefecture study. This gradient in the HTLV-1 subtype distribution suggests that not all HTLV-1 in mainland Japan originated from aboriginal Japanese people.

The distribution of HTLV-1 genotypes among 41 HCs in Taiwan, located approximately 600 km southwest of Okinawa Prefecture, was 70% transcontinental subgroup and 30% Japanese subgroup [43], indicating a higher rate of patients in the transcontinental subgroup than that in Okinawa Prefecture found in our study. Meanwhile, a

recent molecular anthropological study including genome-wide SNP analysis demonstrated that there is genetic differentiation between inhabitants of Okinawa Prefecture and mainland Japan, and among islanders of Okinawa Island and the Yaeyama Islands and Miyako Islands, which are part of the greater Okinawa archipelago [44]. In addition, no genetic relationship between residents in Okinawa Prefecture and aboriginal Taiwanese has been reported [44]. To elucidate the transmission of HTLV-1 in Japan, additional analyses of the differences in distribution of HTLV-1 genotypes are needed not only in each island of Okinawa Prefecture, but also in each area of Southeast and east Asia.

In this study, we followed the clinical courses of 45 patients with aggressive ATL and compared the prognosis between patients with *tax*A and *tax*B genotypes. We found that the overall response and OS rates in 14 patients with the *tax*A genotype were poorer than those in 31 patients with the *tax*B genotype (overall response rate, 50% and 71%; 1-year OS, 35% and 49%, respectively), although the differences were not statistically significant (Fig 3, Table 3). Additionally, in a multivariate analysis, the HR of the *tax*A group compared with the *tax*B group was approximately 2.68 (95% CI, 0.87–8.25, $P = 0.086$, Table 4). Our results suggest that the *tax*A genotype represents a poorer prognostic factor compared with the *tax*B genotype in patients with aggressive ATL, although this study has some limitations including a small number of patients, heterogeneous treatments, and short follow-up periods. A large-scale comparison of survival time between *tax*A and *tax*B genotypes in patients with aggressive ATL is needed to validate the value of the *tax* genotype as a prognostic factor.

Our recent retrospective analysis of 659 patients with aggressive ATL in Okinawa Prefecture revealed that the MST of 6.5 months for the entire cohort [34] was lower than that of 8–13 months observed in previous reports in other areas in Japan [7–9]. Furthermore, among the 217 patients with a clinical status similar to that described in the eligibility criteria

of the JCOG9801 study, 147 who received CHOP had lower MSTs than those in the CHOP-14 arm of JCOG9801 (8 vs. 11 months) [34]. The higher frequency of HTLV-1 patients with the *tax*A genotype (44%) in Okinawa compared with that in mainland Japan (10%) might explain the poor outcome of patients with aggressive ATL in Okinawa.

An integrated molecular analysis of ATL patients has shown that somatic alterations are highly enriched for T-cell receptor (TCR)/NF-κB signaling, the G-protein coupled receptor associated with T-cell migration, and other T-cell-related pathways, as well as immune surveillance-related genes, *PLCG1*, *PRKCB*, *CARD11*, *VAV1*, *IRF4*, *CCR4*, and *CCR7*-activating loci, and *CTLA4-CD28* and *ICOS-CD28* fusion genes [45]. Notably, many somatic alterations were converged on the *Tax* interactome; for instance, genes relating to TCR/NF-κB pathway, activated by *Tax*, had gain-of function mutations, while loss-of-function mutations were found in *TP53* and *CDKN2A*, inactivated by *Tax* [45]. These findings suggest that ATL cells acquired alterations in the *Tax* interactome to compensate for lack of Tax, although the expression of *tax* was inactivated to escape immune surveillance. Meanwhile, the analyses were conducted using ATL cells obtained from institutions in mainland Japan, where *tax*B HTLV-1 carriers have a wide distribution, based on previous reports [31, 32], and the differences in genetic expression profiles among *tax* variations are still unknown. A comparative analysis of the gene profiles of patients with ATL between the *tax*A and *tax*B genotypes might be useful for the detection of genes associated with poor sensitivity to chemotherapy.

In conclusion, we clarified the differences in the distribution of HTLV-1 genotypes between Okinawa and mainland Japan, and revealed the potential value of the *tax* genotype as a prognostic factor for aggressive ATL, despite the small number of patients, heterogeneous treatments, and short follow-up periods. A survey of the distribution of

HTLV-1 genotypes in other areas in the Asian-Pacific region may elucidate the route of HTLV-1propagation. We are currently conducting genotypic studies of HTLV-1 carrier cohorts on each remote island in Okinawa, as well as further investigations in other parts of Southeast Asia.

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Author Contributions

T.F. designed the study. S.S. and M.S. analyzed data. S.S. and T.F. wrote the paper. M.S., M.M., T.T., N.T., T.M., M.H, S.K., S.N., I.T., Y.N., K.T., K.M., J.U., S.M., K.K., Y.T. and H.M. collected data and reviewed the paper.

Disclosure of Conflicts of Interest

The authors report no potential conflict of interest.

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Table 1.

	No. of patients (%)		
	*tax*A	*tax*B	
	60 (44)	76 (56)	*P*
Clinical diagnosis			
HTLV-1 carrier	13 (45)	16 (55)	0.022
ATL	26 (35)	48 (65)	
HAM	21 (64)	12 (36)	
ATL subtype at diagnosis			
Acute	12 (27)	33 (73)	0.196
Lymphoma	5 (42)	7 (58)	
Chronic	3 (43)	4 (57)	
Smoldering	6 (60)	4 (40)	

Table 2.

Clinical features		No. of patients (%) *tax*A (n = 14)	*tax*B (n = 31)	*P*
Age at diagnosis (years)	<70	7 (50)	17 (55)	1.000
	≥70	7 (50)	14 (45)	
	Median (range)	67 (53–82)	69 (42–90)	
Sex	Male	8 (57)	12 (39)	0.336
	Female	6 (43)	19 (61)	
Subtype	Acute	10 (71)	25 (81)	0.700
	Lymphoma	4 (29)	6 (19)	
ECOG PS	0–1	7 (50)	10 (32)	0.326
	2–4	7 (50)	21 (68)	
Ann Arbor stage	III	2 (14)	1 (3)	0.224
	IV	12 (86)	30 (97)	
LDH, IU/L	<230	3 (21)	1 (3)	0.082
	≥230	11 (79)	30 (97)	
Serum albumin, g/dL	<4	12 (86)	24 (77)	0.698
	≥4	2 (14)	7 (23)	
Corrected Ca, mmol/L	<2.75	12 (86)	19 (61)	0.165
	≥2.75	2 (14)	12 (39)	
sIL-2R, U/mL	<20,000	8 (57)	14 (45)	0.53
	≥20,000	6 (43)	17 (55)	
WBC, count/μL	<18000	10 (71)	12 (39)	0.057
	≥18000	4 (29)	19 (61)	
ATL cell subset, %	<30	10 (71)	12 (39)	0.057
	≥30	4 (29)	19 (61)	
JCOG-PI	Moderate	7 (50)	7 (23)	0.088
	High	7 (50)	24 (77)	
ATL-PI	Low	3 (21)	5 (16)	0.915
	Intermediate	6 (43)	15 (48)	
	High	5 (36)	11 (36)	
Regimen of chemotherapy	VCAP-AMP-VECP	3 (21)	7 (23)	0.808
	CHOP	4 (29)	11 (35)	
	THP-COP	4 (29)	5 (16)	
	Etoposide	1 (7)	5 (16)	
	Other	2 (14)	3 (10)	
Follow-up period, month (range)		4.3 (0.7–16.6)	6.2 (0.3–21.5)	

Table 3.

	No. of patients (%)		
	*tax*A (n = 14)	*tax*B (n = 31)	*P*
Response			
CR+PR	7 (50)	22 (71)	0.296
SD+PD	6 (43)	8 (26)	
Not evaluated	1 (7)	1 (3)	

Table 4.

Variables	HR (95% CI)	*P*
Tax genotype		
*tax*B	Reference	
*tax*A	2.68 (0.87–8.25)	0.086
Age		
<70	Reference	
≥70	4.22 (1.51–11.77)	0.006
ECOG PS		
0–1	Reference	
2–4	5.33 (1.38–20.57)	0.015
Corrected calcium		
<2.75 mmol/L	Reference	
≥2.75 mmol/L	1.03 (0.391–2.704)	0.955
sIL-2R		
<20,000 U/mL	Reference	
≥20,000 U/mL	3.57 (1.21–10.53)	0.021